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State of the Art Enamel Remineralization Systems: The Next Frontier in Caries Management

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Abstract
The principles of minimally invasive dentistry clearly dictate the need for clinically effective measures to remineralize early enamel caries lesions. While fluoride-mediated remineralization is the cornerstone of current caries management philosophies, a number of new remineralization strategies have been commercialized or are under development that claim to promote deeper remineralization of lesions, reduce the potential risks associated with high-fluoride oral care products, and facilitate caries control over a lifetime. These non-fluoride remineralizing systems can be broadly categorized into biomimetic enamel regenerative technologies and the approaches that repair caries lesions by enhancing fluoride efficacy. This paper discusses the rationale for non-fluoride remineralization and the mechanism of action, challenges, and evidence behind some of the most promising advances in enamel remineralization therapies.

Dental caries pathophysiology is not simply a continual cumulative loss of tooth minerals, but rather a dynamic process characterized by alternating periods of demineralization and remineralization. Lesion progression or reversal depends on the equilibrium between demineralization-favouring pathological factors (cariogenic bacteria, fermentable carbohydrates, salivary dysfunction) and the protective factors (antibacterial agents, sufficient saliva, remineralizing ions) that tip the balance towards remineralization [Featherstone and Chaffee, 2018]. Remineralization can occur as a natural repair process where plaque/salivary calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions are deposited into crystal voids of the demineralized tooth structure, resulting in net mineral gain. The presence of free fluoride (F^-) ions in the oral environment can drive the incorporation of Ca^{2+} and PO_4^{3-} ions into the crystal lattice, with the ensuing fluorapatite mineral significantly more resistant to a subsequent acid challenge [ten Cate, 1999].

A better understanding of regenerative and physiochemical mechanisms has influenced the development of a number of innovative remineralization technologies that go beyond fluoride-mediated remineralization. While traditional fluoride-based remineralization re-

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mains the cornerstone for caries management with the highest level of supporting evidence, additional remineralizing agents to enhance fluoride effects are often needed in high caries risk individuals and population groups [Amaechi and van Loveren, 2013; Fontana, 2016]. The first International Conference on Novel Anticaries and Remineralizing Agents had suggested that the broad aim of new remineralization therapies should be to "facilitate caries control over a lifetime using evidence-based, clinically effective, multifactorial prevention to keep the caries process in balance" [Pitts and Wefel, 2009]. This paper discusses the rationale for using non-fluoride remineralization systems and the mechanisms, challenges, and evidence underpinning some of the technological advances in enamel remineralization therapies.

Non-Fluoride Remineralization Systems: Are They Needed?

Natural Remineralization Alone Is Not Sufficient

The remineralization potential of saliva is well documented [Stookey, 2008], having evolved to deliver Ca^{2+} and PO_4^{3-} ions in a bioavailable form for hard tissue development and maintenance throughout life [Cochrane and Reynolds, 2012]. At physiological pH, saliva is supersaturated with phosphoprotein-stabilized Ca^{2+} and PO_4^{3-} ions, ensuring that the ions remain bioavailable to diffuse into mineral deficient lesions [Cochrane et al., 2010]. However, longitudinal studies that followed the natural progress of white spot lesions (WSL) found that although some WSL get smaller, the majority are largely unaffected even after 2 years [Mattousch et al., 2007; van der Veen et al., 2007]. Moreover, net salivary remineralization is a slow process [Dowd, 1999], with a tendency for mineral gain only on the surface of the WSL due to the low ion concentration gradient from saliva into the lesion [Silverstone, 1972]. Fluoride-mediated salivary remineralization is also seen to be restricted to the outer 30 μm of the tooth [Schmidlin et al., 2016]. This surface-only remineralization improves neither the aesthetics nor the structural properties of the subsurface lesion [Cochrane et al., 2010]. The presence of additional extrinsic sources of stabilized Ca^{2+} and PO_4^{3-} ions could augment the natural remineralization potential of saliva by increasing diffusion gradients favouring faster and deeper subsurface remineralization.

