Current Developments on Enamel and Dentin Remineralization

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Abstract

Purpose of Review To present an overview of the ongoing research on enamel and dentin remineralization and to describe particle-mediated and biomimetic approaches. The importance of restoring tissue functionality as the ultimate goal of remineralization is emphasized.

Recent Findings Calcium-releasing particles and adjuvants to increase fluoride uptake by enamel are described in the literature. In order to recover the prismatic structure in mineral-depleted enamel, amelogenin-derived peptides and amelogenin analogues have been proposed as templates for apatite deposition. In dentin, mineral deposition per se is not enough to recover the mechanical properties, and the use of biomimetic analogs is necessary to guide apatite formation into the collagen intrafibrillar spaces.

Summary The use of biomimetic analogues associated with ion-releasing materials seems a promising approach for both enamel and dentin remineralization. Clinical translational protocols are still premature and have, so far, only been explored experimentally in vitro, with good outcomes particularly on structural and functional repair of artificial dentin carious lesions.

Keywords Dentin remineralization · Enamel remineralization · Functional remineralization · Biomimetic remineralization · Biomimetic analogues · Fluoride

Introduction

Over the years, clinical and scientific evidence have shown the benefits of minimally invasive dentistry [1, 2••]. One of the cornerstones of this concept is the possibility of remineralizing initial enamel lesions or the caries-affected dentin. The use of fluoride varnishes was shown to remineralize white spot lesions (WSL) in clinical studies [3, 4]. However, it is a consensus that fluoride therapy is effective in the first 10–30 μm of the lesion [5, 6], and alternatives must be developed to allow for remineralization of deeper areas. Though successful

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remineralization of dentin has frequently been claimed, evidence of a mechanical reinforcement of collagen by reincorporation of apatite mineral is lacking, leading to the conclusion that there is currently no commercial product available for dentin caries repair through remineralization.

The majority of the ongoing research on enamel and dentin remineralization focus on (1) increasing the availability of calcium ions to foster apatite precipitation in deeper areas of the demineralized tissue, and (2) the use of biomimetic analogues for ion transport and to guide mineral deposition in order to restore the tissues original organization and functionality. This text presents an overview of the recent literature on the subject.

Enamel

Calcium-Releasing Particles

A higher availability of calcium than what is found in saliva may favor the ionic gradient towards remineralization. Also, an increased calcium concentration in the biofilm was associated with higher fluoride retention and reduced enamel

demineralization [7, 8]. There are several commercially available products developed to deliver high calcium concentrations for enamel remineralization. Casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP) (Recaldent™) has been available for nearly two decades. In spite the large number of studies, there is no consensus among researchers that the use of gels or toothpastes containing CPP-ACP is more efficient than fluoride [9, 10].

Bioactive glasses (sodium-calcium-phosphosilicate, commercial examples NovaMin™, BioMin™) have been the subject of a recent systematic review [11]. According to the authors, in vitro remineralization of WSL with ion-releasing glasses is more efficient than with fluoride or CPP-ACP. However, a toothpaste containing 927 ppm of fluoride and 5% of bioactive glass particles did not show better remineralization results in comparison with a toothpaste containing only fluoride in situ [12].

Experimental resin-based composites containing amorphous calcium phosphate (ACP) or dicalcium phosphate dihydrate (DCPD) were shown to remineralize artificial enamel caries in vitro [13, 14]. This "proof of concept" demonstrates the potential of these materials to prevent or postpone caries lesion development around orthodontic brackets, for instance. Mineral recovery at deeper regions of the lesion is more efficient than with fluoride-releasing materials [14, 15], possibly due to the fact that lesion pores are not obliterated by CaF_2 deposits. The limitation of this strategy, however, is that ion release is reduced overtime (usually around 2–3 months), and the addition of ion-releasing particles to resin-based materials significantly reduce their mechanical properties [16].

Toothpastes containing hydroxyapatite (HA) nanoparticles were shown to improve remineralization [17, 18] and reduce tooth hypersensitivity [19]. However, studies comparing commercially available toothpastes containing CPP-ACP, HA or bioactive glass, and fluoride show conflicting results regarding their efficiency in remineralizing enamel subsurface lesions in relation with those containing only fluoride [20, 21].

