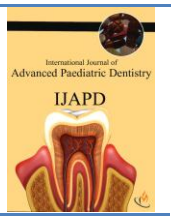
INTERNATIONAL JOURNAL OF ADVANCES IN PEDIATRIC DENTISTRY

Journal homepage: www.mcmed.us/journal/ijapd



NEWER APPROACHES TO ENAMEL REMINERALISATION : A REVIEW

Akhila.B [1]*, Himabindu.B[1], J.V.Shiva Kumar[1], Nusaibah Bari[1], Sushma H[2], SK Srinath[3]

[1] Post Graduate Student, Department of Pedodontics and Preventive Dentistry, Panineeya Mahavidyalaya Institute Of Dental Sciences, Hyderabad, Telangana, India.
[2] Post Graduate Student Department of Pedodontics and Preventive Dentistry Government Dental College Research Institute, Bengaluru, Karnataka, India.
[3]Professor & HOD Department of Pedodontics and Preventive Dentistry Government Dental College Research Institute, Bengaluru, Karnataka, India.

Corresponding Author:- **Akhila.B**
E-mail: akhila.kashyap89@gmail.com

Article Info
Received 15/04/2019
Revised 27/05/2019
Accepted 02/06/2019

Key words:
Remineralisation, Cpp-Acp, Fluoride, White Spot Lesion, Xylitol.

ABSTRACT
A greater understanding of the dental caries process and improved and more diverse methodologies to assess early demineralization and caries has enabled development of a new class of remineralising therapeutics. Hence, an emerging goal of modern dentistry is to manage non- cavitated carious lesions non- invasively through remineralisation in an effort to prevent disease progression and improve strength, aesthetics, and function of teeth. A critical element fundamental to this current therapeutic philosophy is the need for new and highly efficacious technologies for enamel remineralisation. The aim of this article is to provide an overview of new compositions and to review contemporary non-fluoridated systems in terms of their basic composition, mode of delivery, and currently available evidence.

INTRODUCTION

Tooth enamel is the hardest tissue in the human body. Mature enamel is a crystalline structure, containing up to 96% hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) by weight. [1] The outermost region of tooth enamel is in intimate contact with saliva and plaque fluid, and the surfaces of the enamel HAP crystals are in dynamic equilibrium with these adjacent aqueous phases. At pH<5.5, HAP can dissolve in the process known as demineralisation.

The presence of clinically detectable, localized areas of enamel demineralization, observed as white spot lesions (WSL) of different opacity, is a sign that the caries process has begun. Dental caries results in the dissolution of apatite crystals and the loss of calcium, phosphate and other ions, which eventually leads to demineralization of the tooth substrate.[1] The subsurface porosity caused by demineralization gives the lesion a milky appearance that can be found on the smooth surfaces of teeth.[2]

In the minimally invasive dentistry paradigm, incipient enamel carious lesions should be treated with non-invasive remineralisation strategies wherever possible, instead of surgical intervention. For this purpose, topical gels, varnishes, mouthwashes, and dentifrices that contain fluoride are used by dentists for the treatment of WSLs.[3]

Saliva is rich in calcium and phosphate ions. It can act as a natural buffer to neutralise acid and restrict the dissolution process. At pH>5.5 together with the high concentration of calcium and phosphate ions, the equilibrium can be tipped the other way, calcium phosphate can be re-precipitated and demineralised tooth tissues remineralised. It has been suggested that high levels of fluoride are necessary to significantly increase enamel's resistance to erosion.

Classification:

1. Fluoride Remineralising Agents

a) Chitosan

b) Casein Phosphopeptide–Amorphous Calcium Phosphate Fluoride (CPP-ACPF)