Fluoride – Improving Its Efficacy and Safety

The pivotal discovery of fluoride as agent that could prevent dental caries was one of the most important landmarks in dentistry [ten Cate, 2015]. The dramatic decline in caries prevalence rates of developed countries from the latter half of the 20th century has been largely attributed to the widespread use of oral care products containing fluoride [Fejerskov, 2004]. Fluoride remains the gold standard for arresting caries lesions with multiple systematic reviews confirming the role of fluoride products in preventing dental caries [Benson et al., 2013; Marinho et al., 2003, 2015, 2016; Shahid, 2017]. However, emerging epidemiological data are showing a worrying trend, with caries experience plateauing or even increasing in some population groups, despite the regular use of fluoride dentifrices in these countries [Agustsdottir et al., 2010; AIHW, 2018; Dye et al., 2017; Haugejorden and Birkeland, 2005]. This raises questions on whether the earlier reduction in caries prevalence rates has continued into this century [Gimenez et al., 2016]. The reported pause in the decline of dental caries has been attributed to the fact that diets across the world are changing to include more processed and sugar-laden foods, limiting the repair potential of fluoride [Duggal et al., 2001]. While under normal physiological conditions, fluoride and salivary homeostatic mechanisms are often enough to remineralize early lesions, these are not adequate in highly cariogenic oral environments. Other at-risk population groups (xerostomia patients, elderly individuals at risk of root caries) can also benefit from boosters to improve the remineralizing and preventive efficacy of fluoride [Fontana, 2016].

An obvious approach to increase the remineralizing potential of fluoride would be to just add more fluoride to oral care products. Dentifrices with 5,000 ppm fluoride have been found to be more efficacious for remineralization of root caries lesions than 1,000–1,500 ppm fluoride dentifrices [Wierichs and Meyer-Lueckel, 2015]. A dose-response relationship of decreasing caries incidence with increasing dentifrice fluoride concentration has also been observed [Walsh et al., 2010]. However, the recent classification of fluoride as a chemical neurotoxicant could raise safety concerns among the general public regarding the use of high concentration fluoride products [Grandjean and Landrigan, 2014]. More pertinent are the growing concerns that children today are exposed to fluoride from multiple sources, potentially increasing their risk of developing dental fluorosis [Zohoori and Maguire, 2018]. This "halo" effect of fluoride probably accounts for the increased prevalence of permanent tooth mottling being seen in western countries [McGrady et al., 2012; Pendrys, 2000]. The increased risk of dental fluorosis has led the World Health Organization (WHO) to recommend the

need to assess total fluoride exposure of the population before introducing any additional fluoridation for caries prevention [Baez and Marthaler, 2014]. Besides fluorosis in children, the surface-only remineralization that often occurs in the presence of high topical fluoride concentrations can increase the incidence of occult caries ("fluoride syndrome") across all age groups [Ball, 1986]. Considering the narrow "dose gap" between caries reduction benefit and fluoride side effects, regulatory authorities have limited the fluoride concentration in non-prescription toothpastes to within 1,000–1,500 ppm, while for children below 6 years this dose is even lower and probably suboptimal for effective remineralization of early lesions.

Evidently, there is a need for new-age remineralization technologies with an ability to complement fluoride, close the gap in its remineralizing efficacy, and effect a fuller consolidation of carious lesions [Lynch and Smith, 2012]. Effective non-fluoride remineralization systems can also potentially allow dental products to be designed with lower fluoride concentrations, to allay the safety concerns associated with consumer oral care products containing high fluoride concentrations.

Modern Caries Management

Enamel caries presents as a progressive subsurface demineralization that if not reversed will result in mechanical failure and cavitation, often leading to a vicious restoration cycle. Despite long-standing recommendations for adopting a biological approach to caries management [Pitts, 2004], a significant proportion of dentists continue with the restorative-only model that has failed both clinically and economically [Innes and Schwendicke, 2017; Pitts and Zero, 2016]. There is a global consensus that the principal approach to modern-day caries management should be to "preserve the tooth structure and restore only when necessary" [Ismail et al., 2013]. New remineralization systems that either regenerate lesion body structure (e.g., biomimetic peptide scaffolds) or provide ions favouring subsurface mineral gain (e.g., calcium phosphate systems) can significantly reduce the need for traditional restorations and preserve tooth structure.

Disease detection is also increasingly shifting from the conventional DMFT criteria of the WHO to the use of the International Caries Detection and Assessment System (ICDAS), where non-cavitated enamel lesions (ICDAS 1 and 2) are also included. This has increased the proportion of individuals diagnosed with dental caries, providing a significant opportunity for secondary prevention and non-operative care using regenerative medicine-based dental approaches. Modern-day dentistry clearly needs such minimally invasive remineralization measures, not just to enhance clinical outcomes, but also to improve patient experience and well-being [Pitts and Wright, 2018].