Adjuvants to Fluoride Therapies

Some of the current technologies have the purpose of optimizing fluoride uptake by enamel. β-Tricalcium phosphate (β-TCP) particles functionalized with carboxylic acids (to prevent premature interactions with calcium) and surfactants (to increase hydrophilicity) were developed with the purpose of acting synergistically with fluoride, increasing fluoride-based mineral nucleation [22]. Metaphosphates, $M_n(PO_3)_n$ (where M is a monovalent metal) are a subgroup of inorganic condensed phosphates showing cyclic anions [23]. Sodium trimetaphosphate (TMP) and hexametaphosphate (HMP) added to fluoride gels, varnishes, and toothpastes were shown to increase the effect of topical fluoride on enamel in vitro [24–26] and in situ [27–29]. Their negatively charged sites

can bind to the enamel surface and also to Ca^{2+} and Ca-F complexes. In acidic conditions, these cations would be released and form electrically neutral species ($CaHPO_4^0$ and HF^0) capable of diffusing into the enamel much more efficiently than ionic species [25]. Additionally, the presence of metaphosphates was shown to reduce the formation of loosely bound CaF_2 deposits as well as firmly bound fluoride (fluorapatite-like mineral) on the enamel surface, and the opened surface pores contribute for the diffusion process [27, 28].

Biomimetic Approaches

During odontogenesis, ameloblasts secrete proteins of the enamel extracellular matrix responsible for guiding the deposition of hydroxyapatite in its characteristic prismatic structure. Approximately 90% of these proteins are amelogenins, which present a negatively charged structure that interact with calcium ions and control the orientation of the growing crystals [30, 31]. In the maturation stage, enamel mineral content increases while the enamel matrix is degraded by proteolytic enzymes [32]. As a result, while remineralization of initial caries lesions (i.e., increase in mineral content) is possible to a certain extent, biomimetic template analogues of the natural proteins involved in biomineralization are necessary to guide hydroxyapatite deposition and promote regeneration of enamel [33]. Different molecules are being tested as amelogenin analogues, all of them presenting multiple negatively charged side chains along its backbone. These molecules can bind to the enamel surface and also attract calcium ions for apatite nucleation [34].

Extracellular enamel matrix proteins (mostly amelogenins) obtained from immature porcine teeth dispersed in propylene glycol alginate have been used in regenerative periodontal therapy for the past 20 years (Emdogain, Straumann, Basel, Switzerland) [35]. Recently, a mixture of this enamel matrix derivative (EMD) and an agarose hydrogel containing $CaCl_2$ was tested on demineralized human enamel. After 96 h in a fluoride containing (500 ppm) phosphate solution, the enamel treated with EMD presented a microstructure similar to that of natural enamel (not observed in the control samples without EMD); however, mechanical properties were not completely recovered [36].

The amelogenin structure and its role in enamel formation has been extensively investigated over the years [32]. Researchers have been trying to reproduce some of the features developing amelogenin-derived peptides with similar amino acid sequences. For example, the use of a peptide containing five (Glutamine-Proline–X) repeats ("X" representing a different amino acid in each sequence) resulted in enamel remineralization similar to the use of 1000 ppm NaF in vitro [30] and in a rat caries model [37]. A porcine leucine-rich amelogenin peptide (LRAP) applied to bovine enamel

presenting artificial caries lesions showed a 34% reduction in lesion depth (control 8%), 48% mineral gain (control 24%), and 55% increase in enamel nanohardness (control 23%) after 10 days in remineralizing solution [38]. Dentin phosphoprotein (DPP) is an important non-collagenous component of the dentin extracellular matrix involved in dentin biomineralization, constituted by several repeats of aspartate-serine-serine (DSS) sequences. The peptide 8DSS (eight DSS sequences) was tested on enamel lesions both in vitro [34] and in a rat enamel caries model [33] with results similar to the use of 1000 ppm NaF.

Self-assembling peptides have gained attention in the field of bioengineering due to their property of transitioning from low-viscosity oligomers to hierarchically organized fibers forming tridimensional biomimetic scaffolds when exposed to specific triggers. An anionic peptide, P_{11}-4, was designed to penetrate the demineralized enamel and rapidly undergo gelation in pH below 8.0. The pH reduction as well as the presence of Ca^{2+} ions neutralize parts of its negative charges, changing the interaction between adjacent side chains and triggering self-assembling. This 3D scaffold would mimic the role of enamel matrix proteins as a template for hydroxyapatite deposition within the lesion. The distance between adjacent calcium-binding sites was shown to favor hydroxyapatite nucleation [39, 40•]. Though commercially available since 2012 (Curodont Repair, Credentis, Windisch, Switzerland), clinical data are scant. Nevertheless, the available information show positive results [41, 42]. The overall performance of P_{11}-4 in vitro is also satisfactory [43, 44] and suggests that the effect of the peptide scaffold inhibiting mineral loss would be more significant than the actual remineralization [39]. However, an in vitro study comparing P_{11}-4, fluoride solutions (10,000 ppm and 43,350 ppm), and a fluid resin (Icon, DMG, Hamburg, Germany) revealed that only P_{11}-4 failed to prevent further mineral loss of enamel lesions. It has been speculated that severe pH changes could lead to flocculation and inactivation of the gel [45].