2. Non-Fluoride Remineralising Technologies:

Technology		Commercial product
Biomimetic systems	Dentin phosphoprotein 8dss peptides	Not available
	P11-4 peptides	Curodont repair/curodont protect
	Leucine rich amelogenin peptides	
	Poly (amido amine) dendrimers	Not available
	Electrically accelerated and enhanced remineralisation	
	Nano hydroxyapatite	Apagard toothpaste/desensin oral rinse
Fluoride boosters		
Calcium phosphate systems	Stabilised calcium phosphates	Tooth mousse/mi
	Casein phosphopeptide amorphous calcium phosphate	Paste crèmes Recaldent/tridentt white sugar free gum Mi paste one toothpaste
	Crystalline calcium phosphates	
	Functionalised β tricalcium phosphate	Clinpro toothpaste
	Calcium sodium phosphosilicate (novomin^{tm} technology)	Oravive toothpaste
	Unstabilised calcium phosphates	
	Amorphous calcium phosphate (enamelon^{tm}technology)	Enamelontoothaste
Polyphosphate systems	Calcium glycerophosphate	Oral-b pro expert toothpaste
	Sodium trimetaphosphate	
	Sodium beta metaphosphate	

3. <u>Natural products:</u>

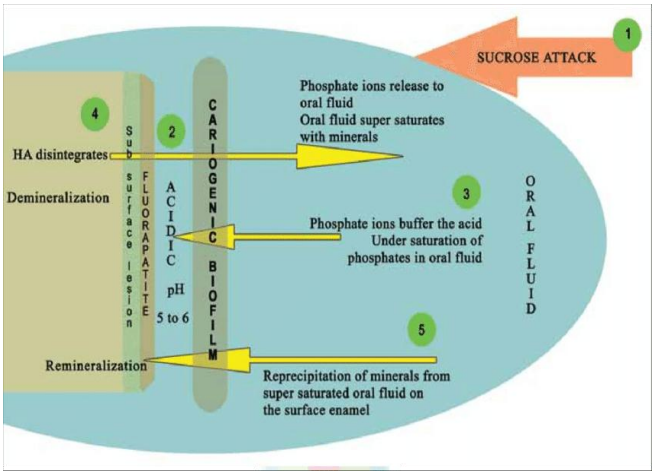
a) Gallachinensis

b) Hesperidin

c) Gum Arabic

d) Grape seed extract

e) Sanguinaria Canadensis



Demineralization of hydroxyapatite (HA) and remineralization with fluorapatite (FA)-depicted as stage 1-5 Stage 1: Fermentable sucrose intake. Stage 2: Microbes in cariogenic plaque metabolisethem releasing acid in the biofilm-tooth interface. The pH in the interface drops below the critical pH of HA Stage 3: Phosphate ions from oral fluid buffer the acidic ions resulting in undersaturation. Stage 4: HA disintegrates to release the phosphate ions back to the oral fluid till it supersaturates-Demineralization Stage 5: Supersaturated oral fluid re precipitates the minerals onto the disintegrated enamel. If fluoride also deposits FA is formed on the superficial layer-Remineralization. Sub surface demineralization remains.

Methods of delivery:

The different ways of delivering fluorides in the oral environment are summarised here:

Community Methods:	Self- Applied Methods:	Professional Methods:
Water fluoridation	Tooth pastes	Solutions
Milk fluoridation	Mouth rinses	Gels
Salt fluoridation	Drops	Foams
	Lozenges and chewing gums	varnishes
		Fluoride releasing dental materials

Personally used Fluorides

Fluoride Dentifrices, Tooth pastes and Mouthrinses

Fluoride is added into toothpastes mostly as sodium fluoride (NaF), sodium monofluorophosphate (MFP), amine fluoride and stannous fluoride. The other ingredients of toothpaste may also affect the availability of fluoride in the oral cavity. This is especially true in the case of calcium containing abrasives due to their potential to inactivate the fluoride. Similarly, fluoride will react with silica to form fluorosilicates if a sufficient amount of detergent is not present. [4] However, the benefits and therapeutic efficacy of using fluoridated tooth pastes may be affected by multiple factors such as the concentration of fluoride, the amount of toothpaste used, and individual variations including the duration and frequency of brushing and rinsing behaviour.[5]

Fluoride mouthrinses are advised for school children over 5 years of age, person with high caries susceptibility and patients with orthodontic and prosthetic appliance.

Professionally Applies Fluorides

Fluoride Solutions

The topical solutions of fluoride used are 2% sodium fluoride, 8% stannous fluoride and 1.23% of acidulated phosphate fluoride solutions.

Fluoride gels

Gels adhere to teeth and eliminate continuous wetting of enamel and it is possible to treat two-four quadrants simultaneously.