Non-Fluoride Enamel Remineralizing Systems: Types and Mechanisms

The development of novel enamel remineralization systems has significantly progressed in recent years with many of them already in clinical use, while others are in various stages of development. The most promising of these remineralizing technologies are briefly summarized in Table 1, categorized into: (i) biomimetic regenerative systems and (ii) approaches that synergize fluoride efficacy.

Biomimetic Remineralization

Oral care products containing fluoride are effective in remineralizing enamel but do not have the potential to promote formation of organized apatite crystals [Ruan and Moradian-Oldak, 2015]. Presently, there is an attempt to shift from reparative to regenerative biomineralization therapies, wherein diseased dental tissues are replaced with biologically similar tissues [Alkilzy et al., 2018b]. Enamel regeneration is however particularly challenging as mature enamel is acellular and does not resorb or remodel itself unlike bone or dentine [Moradian-Oldak, 2012]. Advances in tissue engineering methods have yielded biomimetic methods that have demonstrated a strong potential for regenerating the hierarchical enamel microstructure.

Dentine Phosphoprotein-Derived 8DSS Peptides

Dentine phosphoprotein (DPP) is the most abundant non-collagenous extracellular matrix component in dentine and is known to play a critical role in tooth mineralization [Hsu et al., 2011]. Human DPP contains numerous repetitive aspartate-serine-serine (DSS) nucleotide sequences that are believed to promote hydroxyapatite (HA) formation, with studies showing that DPP can generate HA crystals in calcium phosphate solutions [George et al., 1996; Prasad et al., 2010]. Several short functional peptides based on DPP have been designed as they offer a number of advantages over full-length DPP such as higher purity and better conformational fit on enamel, while avoiding allergies and immunogenicity often associated with animal proteins [Hsu et al., 2011]. Among the DPP-derived peptides, the octuplet repeats of aspartate-

Table 1. Non-fluoride enamel remineralizing technologies

Technology		Commercial product
Biomimetic systems		
1	Dentin phosphoprotein 8DSS peptides	Not available
2	P11-4 peptides	Curodont Repair/Curodont Protect
3	Leucine-rich amelogenin peptides	Not available
4	Poly(amido amine) dendrimers	Not available
5	Electrically accelerated and enhanced remineralization	Not available
6	Nanohydroxyapatite	Apagard toothpaste/Desensin oral rinse
Fluoride boosters		
1	Calcium-phosphate systems	
	Stabilized calcium phosphates	
	– Casein phosphopeptide-amorphous calcium phosphate	Tooth Mousse/MI Paste crèmes Recaldent/Trident White sugar-free gum MI Paste One toothpaste
	Crystalline calcium phosphates	
	– Functionalized β-tricalcium phosphate	ClinPro toothpaste
	– Calcium sodium phosphosilicate (NovoMin™ technology)	Oravive toothpaste
	Unstabilized calcium phosphates	
	– Amorphous calcium phosphate (Enamelon™ technology)	Enamelon toothpaste
2	Polyphosphate systems – Sodium trimetaphosphate – Calcium glycerophosphate – Sodium hexametaphosphate	Oral-B Pro Expert toothpaste
3	Natural products – *Galla chinensis* – Hesperidin – Gum arabic	Not available

serine-serine (8DSS) are the most active in promoting biomineralization [Yarbrough et al., 2010].

8DSS peptides have essentially two mineral-binding surfaces and can strongly bind not only to free Ca^{2+} and PO_4^{3-} ions, but also to the HA surface [George et al., 1996; Yarbrough et al., 2010]. Applying these peptides to enamel can prevent dissolution of Ca^{2+} and PO_4^{3-} ions into the surrounding medium while promoting the capture of these ions from solution. 8DSS peptides thus appear to have a dual mechanism in the mediation of biologically directed mineral deposition. First, they limit the dissolution of Ca^{2+} and PO_4^{3-} ions from demineralized dentine, and second, they promote the capture of these ions to form new mineral deposits on demineralized enamel [Hsu et al., 2011; Yang et al., 2014]. The newly grown mineral had uniform deposition of small apatite crystals with significantly improved properties such as reduced surface roughness, and higher hardness and elastic modulus [Chung et al., 2012; Hsu et al., 2011]. A recent in vitro study also provided strong evidence that the biomimetic 8DSS peptide, besides inhibiting enamel demineralization on its own, could significantly potentiate the ability of fluoride to do the same [Yang et al., 2016]. This synergistic interaction can be useful to lower fluoride concentration for caries prevention in young children reducing their risk of dental fluorosis.