Poly(amidoamines) (PAMAM) are synthetic dendrimers (highly branched polymers) with properties that can be tailored according to their surface groups. When carboxyl-terminated PAMAM dendrimers are immersed in calcium solution, the interaction of their carboxylic groups with the calcium ions trigger their self-assembling into microribbons, mimicking the amelogenin behavior [46]. Its application resulted in well-oriented apatite deposition on acid-etched enamel surfaces after 3 weeks in artificial saliva, with three times thicker layer compared with the control [31].

Dentin

Dentin is a more complex material compared with enamel with regards to its composition as the matrix of the mineralized tissue is proteinaceous by almost 50% in volume and comprised of collagen fibrils. In evolution, the ability of a collagenous tissue to bear weight and to resist deformation has been associated with the appearance of post-translationally modified proteins, mainly through phosphorylation, that allowed for transport of calcium phosphate inside the lumen of collagen fibrils and the formation of intrafibrillar mineral [47]. The weight-bearing properties of dentin and bone largely derive from intrafibrillar mineralization [48], and evidence has been provided by many on the importance of incorporating mineral inside collagen fibrils to remineralize and restore the functionality of the tissue [49–52].

Remineralization Versus Functional Remineralization

An inherent challenge in evaluating a material's ability to remineralize dentin lies in the fact that an increase in mineral content after treatment is not necessarily associated with improved physical properties like elastic modulus, hardness, and strength [52]. Apatite mineral in dentin needs to be bound to the collagenous matrix or ideally be incorporated into the collagen fibrils to mechanically reinforce the tissue [53]. Remineralization that leads to improved tissue mechanics is called functional remineralization (FR). Therefore, it is critical to evaluate remineralized dentin specimens either by a high-resolution imaging method, e.g., TEM, to identify intrafibrillar mineral or by mechanical testing of the stiffness, hardness, and strength in order to determine if the method was successful and that remineralization was indeed functional. The vast majority of remineralization studies published in the literature did not provide such functional analysis and instead relied primarily on the analysis of mineral content examined by dental X-ray, transmicroradiography (TMR), or micro-computed tomography (μCT) [54, 55]. As those studies do not provide information about the effect on the recovery of physical properties with attempted remineralization procedure, a proper decision about treatment success cannot be made. Furthermore, dentin is a hydrated tissue, and its properties alter significantly when dehydrated [56, 57]. Dehydration effect become particularly severe when demineralized or carious dentin is examined as the demineralized collagen matrix collapses with the removal of water. The "floating" collagen layer, as known from adhesive dentistry, has a much lower stiffness compared with the collapsed collagen layer after drying [57]. Hardness measurements should therefore be performed under fully hydrated conditions.

Remineralization of Dentin Using Products Designed for Enamel

The ability to deliver fluoride to tooth structures has been critical in the prevention and reduction of dental caries, clearly

demonstrating an efficacy for protection and repair of caries-affected enamel, as discussed above. Historically, the beneficial aspects of fluoride exposure to tooth structures have been extended to dentin assuming that similar mechanisms applied in enamel with regards to solubility and mineral formation [58, 59]. While recent studies and literature reviews confirmed that fluoride release into demineralized dentin and dentin caries promotes mineral formation, they also raised concerns that the mineral formed in dentin may not be functionally bound to the organic matrix and may not reinforce collagen fibrils [60, 61]. The hypothesis that fluoride release from glass ionomer cements (GIC) restores dentin properties has been rejected almost a decade ago [62]. While mineral formation in dentin lesions was observed with GIC application, electron microscopy could not identify intrafibrillar mineralization, and changes in hardness were insignificant [62]. It has been shown that exposure of carious dentin to saturated calcium phosphate solutions in vitro will enhance the mineral profile of the lesion over time as shown by X-ray analysis [54, 58]. However, these studies did not analyze tissue functionality. Several studies suggest instead that remineralization induced from saturated solution leads to precipitation of apatite nanocrystals on the lesion surface or the surface of collagen fibrils in the matrix in addition to mineral in the dentin tubules [51]. Such mineral contributes little to the mechanics of dentin and is not functional.

Calcium Silicate Cements

Calcium silicate cements (CSCs) are traditionally used as endodontic sealants and largely in non-weight-bearing applications due to the slower setting behavior compared with GIC. The main advantage of CSC with regards to mineralization is the alkaline environment that they create with initial pH in the range of 10 to 11, resulting in high supersaturation leading to calcium phosphate and apatite precipitation [55]. In vitro and clinical studies have shown increased mineral formation in dentin when CSCs were applied to artificial and natural caries lesions, which was largely associated with intratubular and superficial mineral deposition [61, 63]. Such mineral on the lesion surface appeared to be beneficial towards the mechanical and chemical integrity of the dentin-cement interface [61]. Recent studies using mineral trioxide aggregate (MTA) or modified MTA products (for example, Biodentine[TM]) demonstrated that cements without process-directing agents do not induce intrafibrillar mineral in collagen, differently from experimental groups of modified CSCs supplemented with process-directing agents, in which the formation of intrafibrillar mineral was shown by TEM analysis and hardness testing [60, 64].

