**Review articles**

# A novel biomimetic approach in re-mineralizing enamel and dentine - A review

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## ABSTRACT

This study aims at reviewing the various methods on biomimetic remineralization of enamel and dentine. Remineralization of the dentin that is demineralized is important for improving dentin bonding stability and controlling primary and secondary caries. Remineralization of enamel plays a crucial role in the progression of carious process and the management of early caries lesion. This has implications for the management of non-carious tooth loss resulting from dental erosion, attrition, and abrasion. Nevertheless, conventional dentin remineralization strategy is not suitable for re-mineralizing completely demineralized dentin within hybrid layers created by etch-and-rinse and moderately aggressive self-etch adhesive systems, or the superficial part of a caries-affected dentine lesion left behind after minimally invasive caries removal. Biomimetic remineralization represents a different approach to this problem by attempting to backfill the demineralized dentin collagen with liquid-like amorphous calcium phosphate nano-precursor particles that are stabilized by biomimetic analogues of non-collagenous proteins.

**Keywords**: Biomimetic; dentine; remineralization; enamel; calcium; phosphate.

## INTRODUCTION

The human tooth is composed of two layers, the outer layer enamel and the inner dentine-enamel complex (1). Enamel is the hardest mineralised tissue composed of about 95% of mineral content (2). The enamel is composed of Hydroxyapatite crystals of nanoscale level (3). Amelogenin secreted by ameloblasts, are unique set of proteins found in enamel during its formation. The ameloblasts undergo apoptosis after the enamel is formed (4). Processes like remineralization and demineralization coexist during the entire life in the teeth. In pathological conditions, demineralization outweighs remineralization; Dental hard tissue, both the enamel and the underlying dentin, cannot self-heal if damaged. These are treated with synthetic rigid restorative materials. However, this demands removal of healthy surrounding tooth tissues. They have their own disadvantages like hyper-sensitivity to the dentin, interface microleakage between filling materials and dental tissue. These have also resulted in financial problems an example being that in the U.S., more than 100 million dollars is spent annually on dental service (5). Currently it is difficult to remineralize remaining demineralized dentine. Dentine is more difficult to remineralize than enamel. This could be due to the fact that remineralization occurs by growth of residual crystals in the lesions.

### Biomimetic approach

The biomimetic process is to regenerate destroyed enamel and it is an emerging field in regenerative dentistry and the subject of the work of several research groups (6).

### Remineralisation of dentine

Dentine remineralisation is a lot of complicated and fewer effective than enamel remineralization as a result of their square measure residual seed mineral crystals on enamel, however these square measures absent in dentine lesions (7,8). It was reported that underneath a similar re-mineralizing condition, remineralization occurred on the surface of acid-etched enamel however not on the surface of acid-etched dentine (2). This difference could be attributed to the fewer amounts of residual mineral crystals and the exposure of organic matrix on the acid-etched dentine surface. Biomimetic remineralization, a technique that imitates the process of mineralization, represents a special approach to the current downside by making an attempt to backfill the demineralized dentine scleroprotein with liquid-like ACP nano-precursor particles. This does not rely on seed crystallites. This paper may be a systematic review of the various revealed ways that with success achieved biomimetic remineralization of human dentine further because the enamel which might be mentioned later. It is a known fact that the collagen matrix serves as a scaffold for crystal deposition but does not provide a mechanism for nucleation of hydroxyapatite. The biomineralization process is usually modulated (9) by a series of NCPs (non-collagenous proteins). In dentine biomineralization, NCPs with a high affinity for Ca ions and albuminoid

fibrils square measure to blame for control the nucleation and growth of minerals, like dentine matrix super-molecule (DMP1) and dentine phosphophoryn (DPP, DMP2). These NCPs can regulate biomineralization of dentine in vivo by working as nucleator or inhibitor. But it is difficult to extract and purify natural NCPs. Thus, many researchers focus on developing the analogues that can play the role of NCPs in the biomineralization process. NCP analogues like Polyacrylic acid (PAA) and polyvinylphosphonic acid (PVPA) were used in the biomimetic mineralization of dentine. PAA may act like the calcium phosphate binding sites of DMP1, and PVPA simulates the collagen-binding function of DMP1 in guiding the nano-precursors' recruitment to the collagen matrix. STMP, which is used as a chemical phosphorylating reagent in the food industry, can absorb type I collagen via an electrostatic mechanism and a chemical phosphorylation mechanism. The phosphorylated dentine collagen matrix act as a template-molecule to attract ACP nano-precursors and to nucleate apatite within the collagen fibrils resulting in the formation of intrafibrillar and interfibrillar remineralization of dentine. Apart from the PVPA and STMP, phosphorylated chitosan, peptide/oligopeptide, and PAMAM dendrimer also functioned as the template-analogues for the biomimetic re-mineralization of dentine collagen matrix.