To date, the proof of concept of 8DSS peptides has been shown only in in vitro systems and is likely to present some challenges when used clinically. For example, it is not known whether these peptides can survive enzymatic action in the oral cavity, although being short peptides should make them relatively difficult targets for hydrolytic enzymes. Another drawback is that because 8DSS binds calcium strongly it could lead to calculus formation if not controlled. However, if future in vivo studies can confirm the clinical promise of 8DSS and overcome the challenges, it holds great promise as non-fluoride biomineralizing agent [Yang et al., 2014].

Self-Assembling P11-4 Peptides

An ideal enamel regenerative approach would involve substituting the degraded enamel matrix with a biomimetic matrix that favours in-depth remineralization of enamel lesions [Alkilzy et al., 2018a]. An exciting development in this field is a monomeric peptide consisting of 11 amino acids called P11-4. This rationally designed peptide self-assembles into hierarchical 3-dimensional fibrillar scaffolds in response to local conditions such as high ionic strength and acidic pH found in the lesion body [Kirkham et al., 2007]. The P11-4 fibrillar matrix has a high affinity for Ca^{2+} ions and acts as a nucleator for de novo HA formation resulting in remineralization of the lesion body [Kind et al., 2017; Kirkham et al., 2007]. Analysis of in vitro data showed that the presence of P11-4 fibres in the lesion body resulted in faster HA formation, yielding tangentially arranged needle-shaped crystals, with increased microhardness of the remineralized subsurface lesion [Schmidlin et al., 2016; Sousa et al., 2017; Takahashi et al., 2016].

P11-4 has shown promising results as a biomimetic mineralization agent in in vivo and clinical trials. This includes the ability to reverse early occlusal and proximal lesions that are more resistant to fluoride remineralization than smooth surface lesions [Alkilzy et al., 2018a, 2015; Brunton et al., 2013; Schlee et al., 2014, 2018]. The low viscosity isotropic P11-4 when applied on the initial carious lesion rapidly diffuses into the lesion body, where it transforms to an elastomeric nematic gel in the presence of cations and pH <7.4, leading to the 3-dimensional fibre matrix assembly and subsequent biomineralization of the lesion [Brunton et al., 2013]. The P11-4-treated carious lesions showed a significantly improved visual appearance and increased radiographic opacity, remaining stable even 6–12 months after treatment [Schlee et al., 2014, 2018]. A recent randomized controlled trial (RCT) demonstrated that biomineralization facilitated by P11-4 in combination with fluoride is safe and more effective than the present clinical gold standard of fluoride treatment alone [Alkilzy et al., 2018b].

As P11-4 relies on natural remineralization driven by saliva, its effectiveness will depend on the individual's quality of saliva especially its mineral content, pH, and flow rate [Schlee et al., 2018]. This could reduce its efficacy in xerostomia patients. Undoubtedly, P11-4 therapy is a significant step towards the elusive goal of guided enamel regeneration, but more long-term controlled studies are needed to confirm and quantify these findings, as well as to identify additional factors that can potentiate the repair process.

Amelogenin

The amelogenin-rich enamel organic matrix plays a critical role in regulating the growth, shape, and arrangement of HA crystals during enamel mineralization. However, mature enamel lacks matrix proteins and cannot regenerate the mineral loss caused by dental caries or erosion [Ruan and Moradian-Oldak, 2015]. Recently, several promising strategies have been proposed to replicate the complex enamel microstructure using synthetic amelogenin-based systems. Recombinant porcine amelogenin (rP172) was found to stabilize calcium phosphate clusters and promote the growth of hierarchically arranged enamel crystals on acid-etched lesions, significantly improving its hardness and elastic modulus [Fan et al., 2009; Ruan et al., 2013, 2016]. This biomimetic regrowth of HA crystals also generated a robust interface between the newly formed layer and native enamel ensuring efficacy and durability of restorations.

An excellent low-cost and safer alternative to the full-length amelogenin is a leucine-rich amelogenin peptide that is comprised of only 56 amino acids. The non-phosphorylated leucine-rich amelogenin peptide contains only the N- and C-terminal domains of the parent amelogenin, with these domains known to be responsible for directing mineral growth and binding [Le Norcy et al., 2011]. In vitro studies have shown treatment of enamel lesions with leucine-rich amelogenin peptide reduced lesion depth and allowed biomimetic reconstruction of enamel by promoting linear growth of mature enamel crystals along the *c*-axis [Bagheri et al., 2015; Mukherjee et al., 2016; Shafiei et al., 2015]. The addition of mineralization inhibitors such as inorganic pyrophosphate or matrix metalloproteinase to synthetic amelogenin assemblies was able to better regulate size, shape, and orientation of a strongly adherent new mineral layer, while preventing undesirable protein occlusion within newly formed crystals [Kwak et al., 2017; Prajapati et al., 2018].