The use of Bioglass[R] (BG) has also been reported to promote remineralization of dentin. However, while these studies demonstrate mineral formation in the presence of BG, such mineral predominantly occluded dentin tubules. While it may be beneficial for treating dentin hypersensitivity, proof of mechanical reinforcement of affected dentin after the application of BG has not been provided [65].

Process-Directing Agents for Dentin Remineralization

Phosphorylation of proteins in the extracellular matrices was critical to the evolution of dentin and bone and ultimately allowed for locally restricted mineralization of collagen fibrils in intrafibrillar and extrafibrillar spaces [66]. A method that has been able to mimic this process in vitro was developed by Laurie Gower and collaborators, termed the polymer-induced liquid precursor (PILP) approach for collagen mineralization [67]. The PILP method relies on the presence of process-directing agents that stabilize saturated calcium phosphate solutions by forming nanodroplets rich in these ions. When in contact with collagen fibrils, nanodroplets release ions into collagen fibrils and promote formation of amorphous calcium phosphate which gradually turns into oriented apatite crystals similar to mineral in natural bone and dentin [68]. A number of process-directing agents have been identified and have been tested on collagen or on demineralized dentin, e.g., polyaspartic acid (pAsp), polyacrylic acid combined with tripolyphosphate, osteopontin, phosvitin, and cationic polyamide among others [51, 52, 60, 69–73].

While the PILP method has been developed over 15 years ago, there is, so far, no commercial product on the market that applies a process-directing agent to dentinal caries, and only a limited number of studies have reported on a translational approach for the PILP method in dentistry. There are attempts of incorporating process-directing agents into dental adhesives to promote the remineralization of acidic self-etching primers and aid in reducing the incidence of nanoleakage and secondary caries [74, 75]. For applications towards the remineralization of deep caries and the repair of dentin, the incorporation of pAsp and other agents into glass ionomer cements have been investigated with some indication of functional remineralization of artificial lesions in dentin [76•].

Conclusions

Based on the available literature, it is evident that most biomimetic approaches for enamel remineralization are still in their laboratory stage. The only commercial product shows contradictory results in vitro, and the clinical evidence is low. Ion-releasing fillers in enamel only have in situ results. Probably, the best approach is to combine both.

Analysis of dentin mineral content is not a good indicator of treatment success when evaluating dentin remineralization. Clinical translational approaches that include process-directing agents for intrafibrillar collagen mineralization are

still premature and have so far only been explored experimentally in vitro, with good outcomes on structural and functional repair of artificial dentin carious lesions by remineralization.

Compliance with Ethical Standards

Conflict of Interest Dr. Braga declares no conflicts of interest. Dr. Habelitz reports no conflicts of interest. In addition, Dr. Habelitz has a patent on compositions for the remineralization of dentin pending and not licensed.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. Murdoch-Kinch CA, McLean ME. Minimally invasive dentistry. J Am Dent Assoc. 2003;134(1):87–95. https://doi.org/10.14219/jada.archive.2003.0021.

2.•• Innes NPT, Chu CH, Fontana M, Lo ECM, Thomson WM, Uribe S, et al. A century of change towards prevention and minimal intervention in cardiology. J Dent Res. 2019;98(6):611–7. https://doi.org/10.1177/0022034519837252 **This paper summarizes adaptation in caries management and restorative treatments which mostly occurred in the last 20 to 30 years and were leading to the concept of minimally-invasive dentistry which emphasizes tissue repair and conservation over surgical removal.**

3. 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;16:12. https://doi.org/10.1186/s12903-016-0171-6.

4. Lenzi TL, Montagner AF, Soares FZ, de Oliveira Rocha R. Are topical fluorides effective for treating incipient carious lesions?: a systematic review and meta-analysis. J Am Dent Assoc. 2016;147(2):84–91 e1. https://doi.org/10.1016/j.adaj.2015.06.018.

5. Li X, Wang J, Joiner A, Chang J. The remineralisation of enamel: a review of the literature. J Dent. 2014;42(Suppl 1):S12–20. https://doi.org/10.1016/S0300-5712(14)50003-6.

6. Philip N. State of the art enamel remineralization systems: the next frontier in caries management. Caries Res. 2018;53(3):284–95. https://doi.org/10.1159/000493031.