Silva classified the synthesis of biomaterials as top-down or bottom-up approaches (10). The top-down approach or classical ion-based mineralization strategy occurs by epitaxial growth over existing seed crystallites, which cannot occur by spontaneous nucleation of minerals on the organic matrix, such as demineralized dentine. In contrast to the top-down approach, the bottom-up approach starts with one or more defined molecular species, which undergoes certain processes that result in a higher-ordered and organized structure. This non-classical particle-based crystallization pathway involves a multistage process. The calcium and phosphate ions would self-assemble into prenucleation clusters. These prenucleation clusters would aggregate into amorphous ACP Nano-precursors in the presence of NCP analogues. Then these precursors penetrate into the gap zones of collagen fibrils and further grow into apatite crystals along the intrafibrillar space of collagen. The intrafibrillar remineralization of collagen fibrils would lead to inter-fibrillar remineralization between adjacent collagen fibrils.

**Remineralisation of enamel**

The critical pH-value for enamel dissolution is around 5.5. Depending upon the amount of phosphate and calcium ions in plaque and saliva; the solubility of the enamel changes. Supersaturation of the saliva is generated by the abundance of phosphate and calcium; and thus, the HAP constitutes the enamel. By means of precipitating calcium phosphate, the enamel surfaces are re-mineralized. However, this remineralization from the saliva cannot resemble the enamel's complex microstructure.

Fluorides are for the rescue of such issues. Fluoride compounds like Sodium Fluoride (NaF) or Stannous Fluoride (Sn (II) F2) are currently the most prominent remineralization systems in oral care (11). Fluoride ions, F-, are believed to enhance the natural enamel remineralization process and to inhibit de-mineralization (12).

Caries preventive property of topically applied fluorides is based on accelerating the reintegration of calcium phosphate mostly derived from saliva into demineralized surface lesions at the tooth-bacterial biofilm interface. However, exposure to extraordinary high fluoride levels can cause side effects like fluorosis and bone weakening. Therefore, the concentration of fluoride in oral care creations is strictly regulated worldwide. For example, in the EU, toothpastes are classified as cosmetic products and a maximum of 1500 ppm fluoride is allowed, and on average toothpastes with fluoride usually sold over the counter contain levels of around 1000 ppm (13). While the safety of the proper use of fluoridated toothpastes has been firmly established by numerous studies, dosage and toxicity aspects always have to be considered, particularly in children, where fluoride overdosing may result in the manifestation of mottled enamel or other signs of chronic fluorosis (14, 15).

Fluorides require salivary calcium and phosphate ions to improve the natural remineralization. Hyposalivation therefore, a common problem in elderly subjects, may significantly impair the preventive efficacy of topically applied fluorides (16). For xerostomia patients (e.g. induced by medications), oral care goods with the addition of calcium and phosphate are suggested in order to recompense a calcium phosphate deficiency (17). To overcome these drawbacks, increasing attention is given to the development of alternative non-fluoride agents that improve remineralization without having any possible side effects on the human body (18,19). In the recent years, biomimetic concepts along these lines have been developed in oral care with the aim to address these issues.

**Hydroxyapatite (HAP)**

Out of all calcium phosphate phases, HAP, $Ca_5(PO_4)_3(OH)$, has the highest similarity to the natural enamel (20,21). It also has the lowest solubility of all calcium phosphates. HAP is synthesized in different

crystallite morphologies and particle sizes, i.e. from nanometre to micrometre size. Commonly used HAP particles in oral care applications are organized in micro clusters. Synthetic HAP particles were shown to interact both with enamel and dentin surfaces where they can unfold their effects such as the reduction of initial bacterial colonization. HAP particles show equivalent performance compared to the standard use of fluorides in oral care.

**B-Tricalcium phosphate (B-TCP)**

There are two different tricalcium phosphates, $Ca_3(PO_4)_2$, known, both of which cannot be found in pure form in nature (22). While α-tricalcium phosphate can only exist at high temperatures (above 1125 °C), β-TCP is stable at room temperature. β-TCP shows merely a moderate solubility in water (25 mg/L at 25°C). The size of β-TCP powders differs depending on the milling-procedures, but mostly ranges between 0.01-5 μm. β-TCP is the bio form of tricalcium phosphate that is used in inventions for medicine and oral care.