A disadvantage of amelogenin-mediated enamel regeneration is that not only is the protein difficult to extract and store, but the growth of the repaired enamel layer also takes an extended amount of time, making it potentially unsuitable for clinical use. Furthermore, while amelogenin has been seen to promote apatite nucleation in vitro, there is as yet no direct evidence that similar biomineralization occurs in vivo [Ruan and Moradian-Oldak, 2015].

Poly(Amido Amine) Dendrimers

Poly(amido amine) (PAMAM) dendrimers are highly branched polymers characterized by the presence of in-

ternal cavities, a number of reactive end groups, and a well-defined size and shape [Chen et al., 2013]. These amelogenin-inspired dendrimers have been referred to as "artificial proteins" as they can mimic the functions of organic matrices in modulating the biomineralization of tooth enamel. Several in vitro studies have demonstrated that amphiphilic, carboxyl-terminated, and phosphate-terminated PAMAM dendrimers exhibited a strong tendency to self-assemble into hierarchical enamel crystal structures [Chen et al., 2013, 2014, 2015; Wu et al., 2013; Yang et al., 2011]. The new crystals created by the PAMAM organic templates had the same structure, orientation, and mineral phase of the intact enamel, with the HA nanorods closely paralleling the original prisms [Chen et al., 2013].

The synthetic PAMAM dendrimers have the potential to act as amelogenin analogues for biomineralization, overcoming the difficulty associated with extracting, purifying, and storing the natural protein. However, they are still far from clinical translation with in vivo studies so far limited to only animal experiments. Furthermore, like amelogenin, PAMAM-mediated enamel remineralization is also a time-consuming process, and unless this can be potentiated their clinical application may not be practical. Recently, there have been suggestions that lasers could be used to speed up the biomineralization process and control the crystal growth precisely where needed [Sun et al., 2017].

Electrically Accelerated and Enhanced Remineralization

Electrically accelerated and enhanced remineralization (EAER) is a recently developed remineralization technology targeted at initial and moderate enamel lesions with the treatment objectives of preserving all healthy tissue, restoring the full depth of the caries lesion, and improving mechanical properties of the treated enamel [Pitts and Wright, 2018]. It utilizes iontophoresis to accelerate the flow of remineralizing ions into the deepest part of the subsurface caries lesion. This creates an environment that favours remineralization of the lesion that then matures to give the repaired lesion optimal hardness and mineral density. Unlike the biomimetic peptides, EAER does not "regenerate" lost enamel via matrix proteins or the organic capture of Ca^{2+} and PO_4^{3-} ions. However, the EAER-treated lesions have a very similar appearance to healthy enamel, with no broken rods or degraded prisms visible under scanning electron microscopic examination [Pitts and Wright, 2018]. An advantage that the EAER technology will have over synthetic biomimetic peptides is that it proposes to utilize tools and chemicals commonly available in most dental practices. The early in vitro results using the EAER technology are very promising, although a thorough evaluation of its remineralization potential will depend on results from in vivo studies, as well as studies independent of the technology developers.

Nanohydroxyapatite

Synthetic nanohydroxyapatite (nHA) is considered one of the most biocompatible and bioactive materials having similar morphology, structure, and crystallinity to the apatite crystal within enamel [Hanning and Hanning, 2010]. The nano-sized particles can strongly bind to enamel surfaces and with fragments of plaque and bacteria. The small size of the particles that compose nHA considerably increase its surface area for binding as well as allowing it to act as a filler to repair small holes and depressions on the enamel surface [Pepla et al., 2014]. In vitro dynamic pH-cycling experiments have shown that nHA had the potential to remineralize initial enamel lesions with a comparable or even superior efficacy to that of fluoride [Huang et al., 2009, 2011; Najibfard et al., 2011; Tschoppe et al., 2011]. Another in vitro study found that nHA gel had significant potential for enamel remineralization around restoration margins [Juntavee et al., 2018]. The mechanism of nHA biomimetic function is not clear with some researchers suggesting that it promotes remineralization through the creation of a new layer of synthetic enamel around the tooth or by depositing apatite nanoparticles in the enamel defects [Li et al., 2008; Pepla et al., 2014]. However, others have proposed that nHA acts as calcium phosphate reservoir maintaining a state of supersaturation with respect to enamel minerals, thereby inhibiting demineralization and enhancing remineralization [Huang et al., 2011].