7. Melo MA, Weir MD, Rodrigues LK, Xu HH. Novel calcium phosphate nanocomposite with caries-inhibition in a human in situ model. Dent Mater. 2013;29(2):231–40. https://doi.org/10.1016/j.dental.2012.10.010.

8. Souza JG, Tenuta LM, Del Bel Cury AA, Nobrega DF, Budin RR, de Queiroz MX, et al. Calcium prerinse before fluoride rinse reduces enamel demineralization: an in situ caries study. Caries Res. 2016;50(4):372–7. https://doi.org/10.1159/000446407.

9. Meyer-Lueckel H, Wierichs RJ, Schellwien T, Paris S. Remineralizing efficacy of a CPP-ACP cream on enamel caries lesions in situ. Caries Res. 2015;49(1):56–62. https://doi.org/10.1159/000363073.

10. Gonzalez-Cabezas C, Fernandez CE. Recent advances in remineralization therapies for caries lesions. Adv Dent Res. 2018;29(1):55–9. https://doi.org/10.1177/0022034517740124.

11. Taha AA, Patel MP, Hill RG, Fleming PS. The effect of bioactive glasses on enamel remineralization: a systematic review. J Dent. 2017;67:9–17. https://doi.org/10.1016/j.jdent.2017.09.007.

12. Parkinson CR, Siddiqi M, Mason S, Lippert F, Hara AT, Zero DT. Anticaries potential of a sodium monofluorophosphate dentifrice containing calcium sodium phosphosilicate: exploratory in situ randomized trial. Caries Res. 2017;51(2):170–8. https://doi.org/10.1159/000453622.

13. Alania Y, Natale LC, Nesadal D, Vilela H, Magalhaes AC, Braga RR. In vitro remineralization of artificial enamel caries with resin composites containing calcium phosphate particles. J Biomed Mater Res B Appl Biomater. 2018. https://doi.org/10.1002/jbm.b.34246.

14. Langhorst SE, O'Donnell JN, Skrtic D. In vitro remineralization of enamel by polymeric amorphous calcium phosphate composite: quantitative microradiographic study. Dent Mater. 2009;25(7):884–91. https://doi.org/10.1016/j.dental.2009.01.094.

15. Pinto MFC, Alania Y, Natale LC, Magalhaes AC, Braga RR. Effect of bioactive composites on microhardness of enamel exposed to carious challenge. Eur J Prosthodont Restor Dent. 2018;26(3):122–8. https://doi.org/10.1922/EJPRD_01781Pinto07.

16.• Braga RR. Calcium phosphates as ion-releasing fillers in restorative resin-based materials. Dent Mater. 2019;35(1):3–14. https://doi.org/10.1016/j.dental.2018.08.288 **A recent review written by one of the authors discussing the use of calcium orthophosphates particles as additives in restorative resin-based materials.**

17. Krishnan V, Bhatia A, Varma H. Development, characterization and comparison of two strontium doped nano hydroxyapatite molecules for enamel repair/regeneration. Dent Mater. 2016;32(5):646–59. https://doi.org/10.1016/j.dental.2016.02.002.

18. Souza BM, Comar LP, Vertuan M, Fernandes Neto C, Buzalaf MA, Magalhaes 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. https://doi.org/10.1159/000438466.

19. Lelli M, Putignano A, Marchetti M, Foltran I, Mangani F, Procaccini M, et al. Remineralization and repair of enamel surface by biomimetic Zn-carbonate hydroxyapatite containing toothpaste: a comparative in vivo study. Front Physiol. 2014;5:333. https://doi.org/10.3389/fphys.2014.00333.

20. Shen P, Walker GD, Yuan Y, Reynolds C, Stanton DP, Fernando JR, et al. Importance of bioavailable calcium in fluoride dentifrices for enamel remineralization. J Dent. 2018;78:59–64. https://doi.org/10.1016/j.jdent.2018.08.005.

21. Lippert F, Gill KK. Carious lesion remineralizing potential of fluoride- and calcium-containing toothpastes: A laboratory study. J Am Dent Assoc. 2019. https://doi.org/10.1016/j.adaj.2018.11.022.

22. Karlinsey RL, Pfarrer AM. Fluoride plus functionalized beta-TCP: a promising combination for robust remineralization. Adv Dent Res. 2012;24(2):48–52. https://doi.org/10.1177/0022034512449463.

23. Thilo E. The structural chemistry of condensed inorganic phosphates. Angew Chem Int Ed Engl. 1965;4(12):1061–71. https://doi.org/10.1002/anie.196510611.

24. Danelon M, Takeshita EM, Peixoto LC, Sassaki KT, Delbem ACB. Effect of fluoride gels supplemented with sodium trimetaphosphate in reducing demineralization. Clin Oral Investig. 2014;18(4):1119–27. https://doi.org/10.1007/s00784-013-1102-4.