Fluoride varnish

Topical fluoride reagents have the disadvantage of rapid loss of soluble fluoride formed on teeth. To combat this problem, waterproof sealant in the form of fluoride varnish was introduced. This procedure improves reaction time between fluoride and enamel providing long term effect. Currently, sodium fluoride based varnish (Duraphat) and polyurethane based varnish (Fluorprotector) are commonly used.

Fluoride Releasing Restorative Materials

Glass ionomer cement releases fluoride which is found to be incorporated in enamel, cavity walls and bacteria inhibiting acid production. Further fluoride recharge ability of glass ionomer cement helps in long term inhibition of caries.

Compomers consists of silicate glass particles, sodium fluoride and polyacid-modified monomer without any water. Compomers release less fluoride compared to glass and hybrid ionomers. [6]

Giomers consists of pre-reacted glass ionomer particles and composites thus exhibiting properties of fluoride release and recharging with excellent aesthetics. Giomers released more fluoride than compomer or composites.

Pit-and-fissure sealants contain strontium-fluoride-aluminosilicate glass as filler which undergoes hydrolysis and releases fluoride through external and internal diffusion

Fluoride remineralisation:

Fluoride ions promote the formation of Fluor apatite in enamel in the presence of calcium and phosphate ions produced during enamel demineralization by plaque bacterial organic acids. Fluoride ions can also drive the remineralisation of previously demineralized enamel if enough salivary or plaque calcium and phosphate ions are available when the fluoride is applied. However, for every two fluoride ions, 10 calcium ions and six phosphate ions are required to form one unit cell of Fluor apatite ($Ca_{10}(PO_4)_6F_2$). Hence on topical application of fluoride ions, the availability of calcium and phosphate ions can be the limiting factor for net enamel remineralisation to occur. [7]There has been a marked decline in the prevalence of dental caries in many industrialised countries over the past three decades. This has been attributed to the widespread use of fluoride in various forms and particularly to the use of fluoride toothpastes.

Resting fluoride levels in the plaque and saliva of individuals brushing twice-daily with a fluoride toothpaste are typically elevated twofold as compared to those of non-fluoride toothpaste users.[8-10] Fluoride has the potential to affect the demineralisation/remineralisation balance and hence caries, largely via three mechanisms.

1. Inhibition of demineralisation
2. Promotion of remineralisation
3. Interference with bacterial metabolism

It is well established that levels of fluoride in the range 0.01- 0.2ppm are sufficient to induce hydroxyapatite seeded growth. Gibbs *et al*[11] reported that fluoride concentrations between 0.058 and 0.138ppm, when present in the neutral buffers of a pH-cycling system, promoted significantly more calcium-uptake than the water control and that uptake increased with increasing fluoride concentration.

Chitosan:

Chitosan causes a significant reduction in formation of the dental plaque biofilm through inhibition of the growth of mutans streptococci due to the positively-charged chitosan binding to negatively-charged S. mutans cell surfaces.The positive charge of chitosan allows it to adhere to negatively-charged surfaces, including demineralised enamel. [12]Chitosan-amelogenin (CS-AMEL) hydrogel was shown to induce in-vitro biomimetic remineralisation on either etched enamel or artificial enamel lesions. [13]Enamel-like crystals formed, and lesion depth decreased. Remineralisation did not cease even when the pH decreased below 6.5 due to adhesion provided by the amino groups of chitosan through electrostatic interactions.

CPP-ACPF

Fluoride has been added to the CCP-ACP formulation to increase its remineralization efficacy. CPP-ACPF appeared to have a specific remineralising effect on smooth surface caries lesions but not on pit and fissure

lesions, while another study showed that CPP-ACPF was more effective than a NaF control in remineralising incipient caries lesions. [14]

Ozone

It operates by attacking thiol groups of cysteine amino acid and decimates the cellular membrane of carious bacteria. Ozone is capable of altering acidogenic and aciduric microorganisms to normal commensals permitting remineralisation to occur. Currently HealOzone remineralising solution containing xylitol, fluoride, calcium, phosphate and zinc is recognised for the treatment of caries.[15] It can be used as 2100 ppm of ozone ±5% at a flow rate of 615cc/minute for 40 seconds. Tooth remineralisation might be promoted with the assistance of salivary minerals and useable fluorides or remineralising chemicals bringing about a tooth surface that is more resistant to future acid attacks.