Although nHA products have been available since the 1980s, there are as yet no well-designed RCTs that prove its superior efficacy to fluoride toothpastes. Moreover, under neutral conditions, nHA is seen to promote preferential remineralization of the outer enamel caries lesion, with full remineralization of the lesion not observed [Huang et al., 2011]. Further evidence is required before clinicians can recommend nHA oral products as a substitute to fluoride dentifrices or mouthwashes.

Fluoride Boosters

Calcium Phosphate Systems

Biomimetic-guided enamel regeneration could well be the future of non-fluoride remineralization; however,

their widespread clinical application is still a few years away. Presently, the need to enhance the remineralizing efficacy of fluoride in high caries risk patients is largely met by calcium phosphate systems. The bioavailability of Ca^{2+} and PO_4^{3-} ions is often the limiting factor for net remineralization to occur on topical fluoride application, and this is especially exacerbated under hyposalivation conditions [Reynolds et al., 2008; Vogel et al., 2008]. The presence of extrinsic sources of Ca^{2+} and PO_4^{3-} ions can increase diffusion gradients and augment the F^- ion-mediated remineralization. A number of unique calcium phosphate remineralization systems have been commercialized in recent years, and Cochrane et al. [2010] categorized them into 3 types: (i) stabilized amorphous calcium phosphate systems; (ii) crystalline calcium phosphate systems; and (iii) unstabilized amorphous calcium phosphate systems (Table 1).

Casein Phosphopeptide-Amorphous Calcium Phosphate. This remineralization system was developed based on the idea that the tryptic digestion of milk caseinate produced multiphosphorylated casein phosphopeptides (CPP), substantially increasing the milk protein's solubility and ability to stabilize Ca^{2+} and PO_4^{3-} ions [Reynolds, 1987]. CPP is a saliva biomimetic but with a significantly greater calcium-stabilizing capacity than salivary proteins due to the higher content of its phosphoseryl residues [Cochrane and Reynolds, 2012]. CPP-amorphous calcium phosphate (ACP) nanocomplexes are readily soluble in saliva, creating a diffusion gradient that allows them to localize in supragingival plaque. Low pH conditions that arise during a cariogenic attack facilitate the release of Ca^{2+} and PO_4^{3-} ions, inhibiting demineralization and favouring the remineralization of the incipient lesion by precipitation of the released ions [Reynolds, 2009]. The subsurface remineralization pattern produced by CPP-ACP has been shown to significantly improve the aesthetics, strength, and acid resistance of the remineralized WSL [Cochrane et al., 2010; Mayne et al., 2011].

CPP-ACP is probably the most studied non-fluoride remineralizing agent, although there is considerable variability in the reported results. Many RCTs have demonstrated significantly better remineralizing and anticaries effects for CPP-ACP products compared to a placebo or a fluoride-containing product [Bailey et al., 2009; Guclu et al., 2016; Heravi et al., 2018; Juarez-Lopez et al., 2014; Krithikadatta et al., 2013; Llena et al., 2015; Morgan et al., 2008; Rao et al., 2009; Robertson et al., 2011]. However, other RCTs contradict the above studies as they did not report any superior added effect for CPP-ACP [Beerens et al., 2010; Brochner et al., 2011; Huang et al., 2013; Plonka et al., 2013; Singh et al., 2016; Sitthisettapong et al., 2012, 2015]. Published literature and systematic reviews also reach conflicting conclusions, with some reviews suggesting that CPP-ACP had significant remineralizing and caries preventive effects [Llena et al., 2009; Wang et al., 2017; Yengopal and Mickenautsch, 2009], while others conclude that the evidence to support its long-term remineralizing or synergistic effect with fluoride is limited [Azarpazhooh and Limeback, 2008; Fontana, 2016; Li et al., 2014; Raphael and Blinkhorn, 2015; Zero, 2009].