25. Manarelli MM, Delbem AC, Lima TM, Castilho FC, Pessan JP. In vitro remineralizing effect of fluoride varnishes containing

sodium trimetaphosphate. Caries Res. 2014;48(4):299–305. https://doi.org/10.1159/000356308.

26. Goncalves FMC, Delbem ACB, Pessan JP, Nunes GP, Emerenciano NG, Garcia LSG, et al. Remineralizing effect of a fluoridated gel containing sodium hexametaphosphate: an in vitro study. Arch Oral Biol. 2018;90:40–4. https://doi.org/10.1016/j.archoralbio.2018.03.001.

27. Manarelli MM, Delbem AC, Binhardi TD, Pessan JP. In situ remineralizing effect of fluoride varnishes containing sodium trimetaphosphate. Clin Oral Investig. 2015;19(8):2141–6. https://doi.org/10.1007/s00784-015-1492-6.

28. 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. https://doi.org/10.1159/000449358.

29. Danelon M, Garcia LG, Pessan JP, Passarinho A, Camargo ER, Delbem ACB. Effect of fluoride toothpaste containing nano-sized sodium hexametaphosphate on enamel remineralization: an in situ study. Caries Res. 2018;53(3):260–7. https://doi.org/10.1159/000491555.

30. Lv X, Yang Y, Han S, Li D, Tu H, Li W, et al. Potential of an amelogenin based peptide in promoting remineralization of initial enamel caries. Arch Oral Biol. 2015;60(10):1482–7. https://doi.org/10.1016/j.archoralbio.2015.07.010.

31. Chen M, Yang J, Li J, Liang K, He L, Lin Z, et al. Modulated regeneration of acid-etched human tooth enamel by a functionalized dendrimer that is an analog of amelogenin. Acta Biomater. 2014;10(10):4437–46. https://doi.org/10.1016/j.actbio.2014.05.016.

32. Brookes SJ, Robinson C, Kirkham J, Bonass WA. Biochemistry and molecular biology of amelogenin proteins of developing dental enamel. Arch Oral Biol. 1995;40(1):1–14. https://doi.org/10.1016/0003-9969(94)00135-X.

33. Zheng W, Ding L, Wang Y, Han S, Zheng S, Guo Q, et al. The effects of 8DSS peptide on remineralization in a rat model of enamel caries evaluated by two nondestructive techniques. J Appl Biomater Funct Mater. 2019;17(1):2280800019827798. https://doi.org/10.1177/2280800019827798.

34. Yang Y, Lv XP, Shi W, Li JY, Li DX, Zhou XD, et al. 8DSS-promoted remineralization of initial enamel caries in vitro. J Dent Res. 2014;93(5):520–4. https://doi.org/10.1177/0022034514522815.

35. Hammarstrom L, Heijl L, Gestrelius S. Periodontal regeneration in a buccal dehiscence model in monkeys after application of enamel matrix proteins. J Clin Periodontol. 1997;24(9 Pt 2):669–77.

36. Cao Y, Mei ML, Li QL, Lo EC, Chu CH. Enamel prism-like tissue regeneration using enamel matrix derivative. J Dent. 2014;42(12):1535–42. https://doi.org/10.1016/j.jdent.2014.08.014.

37. Han S, Fan Y, Zhou Z, Tu H, Li D, Lv X, et al. Promotion of enamel caries remineralization by an amelogenin-derived peptide in a rat model. Arch Oral Biol. 2017;73:66–71. https://doi.org/10.1016/j.archoralbio.2016.09.009.

38. Bagheri GH, Sadr A, Espigares J, Hariri I, Nakashima S, Hamba H, et al. Study on the influence of leucine-rich amelogenin peptide (LRAP) on the remineralization of enamel defects via micro-focus x-ray computed tomography and nanoindentation. Biomed Mater. 2015;10(3):035007. https://doi.org/10.1088/1748-6041/10/3/035007.

39. Kirkham J, Firth A, Vernals D, Boden N, Robinson C, Shore RC, et al. Self-assembling peptide scaffolds promote enamel remineralization. J Dent Res. 2007;86(5):426–30. https://doi.org/10.1177/154405910708600507.

40.• Kind L, Stevanovic S, Wuttig S, Wimberger S, Hofer J, Muller B, et al. Biomimetic remineralization of carious lesions by self-assembling peptide. J Dent Res. 2017;96(7):790–7. https://doi.org/10.1177/0022034517698419 **A well-conducted investigation on the use of self-assembling peptides for enamel remineralization.**

41. Alkilzy M, Tarabaih A, Santamaria RM, Splieth CH. Self-assembling peptide P11-4 and fluoride for regenerating enamel. J Dent Res. 2018;97(2):148–54. https://doi.org/10.1177/0022034517730531.