Non fluoride remineralisation:

Commercially available calcium phosphate-based remineralization technologies:

CPP-ACP (Recaldent)

CPP-ACP is a stabilized system of calcium and phosphate that has been considerably studied the last 20 years. When CPP-ACP is applied to the oral environment, the sticky CPP part of the CPP-ACP complex binds readily to the enamel, biofilm, and soft tissues, delivering the calcium and phosphate ions exactly where it is needed. The free calcium and phosphate ions move out of the CPP, enter the enamel rods, and reform the apatite crystalsRecent studies have shown the CPP-ACP remineralisation potential on non-orthodontics lesions, with lesion fluorescence changes as the main outcome. Better clinical evidence is still needed to demonstrate that the CPP-ACP therapy is not inferior to fluoride therapies.

Sodium Trimetaphosphate(TMP)

TMP is a cyclic polyphosphate salt that adsorbs on the enamel surface. It was clinically studied in the 1990s and currently is being tested for its potential to enhance the effect of fluoride. In situ data have shown that TMP nanoparticles added to a 1100-ppm fluoride NaF-containing toothpaste could increase its remineralisation ability.[16] TMP added to low- fluoride concentration toothpaste showed a remineralisation ability similar to that of a toothpaste with standard fluoride concentration in situ.It has been clearly shown the potential of this technology; however, recommendation for clinical use should be done after these results have been confirmed in clinical trials.

Xylitol

Sugar-free chewing gums containing polyols have been demonstrated to reduce caries experience when compared with no gum chewing in randomised clinical trials.[17-19] The anti-cariogenic effect of chewing the sugar-free gum has been attributed to the stimulation of saliva. Recently the anti-cariogenic efficacy of sugar-free gum has been enhanced by the addition of the salivary biomimetic CPP-ACP which significantly increases the buffering and remineralisation capacity of saliva by providing bioavailable calcium and phosphate ions. In a randomised controlled clinical trial a sugar-free chewing containing CPP-ACP with a sorbitol-mannitol (polyol) blend was significantly better than a control chewing gum containing only the polyols in slowing the progression of dental caries and enhancing regression of early lesions in children.[20]

Calcium Carbonate Carrier (Sensistat)

Dr. Israel Kleinberg developed SensiStat which was first introduced commercially in 2003 in Ortek's preclude desensitising prophylactic paste and later in Denclude, a professionally dispensed sensitivity paste for home use launched in 2004. It consists of highly soluble arginine bicarbonate component of SensiStat or is surrounded by particles of the poorly soluble calcium carbonate component and because of the adhesive properties of the composition forms a paste like plug that not only fills the open tubules but also adheres to the dentinal tubule walls. Due to its alkalinity, SensiStat also reacts with the calcium and phosphate ions of the dentinal fluid to make the plug chemically contiguous with the dentinal walls and therefore more secure.

Biomimetically Modified Mineral Trioxide Aggregate

It is more difficult to remineralise dentin than enamel due to the paucity of apatite seed crystallites along the lesion surface for heterogeneous crystal growth. The remineralising potential of mineral trioxide aggregate (MTA) in phosphate containing simulated body fluid (SBF) by incorporating polyacrylic acid and sodium tripolyphosphate as biomimetic analogs of matrix proteins for remineralising artificial caries like dentin was evaluated and it revealed at the end of 6 weeks that biomimetic analogs in modified MTA provides a potential delivery system for remineralisation of dentin thus widening the scope and applications of MTA in dentistry. Inculcation of polyphosphate in MTA may serve as an accessory source of phosphate in critical times.[21]

Self assembling peptides:

Peptide treatment significantly increases net mineral gain due to a combined effect of increased mineral gain and inhibition of mineral loss. Rationally designed β-sheet forming peptides P11-4 that self-assemble

themselves to form three-dimensional scaffolds under defined environmental conditions have been shown to nucleate hydroxyapatite de novo and to have potential applications in mineralized tissue regeneration, mimicking the action of enamel matrix proteins during tooth development. Results suggest that a single application of P11-4 can be beneficial in the treatment of early caries lesions and that self-assembling peptides are candidate materials for mineralized tissue regeneration and repair.[22]