The reasons for the conflicting results from CPP-ACP remineralization studies can partly be due to a poor understanding of CPP-ACP technology. Many RCTs that concluded that CPP-ACP did not provide any superior remineralization to fluoride have not accounted for the fact that remineralization patterns produced by CPP-ACP and fluoride are different. CPP-ACP enhanced remineralization of enamel subsurface lesions compared to predominantly surface-only remineralization produced by fluoride alone products [Shen et al., 2011]. Fully remineralized WSL not only have better aesthetics and strength, but are also more resistant to a subsequent acid challenge. Furthermore, the ability of CPP-ACP to provide high concentrations of stabilized Ca^{2+} and PO_4^{3-} ions could be especially important in highly cariogenic environments (e.g., xerostomia, >6 sugar exposures/day), where fluoride and salivary homeostatic mechanisms alone will not be enough to repair developing lesions. Reasons for inconsistent conclusions from systematic reviews can be attributed to inclusion of studies with inadequate statistical power and possible conflict of interest between competing product manufacturers. Clearly, there is a need for more independent long-term longitudinal studies focussing on high-risk population groups to demonstrate whether CPP-ACP therapy can effect superior remineralization of early lesions compared to fluoride-based products [Gonzalez-Cabezas and Fernandez, 2018].

Functionalized β-Tricalcium Phosphate. Crystalline β-tricalcium phosphate (β-TCP) was modified by coupling it with carboxylic acids and surfactants to yield functionalized β-tricalcium phosphate (fTCP) [Karlinsey et al., 2010]. The purpose of functionalizing β-TCP was to create barriers preventing premature fluoride-calcium interactions, thereby allowing it to act as a targeted low-dose delivery system when applied to teeth via dentifrices or mouthwashes [Karlinsey and Pfarrer, 2012]. It was designed primarily to boost F^- ion activity on the tooth surface, with remineralization driven mostly by salivary Ca^{2+} and PO_4^{3-} ions.

Although already available as a commercial product, data on its remineralizing efficacy are sparse and limited to in vitro studies that do not fully reflect the complex biological process involved in lesion remineralization. Purely based on the mechanisms involved, it does seem that CPP-ACP will have a significant advantage over fTCP in remineralizing early lesions. While the pH-responsive CPP-ACP nanocomplexes can deliver stabilized Ca^{2+} and PO_4^{3-} ions over an extended time, fTCP appears to supply only a small amount of unbound ions during the short period of brushing before being expectorated from the mouth [Walsh, 2009]. Clinical recommendation on using fTCP products will be premature without evidence from well-designed RCTs.

Calcium Sodium Phosphosilicate. Calcium sodium phosphosilicate is a bioactive glass material originally developed as a biocompatible bone regenerative agent. When introduced into the aqueous oral environment, it releases Na^+, Ca^{2+}, and PO_4^{3-} ions, which then interact with saliva and deposit a crystalline hydroxycarbonate apatite layer that is structurally and chemically similar to tooth mineral [Burwell et al., 2009]. Calcium sodium phosphosilicate was initially incorporated into a dentifrice for the treatment of dentine hypersensitivity but there have been suggestions it could be useful for enamel remineralization too [Wefel, 2009]. However, evidence from in vitro and in situ data is weak and contradictory [Parkinson et al., 2017; Wang et al., 2016], while there are no clinical data from RCTs to prove its remineralizing efficacy.

Amorphous Calcium Phosphate. ACP is an unstabilized calcium phosphate system that has been incorporated into a dual-chamber fluoride toothpaste with the intention of separately delivering Ca^{2+} and PO_4^{3-} ions into the mouth [Tung and Eichmiller, 2004]. On brushing, the intraoral mixing of Ca^{2+} and PO_4^{3-} ions results in the immediate precipitation of ACP or amorphous calcium fluoride phosphate. Both ACP and amorphous calcium fluoride phosphate are unstable and rapidly transform into more stable HA or fluorhydroxyapatite. Before their phase transformation, the Ca^{2+} and PO_4^{3-} ions should be transiently bioavailable for subsurface lesion remineralization [Cochrane et al., 2010].

Evidence for the ACP technology is available only from a single RCT in radiation patients where it was found to be superior to a conventional fluoride dentifrice in lowering root caries increment, although there were no significant differences in its ability to control coronal caries [Papas et al., 2008]. One of the main concerns with using an unstabilized calcium phosphate system is that it can promote dental calculus deposition on teeth. Moreover, ACP also tends to rapidly sequester free F^- ions in the oral environment, reducing their availability for lesion remineralization. Considering the limited evidence and better alternatives available, oral products based on the ACP remineralization technology have limited clinical applicability.

Polyphosphates

Sodium Trimetaphosphate. One way to reduce the potential risk of fluorosis while maintaining the anticaries efficacy of conventional dentifrices is to partly replace fluoride with polyphosphate salts like sodium trimetaphosphate (STMP), calcium glycerophosphate, or hexametaphosphate [da Camara et al., 2016; Takeshita et al., 2016; Zaze et al., 2014]. Among the polyphosphates, STMP is seen to be the most effective anticaries agent with an ability to not only inhibit demineralization, but also to enhance remineralization [Freire et al., 2016; Takeshita et al., 2011].