Re-mineralization of corroded enamel is performed by calcium and phosphate originating from saliva. By using β-TCP, the concentration of calcium in the saliva can be increased (β-TCP is soluble at pH < 6). An in vivo study showed an increase of calcium in the saliva as consequence of acidic attacks and an acidic plaque-pH, when 2.5% β-TCP is used as additional compound in chewing gums. Compared to the control (conventional gum, without an additional calcium source), the pH increased (buffering of acidic attacks) as well as the concentrations of free calcium and free phosphates. Both can be used for remineralization, when the enamel is damaged by acidic attacks. This study also showed a deposition of β-TCP in plaque and saliva becoming available as soon as an acidic attack occurs (23, 24).

**Amorphous calcium phosphate (ACP)**

Amorphous Calcium Phosphates (ACP), $Cax\ (PO_4)\ y \cdot n\ H_2O$, are mostly synthesized from the aqueous precipitation of calcium phosphates. ACP mostly occurs as spherical particles with an average diameter of 20-200 nm that can be visualized by SEM. It is suggested that ACP has apatite-like structures. ACP is well studied and largely combined with Casein Phospho peptides (CPP), which stabilize calcium in aqueous solutions. CPP is a natural peptide which can be obtained from bovine milk by tryptic digestion of casein containing the protein-sequence Pse-Pse-Pse-Glu-Glu. Furthermore, CPP has been shown to increase the plaque-pH due to enzymatic breakdown of casein and a resulting increase of ammonia.

ACP seem like to be a promising challenger for re-mineralizing initial caries lesions and enamel corroded by acidic assaults, especially if applied directly before, during or after acid-intervention. However, the conditions of ACP synthesis/precipitation and consequently the use in toothpastes, mouth rinses and chewing gums need to be standardized to have the same quality in each application.

**Calcium phospho silicate (CSPS)**

Calcium phospho silicate (CSPS) is a bioactive glass comprising 45% SiO2, 24.5% Na2O, 24.5% CaO and 6% $P_2O_5$. This mineral was originally developed as bone-regenerative material due to its high bio-compatibility and the ability to release calcium and phosphate ions. Besides occluding dentinal tubules and consequently desensitizing effects of this calcium phosphor silicate, there are also studies describing re-mineralizing potential as well as caries prevention and antiplaque characteristics. The active mechanism seems to be the delivery and deposition of calcium- and phosphate-ions that form a crystalline carbonated-apatite layer.

Tai *et al.,* investigated the Plaque Index (PLI) and Bleeding Index (BLI) within a study with 100 subjects in a RCT over a six-week time period (25). In this study, CSPS was tested against a placebo-control and showed significant improvement of oral health measured by a reduction of PLI and GBI. Nevertheless, the mode of action was not clarified by this study.

Additionally, two *in situ* studies both from Parkinson *et al.,* in 2017 used sodium mono-fluorophosphate with different concentrations as positive control and 5% CSPS as test dentifrice (26). They also utilized different concentrations of sodium mono-fluoro-phosphate in both studies (ranged from 0% to 0.15%). The authors conclude in both the studies no detectable developments of 5% CSPS (alone or in comparison with sodium mono-fluorophosphate) compared to the positive (0.15 ppm sodium mono-fluorophosphate, 0% CSPS) or the negative control (0 ppm sodium mono-fluorophosphate, 0% CSPS). CSPS as active compound in toothpastes seems to act as a calcium-reservoir that can be used for remineralization of demineralized enamel or dentine. Clinical studies with CSPS alone (not in combination with any fluorides) are needed to test the outcome for caries prevention *in vivo*. Plaque reduction and clear improvement of oral (gingival) health can be noticed, when CSPS is used. In-situ studies were able to show non-inferiority to fluorides in case of re-mineralization.

Unlike the bio-glass explained above, which required the addition of fluoride compounds such as sodium monofluorophosphate into the toothpaste formulation,

a fluoride-containing bioactive glass was recently introduced as a caries re-mineralizing and preventive additive in toothpastes. Fluoridated bioglass (f-BG) has fluoride, strontium, potassium and zinc incorporated within the glass itself, thus enabling simultaneous delivery of $Sr^{2+,}$ $Ca^{2+}$, $PO_4^{3-}$ and $F^-$ ions into the initial caries lesions to promote remineralization by formation of a partially fluoridated crystal structure, zinc ions for bactericidal function, and potassium as a desensitizing agent. Having the fluoride incorporated within the glass prevents the risk of premature reaction of fluoride and calcium ions to Calcium Fluoride ($CaF_2$), which reduces the bioavailability of the two ions. However, the lack of clinical studies does not permit any firm conclusions on their effectiveness.