42. Schlee M, Schad T, Koch JH, Cattin PC, Rathe F. Clinical performance of self-assembling peptide P11 -4 in the treatment of initial proximal carious lesions: a practice-based case series. J Investig Clin Dent. 2018;9(1). https://doi.org/10.1111/jicd.12286.

43. Silvertown JD, Wong BPY, Sivagurunathan KS, Abrams SH, Kirkham J, Amaechi BT. Remineralization of natural early caries lesions in vitro by P11-4 monitored with photothermal radiometry and luminescence. J Investig Clin Dent. 2017;8(4):e12257. https://doi.org/10.1111/jicd.12257.

44. Takahashi F, Kurokawa H, Shibasaki S, Kawamoto R, Murayama R, Miyazaki M. Ultrasonic assessment of the effects of self-assembling peptide scaffolds on preventing enamel demineralization. Acta Odontol Scand. 2016;74(2):142–7. https://doi.org/10.3109/00016357.2015.1066850.

45. Wierichs RJ, Kogel J, Lausch J, Esteves-Oliveira M, Meyer-Lueckel H. Effects of self-assembling peptide P11-4, fluorides, and caries infiltration on artificial enamel caries lesions in vitro. Caries Res. 2017;51(5):451–9. https://doi.org/10.1159/000477215.

46. Chen L, Yuan H, Tang B, Liang K, Li J. Biomimetic remineralization of human enamel in the presence of polyamidoamine dendrimers in vitro. Caries Res. 2015;49(3):282–90. https://doi.org/10.1159/000375376.

47. Deshpande AS, Fang PA, Zhang X, Jayaraman T, Sfeir C, Beniash E. Primary structure and phosphorylation of dentin matrix protein 1 (DMP1) and dentin phosphophoryn (DPP) uniquely determine their role in biomineralization. Biomacromolecules. 2011;12(8):2933–45. https://doi.org/10.1021/bm2005214.

48. Kinney JH, Habelitz S, Marshall SJ, Marshall GW. The importance of intrafibrillar mineralization of collagen on the mechanical properties of dentin. J Dent Res. 2003;82(12):957–61. https://doi.org/10.1177/154405910308201204.

49. Olszta MJ, Cheng XG, Jee SS, Kumar R, Kim YY, Kaufman MJ, et al. Bone structure and formation: a new perspective. Mater Sci Eng R-Rep. 2007;58(3-5):77–116.

50. Nudelman F, Pieterse K, George A, Bomans PH, Friedrich H, Brylka LJ, et al. The role of collagen in bone apatite formation in the presence of hydroxyapatite nucleation inhibitors. Nat Mater. 2010;9(12):1004–9. https://doi.org/10.1038/nmat2875.

51. Niu LN, Zhang W, Pashley DH, Breschi L, Mao J, Chen JH, et al. Biomimetic remineralization of dentin. Dent Mater. 2014;30(1):77–96. https://doi.org/10.1016/j.dental.2013.07.013.

52. Burwell AK, Thula-Mata T, Gower LB, Habelitz S, Kurylo M, Ho SP, et al. Functional remineralization of dentin lesions using polymer-induced liquid-precursor process. PLoS One. 2012;7(6):e38852. https://doi.org/10.1371/journal.pone.0038852.

53. Bertassoni LE, Habelitz S, Marshall SJ, Marshall GW. Mechanical recovery of dentin following remineralization in vitro–an indentation study. J Biomech. 2011;44(1):176–81. https://doi.org/10.1016/j.jbiomech.2010.09.005.

54. ten Cate JM. Remineralization of caries lesions extending into dentin. J Dent Res. 2001;80(5):1407–11. https://doi.org/10.1177/00220345010800050401.

55. Prati C, Gandolfi MG. Calcium silicate bioactive cements: biological perspectives and clinical applications. Dent Mater. 2015;31(4):351–70. https://doi.org/10.1016/j.dental.2015.01.004.

56. Bertassoni LE, Habelitz S, Kinney JH, Marshall SJ, Marshall GW Jr. Biomechanical perspective on the remineralization of dentin. Caries Res. 2009;43(1):70–7. https://doi.org/10.1159/000201593.

57. Ryou H, Turco G, Breschi L, Tay FR, Pashley DH, Arola D. On the stiffness of demineralized dentin matrices. Dent Mater. 2016;32(2):161–70. https://doi.org/10.1016/j.dental.2015.11.029.

58. Mukai Y, ten Cate JM. Remineralization of advanced root dentin lesions in vitro. Caries Res. 2002;36(4):275–80. https://doi.org/10.1159/000063924.

59. Ten Cate JM, Buzalaf MAR. Fluoride mode of action: once there was an observant dentist. J Dent Res. 2019;98(7):725–30. https://doi.org/10.1177/0022034519831604.