Bioactive glass

Bioactive glass (BAG) was first invented by Larry Hench in 1969.[23] It can bind to both soft and hard tissues and stimulate tissue mineralization owing to its high bioactivity index (IB=12.5). It has been used for monolithic medical devices, bone regeneration, and toothpaste.[24]

When BAG is incorporated in a dentin desensitizer, it can reduce the movement of fluid across the dentin surface. The adhesion of resin-modified glass ionomer to dentin has also been shown to be enhanced by BAG application.[24] A BAG-containing dentin adhesive improves the nano-mechanical properties of demineralised dentin.[25] As a composite filler, BAG releases calcium, and in certain formulations fluoride ions, increasing the elastic modulus of demineralized dentin.[26]

Nano hydroxyl apatite(n-HAp)

n-HAp is considered one of the most biocompatible and bioactive materials, and has gained wide acceptance in medicine and dentistry in recent years.[27]Compared to the standard fluoride control, the nano-HAP fluoride dentifrice produced directional increase in surface hardness and statistically significant decrease in surface roughness. Those additive effects to fluoride could provide supplemental repair or protection against further caries development.

It would be expected that deposition of apatites initiated by the remineralization process caused the development of the highly mineralized external layer. This zone might be hypothesized to reduce demineralization progress, inhibiting diffusion of acids into deeper areas of enamel. Naturally, this deposition could block diffusion of mineral ions, thus constraining the subsurface enamel zone recrystallization

Electrically Accelerated and Enhanced Remineralization(EAER)

This is a recently developed remineralization technology that utilizes iontophoresis to accelerate the flow of remineralising ions into the deepest part of the subsurface caries lesion. This creates an environment that favours remineralisation 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.[28] 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.

Dentine Phosphoprotein-Derived 8DSS Peptides

Human dentine phosphoprotein (DPP) is the most abundant non-collagenous extracellular matrix component in dentine that contains numerous repetitive aspartate-serine-serine (DSS) nucleotide sequences that are believed to promote HAP formation, with studies showing that DPP can generate HA crystals in calcium phosphate solutions. 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. Among the DPP-derived peptides, the octuplet repeats of aspartate-serine-serine (8DSS) are the most active in promoting biomineralization.[29]

Amelogenin

The amelogenin-rich enamel organic matrix plays a critical role in regulating the growth, shape, and arrangement of HA crystals during enamel mineralization. Recombinant porcine amelogenin (rP172) was found to stabilize calcium phosphate clusters and promote the growth of hierarchically arranged enamel crystals on acid-etched lesions. 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.[28]

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.

Poly Amido Amine (PAMAM)Dendrimers

These are highly branched polymers characterized by the presence of internal cavities, a number of reactive end groups, and a well-defined size and shape. 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. 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.[29]

Herbal remineralisation:

Grape seed extract

Root caries notably prevails among the elder individuals due to gingival recession and exposure of susceptible root surface. Polyphenols are plant based

substances having anti-oxidant and anti-inflammatory properties. They interact with microbial membrane proteins, enzymes and lipids thereby altering cell permeability and permitting the loss of proteins, ions and macromolecules. Proanthocyanidin (PA) is one such polyphenol which is a bioflavanoid- containing benzene-pyran-phenolic acid molecular nucleus. Proanthocyanidin expedites the conversion of soluble collagen to insoluble collagen during development and increases collagen synthesis. Grape seed extract (GSE) is rich in PA content. Inhibition of glucosyltransferases by PA inturn inhibits caries. Grape seed extract can act as a promising and potent supplement or substitute to fluoride in minimally invasive management of caries.[30]

Gum Arabica

Gum arabica consists of calcium, magnesium, and potassium salts of polysaccharides. A high concentration of Ca2+ and possibly phosphate in the remineralization solution composed of gum arabic might enhance the stimulated remineralization of caries-like lesions in the tooth enamel. Gum arabic also contains cyanogenic glycosides and several different types of enzymes, such as oxidases, peroxidases, and pectinases, that exhibit antimicrobial properties.[31]The growth of Porphyromonasgingivalis and Prevotellaintermedia was reported to be inhibited by an Acacia arabica gum,and early plaque deposition was also found to be reduced by Acacia arabica type of chewing gum.These findings suggest that gum arabic may inhibit plaque formation and, together with its remineralization effects, indicate that gum arabic is a potential caries preventive agent.