STMP ($Na_3P_3O_9$) is a condensed inorganic phosphate that is able to strongly bind to phosphate sites on enamel surface and remain adsorbed for a longer time compared to other phosphates [McGaughey and Stowell, 1977]. This leads to the formation of a protective layer on the enamel surface that limits acid diffusion of ions during a cariogenic challenge [McGaughey and Stowell, 1977]. The fact that STMP can minimize mineral loss even in the presence of low fluoride concentrations has been confirmed in several in vitro and in situ studies [Danelon et al., 2014; Favretto et al., 2013; Takeshita et al., 2011, 2015]. The protective barrier against acid diffusion created by the adsorption of STMP on enamel does not seem to hinder the diffusion of Ca^{2+} and F^- ions into the enamel. In situ models have shown that supplementation of a low-fluoride product with STMP produced similar remineralization effects to a 1,100-ppm fluoride formulation [Danelon et al., 2013; Takeshita et al., 2016], while the addition of STPM to conventional fluoride dentifrices and varnishes significantly enhanced their remineralization of artificial caries lesions [Danelon et al., 2015; Manarelli et al., 2015].

While earlier clinical trials evaluating caries preventive effects of STMP produced conflicting results [O'Mullane et al., 1997; Stadtler et al., 1996], a recent 18-month double-blinded RCT showed that a 500-ppm low-fluoride dentifrice supplemented with STMP was significantly superior to a 1,100-ppm fluoride dentifrice in lowering the caries increment of children [Freire et al., 2016]. There is a need for additional clinical studies to ascertain whether STMP can influence the reversal of non-cavitated lesions.

Natural Products

An interesting addition to remineralizing agents are plant-derived natural products that have demonstrated the ability to beneficially shift the de-/remineralization caries equilibrium. Among the most promising is *Galla chinensis*, a leaf gall produced by parasitic aphids, which has been found to be effective in inhibiting demineralization, enhancing remineralization, and increasing the efficacy of fluoride [Cheng et al., 2008, 2010]. The mechanisms are still not fully clear, but it is hypothesized that polyphenols present in *G. chinensis* interact with and stabilize the organic matrix remnants, thereby blocking the ion diffusion pathways, and slowing demineralization [Huang et al., 2017; Zhang et al., 2015]. *G. chinensis* remineralization is believed to be mediated through different polyphenol compounds that act as Ca^{2+} ion carriers into the lesion body [Cheng et al., 2015]. Hesperidin, a citrus flavonoid, and gum arabic, an *Acacia* exudate, are other natural products that have been found to suppress acid-dependent demineralization and boost remineralization even under fluoride-free conditions [Islam et al., 2012; Onishi et al., 2008].

Natural remineralizing agents could find greater acceptability among the general public compared to fluoride-based remineralizing systems. However, chemical characterization and standardization of the natural products will be required before further application in clinical trials.

Conclusions

The era of preventive and minimally invasive dentistry clearly dictates the need for developing newer approaches to remineralize enamel caries lesions. While fluoride-mediated natural repair of early lesions can occur by influencing oral hygiene and diet, this is dependent on variables such as saliva quality and patient compliance. Non-fluoride remineralization systems are less reliant on such factors and can also significantly improve the structure, aesthetics, and acid resistance of the remineralized lesion. Furthermore, effective non-fluoride remineralizing strategies can prevent a non-cavitated lesion from being subjected to a "death spiral of restorations" due to secondary caries at the enamel-restoration interface [Qvist, 2008]. Currently, most commercially available non-fluoride remineralizing systems are aimed at enhancing fluoride efficacy and minimizing the potential risks associated with fluoride. However, a biomimetic strategy for enamel regeneration may well be the future, where organized enamel apatite crystals with robust attachment to the tooth surface are grown to replace demineralized tissue. Guided enamel regeneration is the holy grail of remineralizing therapeutic approaches, and some of the biomimetic technologies discussed here are bringing us a step closer to the reality of growing artificial enamel.

Although highly promising, the currently available clinical evidence for most of the non-fluoride enamel remineralizing systems is either poor, equivocal, or limited to a few early studies. Well-designed RCTs are vital to clarify whether these new-age remineralizing approaches provide any additional benefit over traditional fluoride remineralization, and these studies are especially needed for the products already in the market.

Disclosure Statement

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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