60. Qi YP, Li N, Niu LN, Primus CM, Ling JQ, Pashley DH, et al. Remineralization of artificial dentinal caries lesions by biomimetically modified mineral trioxide aggregate. Acta Biomater. 2012;8(2):836–42. https://doi.org/10.1016/j.actbio.2011.10.033.

61. Watson TF, Atmeh AR, Sajini S, Cook RJ, Festy F. Present and future of glass-ionomers and calcium-silicate cements as bioactive materials in dentistry: biophotonics-based interfacial analyses in health and disease. Dent Mater. 2014;30(1):50–61. https://doi.org/10.1016/j.dental.2013.08.202.

62. Kim YK, Yiu CK, Kim JR, Gu L, Kim SK, Weller RN, et al. Failure of a glass ionomer to remineralize apatite-depleted dentin. J Dent Res. 2010;89(3):230–5. https://doi.org/10.1177/0022034509357172.

63. Hashem D, Mannocci F, Patel S, Manoharan A, Brown JE, Watson TF, et al. Clinical and radiographic assessment of the efficacy of calcium silicate indirect pulp capping: a randomized controlled clinical trial. J Dent Res. 2015;94(4):562–8. https://doi.org/10.1177/0022034515571415.

64. Schwendicke F, Al-Abdi A, Pascual Moscardo A, Ferrando Cascales A, Sauro S. Remineralization effects of conventional and experimental ion-releasing materials in chemically or bacterially-induced dentin caries lesions. Dent Mater. 2019;35(5):772–9. https://doi.org/10.1016/j.dental.2019.02.021.

65. Sauro S, Watson T, Moscardo AP, Luzi A, Feitosa VP, Banerjee A. The effect of dentine pre-treatment using bioglass and/or polyacrylic acid on the interfacial characteristics of resin-modified glass ionomer cements. J Dent. 2018;73:32–9. https://doi.org/10.1016/j.jdent.2018.03.014.

66. Deshpande AS, Beniash E. Bio-inspired synthesis of mineralized collagen fibrils. Cryst Growth Des. 2008;8(8):3084–90.

67. Olszta MJ, Odom DJ, Douglas EP, Gower LB. A new paradigm for biomineral formation: mineralization via an amorphous liquid-phase precursor. Connect Tissue Res. 2003;44(Suppl 1):326–34.

68. Gower LB. Biomimetic model systems for investigating the amorphous precursor pathway and its role in biomineralization. Chem Rev. 2008;108(11):4551–627.

69. Saeki K, Chien YC, Nonomura G, Chin AF, Habelitz S, Gower LB, et al. Recovery after PILP remineralization of dentin lesions created with two cariogenic acids. Arch Oral Biol. 2017;82:194–202. https://doi.org/10.1016/j.archoralbio.2017.06.006.

70. Rodriguez DE, Thula-Mata T, Toro EJ, Yeh YW, Holt C, Holliday LS, et al. Multifunctional role of osteopontin in directing intrafibrillar mineralization of collagen and activation of osteoclasts. Acta Biomater. 2014;10(1):494–507. https://doi.org/10.1016/j.actbio.2013.10.010.

71. Niu LN, Jee SE, Jiao K, Tonggu L, Li M, Wang L, et al. Collagen intrafibrillar mineralization as a result of the balance between osmotic equilibrium and electroneutrality. Nat Mater. 2017;16(3):370–8. https://doi.org/10.1038/nmat4789.

72. Ryou H, Niu LN, Dai L, Pucci CR, Arola DD, Pashley DH, et al. Effect of biomimetic remineralization on the dynamic nanomechanical properties of dentin hybrid layers. J Dent Res. 2011;90(9):1122–8. https://doi.org/10.1177/0022034511414059.

73. Sarem M, Ludeke S, Thomann R, Salavei P, Zou Z, Habraken W, et al. Disordered conformation with low Pii helix in phosphoproteins orchestrates biomimetic apatite formation. Adv Mater. 2017;29(35). https://doi.org/10.1002/adma.201701629.

74. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res. 2011;90(8):953–68. https://doi.org/10.1177/0022034510391799.

75. Tjaderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, et al. Strategies to prevent hydrolytic degradation of the hybrid layer-a review. Dent Mater. 2013;29(10):999–1011. https://doi.org/10.1016/j.dental.2013.07.016.

76.• Bacino M, Girn V, Nurrohman H, Saeki K, Marshall SJ, Gower L, et al. Integrating the PILP-mineralization process into a restorative dental treatment. Dent Mater. 2019;35(1):53–63. https://doi.org/10.1016/j.dental.2018.11.030 **This study proposes methods on how to incorporate process-directing agents into a restorative material to induce functional remineralization of dentin caries.**