Sanguinaria Canadensis (bloodroot)

Sanguinarine, an alkaloid possessing benzophenthridine extracted from Sanguinaria Canadensis, has been found to be effective on antiplaque and antigingivitis activity with varying degrees of efficacy. Mallatt et al. reported that the groups treated with dentifrice containing 0.075% sanguinaria and 0.05% zinc chloride had significantly less plaque and gingivitis than the group which rinsed daily with a 0.05% NaF solution but there were no significant differences between sanguinaria-zinc chloride group and the 0.24% NaF dentifrice group. Kopczyk et al. reported that the groups treated with dentifrice containing fluoride and sanguinaria had better results than the comparison dentifrice in terms of plaque and gingivitis index scores.[31,32]

Conclusion:

The importance of oral health for patients and consumers has seen a steady increase in the number of tooth remineralisation agents, products and procedures over recent years. Continued efforts to increase the efficacy of fluoride have been made, in particular, by the addition of calcium salts or calcium containing materials to oral care products which may enhance the delivery and retention of fluoride into the oral cavity. The goal of modern dentistry is to manage non-cavitated caries lesions non-surgically through remineralisation in an attempt to prevent disease progression and improve aesthetics, strength, and function. It is important for dental professionals to be aware of the different modes of actions of remineralising agents and know, that it takes significant time to establish the effectiveness of a new technology like this.

REFERENCES:

1. Mann A, Dickinson M. Nanomechanics, chemistry and structure at the enamel surface. In: Duckworth RM, editor. The teeth and their environment. Monogr Oral Sc2006;19:105-31.
2. Jones FH. Teeth and bones: applications of surface science to dental materials and related biomaterials. Surf Sci Rep2001;42:75-205.
3. Ren Y-F, Zhao Q, Malmstrom H, Barnes V, Xu T. Assessing fluoride treatment and resistance of dental enamel to soft drink erosion in vitro: applications of focus variation 3D scanning microscopy and stylus profilometry. J Dent 2009;37:167–76.
4. 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.
5. Ferrazzano GF, Amato I, Cantile T, Sangianantoni G, Ingenito A. In vivo remineralising effect of GC tooth mousse on early dental enamel lesions: SEM analysis. Int Dent Ji . 2011;61(4):210-6.
6. Mathews MS, Amaechi BT, Ramalingam K, Ccahuana-Vasquez RA, Chedjieu IP, Mackey AC, Karlinsey RL. In situ remineralisation of eroded enamel lesions by NaF rinses. Arch Oral Biol. 2012;57(5):525-30.
7. Souza BM, Comar LP, Vertuan M, Neto CF, Buzalaf MA, Magalhães AC. Effect of an experimental paste with hydroxyapatite nanoparticles and fluoride on dental demineralisation and remineralisation in situ. Caries Res. 2015;49(5):499-507.
8. Garry AP, Flannigan NL, Cooper L, Komarov G, Burnside G, Higham SM. A randomised controlled trial to investigate the remineralising potential of Tooth Mousse™ in orthodontic patients. J Orthod. 2017;44(3):147-56.
9. Quarto M, Nitride C, Ferranti P, Mauriello R, Garro G, Di Stasio M, Volpe MG, Ferrazzano GF, Chianese L. Peptidomic study on in vitro and in vivo phosphopeptide release during the chewing of gum fortified with a commercial casein hydrolysate. Int Dairy J. 2018;79:78-84.
10. Tyagi SP, Garg P, Sinha DJ, Singh UP. An update on remineralising agents. J Interdiscip Dentistry. 2013; 3:151-8.

11. Ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. ActaOdontolScand1999 57: 325-329.
12. Brading MG, Green AK, de Brabander S *et al*. The role of Triclosan in dentifrice formulations, with particular reference to a new 0.3% Triclosan calcium carbonate-based system. Int Dent J2004 54: 291-298.
13. Robinson C, Kirkham J, Weatherall A. Fluoride in teeth and bone. *In*: Fejerskov O, Ekstrand J, Burt BA (Eds.) Fluoride in dentistry. 2nd ed. pp. 69- 87. Copenhagen: Munksgaard, 1996.
14. Lynch RJM, Mony U, ten Cate JM. De and remineralisation of enamel lesions at low pH under simulated plaquefluid conditions. Caries Res2004 38: 399-400
15. Guclu ZA, Alacam A, Coleman NJ. A 12-Week assessment of the treatment of white spot lesions with CPP-ACP paste and/or fluoride varnish. BioMed Res Int. 2016;17(1):35-9.
16. Sitthisettapong T, Doi T, Nishida Y, Kambara M, Phantumvanit P. Effect of CPP-ACP paste on enamel carious lesion of primary upper anterior teeth assessed by quantitative light-induced fluorescence: a one-year clinical trial. Caries Res. 2015;49(4):434-41.
17. Ruan Q, Zhang Y, Yang X, Nutt S, Moradian-Oldak J. Anamelogenin-chitosan matrix promotes assembly of an enamel-like layer with a dense interface. ActaBiomater2013;9:7289–97.
18. Gao SS, Zhang S, Mei ML, Lo EC, Chu CH. Caries remineralisation and arresting effect in children by professionally applied fluoride treatment–a systematic review. BMC Oral Health. 2016;16(1):12.
19. Chu CH, Gao SS, Li SK, Wong MC, Lo EC. The effectiveness of the biannual application of silver nitrate solution followed by sodium fluoride varnish in arresting early childhood caries in preschool children: study protocol for a randomised controlled trial. Trials. 2015;16(1):426.
20. Fernández CE, Fontana M, Samarian D, Cury JA, Rickard AH, González-Cabezas C. Effect of fluoride-containing toothpastes on enamel demineralization and streptococcus mutans biofilm architecture. Caries Res. 2016;50(2):151-8.
21. Llena C, Leyda AM, Forner L. CPP-ACP and CPP-ACFP versus fluoride varnish in remineralisation of early caries lesions. A prospective study. Eur J Paediatr Dent. 2015;16(3):181-6.
22. Takeshita EM, Danelon M, Castro LP, Cunha RF, Delbem AC. Remineralizing potential of a low fluoride toothpaste with sodium trimetaphosphate: an in situ study. Caries Res. 2016;50(6):571-8.
23. Miake Y, Saeki Y, Takahashi M, Yanagisawa T. Remineralization effects of xylitol on demineralized enamel. J Electron Microsc Tech. 2003;52(5):471-6.
24. Kirkham J, Firth A, Vernals D et al. Self-assembling peptide scaffolds promote enamel remineralization. J Dent Res. 2007; 86: 426–430.
25. Bakry AS, Tamura Y, Otsuki M, Kasugai S, Ohya K, Tagami J. Cytotoxicity of 45S5 bioglass paste used for dentine hypersensitivity treatment. J Dent. 2011;39(9):599-603.
26. Salehi S, Davis HB, Ferracane JL, Mitchell JC. Sol-gel-derived bioactive glasses demonstrate antimicrobial effects on common oral bacteria. American J Dent. 2015;28(2):111-5.
27. R. Osorio, M. Yamauti, S. Sauro, T.F. Watson, M. Toledano, Experimental resin cements containing bioactive fillers reduce matrix metalloproteinase-mediated dentin collagen degradation, J. Endod.2012;1227–32.
28. Philip N. State of the art enamel remineralization systems: the next frontier in caries management. Caries Res. 2019;53(3):284-95.
29. Yang Y, Lv X, Shi W, Zhou X, Li J, Zhang L: Synergistic inhibition of enamel demineralization by peptide 8DSS and fluoride. Caries Res 2016; 50: 32–39.
30. Huang S, Gao S, Cheng L, Yu H. Combined effects of nanohydroxyapatite and Gallachinensis on remineralisation of initial enamel lesion in vitro. J. Dent.2010;38:811–9.
31. Sarni-Manchado P, Cheynier V, Moutounet M: Interactions of grape seed tannins with sali-vary proteins. J Agric Food Chem 1999; 47: 42–47.
32. More G, Tshikalange TE, Lall N, Botha F, Meyer JJ: Antimicrobial activity of medicinal plants against oral microorganisms. J Ethnopharmacol 2008; 119: 473–477.