**Calcium glycerophosphate (CGP)**

Calcium Glycerophosphate, $C_3H_7CaO_6P$, (CGP) is a salt of glycerol phosphoric acid. It is typically used as a food ingredient and a nutrition supplement. The first studies from 1972 evaluating the cariostatic impact of this organic calcium phosphate by Bowen used CGP as a nutrient supplement. Brook *et al.*,(27,28) used CGP as a nutrient supplement too. Within a cohort of 14 children, the mean plaque levels were estimated between a group of children receiving 1% CGP 4 times daily and a group without any specific treatment. While plaque levels were increased in the experimental group, $Ca^{2+}$ was not different. An even smaller study sample (n=8) did not clean their teeth for 18 days and rinsed with a 50%-sucrose solution. Optical changes were characterized as demineralization and could not be reduced by the addition of CGP (1%). Even topical applications of sodium fluoride (2%) were not able to inhibit these changes. Another study determined the accumulation of CGP in the dental plaque by having three different mouth rinse-interventions: (1) No CGP, (2) 0.5% CGP and (3) 1.5% CGP. The concentration of phosphate was significantly greater in the plaque in (3) compared to (1), indicating a higher potential for buffering acidic attacks.

**DISCUSSION**

Hydroxyapatite, in enamel and in bone, is accountable for the mechanical behavior of the calcified tissues. Unlike bone, when the enamel hydroxyapatite is dissolved or scratched, it cannot spontaneously re-mineralize, because enamel contains no cells. Biomimetic carbonate hydroxyapatite (CHA) nanocrystals have been synthesized with a stoichiometric Ca/P molar ratio of around 1.7 ± 0.1, containing 4 ± 1 wt.% of carbonate ions, prevalently replacing phosphate groups, while 1% of $Ca^{2+}$ions are substituted by $Zn^{2+}$. The nanostructured Zn-CHA microcrystals, made in laboratory according to the original methodology, represent the active component of the experimental Zn-CHA toothpaste. The micrometric dimension of the crystal clusters allows avoiding any suspicion about the *in vivo* utilization of Nano-dimensioned particles. Nevertheless, the nanostructured surface of the micro clusters is responsible for the high surface area that is crucial for their chemical reactivity.

Synthesized biomimetic CHA nanocrystals and human enamel apatite not only contain a similar carbonate amount, but also have been shown to promote carbonate substitution to the phosphate and/or hydroxyl group, which is very similar to the synthetic and biological CHA nanocrystals. The synthetic experimental CHA nanocrystals have a plate-like morphology and a structure very close to that of the enamel, dentine the use of a toothpaste containing Zn-substituted CHA nanocrystals can produce a biomimetic coating on the enamel surface, thus mimicking the composition, structure, morphology and surface reactivity of the biological enamel hydroxyapatite. the other hand, the re-mineralizing/ repairing effect of the enamel surface treated using synthetic nanostructured CHA microcrystals is consistent with a mineral biomimetic apatitic deposition, which does not alter the chemical-physic properties of the enamel. The biomimetic CHA coating can appear of different thickness, probably due to the underside different enamel surface morphology, which can change in function of the degree of enamel damage. However, the EDAX analysis reveals that the Ca/P molar ratio of CHA crystals (about 1.7) is homogeneously constant on the enamel surface. This finding assures a uniform enamel protection against the enamel wear and loss phenomena, thus preventing dentine exposure. Results of the first clinical randomized trial by Orsini *et al.*, (2010) have already demonstrated the efficacy of Zn-CHA toothpastes in reducing DH (29). Moreover, a further very recent randomized trial by the same authors showed that this effect could be exerted after only 3 days of treatment (30). The results of this *in vivo* morphological and chemical-physic study might in part explain the beneficial effect of Zn-CHA toothpastes in reducing DH, since the deposition of a synthetic nanostructured CHA microcrystals-rich coating could lead to a re-mineralizing/repairing effect of the enamel surface, in the teeth treated using Zn-CHA toothpaste. Therefore, the principal finding of this study is that: the re-mineralizing mechanism of the nanostructured CHA microcrystals, largely documented by previous *in vitro* reports can be also confirmed *in vivo*. Moreover, it can be suggested that this synthetic CHA deposition mainly occurs on the enamel areas characterized by enamel loss and/or damage (probably due to erosion effects), thus being considered as a real enamel repair. In

contrast, the use of a toothpaste containing KNO3/NaF may form only a deposition, consisting of silica, as an abrasive phase on the enamel surface, which, however, does not remineralize the damaged enamel area, but it is generally deposed in correspondence of natural concavities proper of the natural tooth morphology. Furthermore, no deposition on the enamel surfaces has been observed after treatment using the fluoride and the KNO3/NaF toothpastes, which may lead only to a partial substitution of the hydroxyl groups with fluoride ions in the native enamel hydroxyapatite. The CHA formed coating is generally insoluble in physiological mouth pH, but it may undergo to solubilization when, for instance, a bacterial biofilm covers the teeth and its products decrease the pH value. During the CHA coating solubilization, Ca ions, phosphates, and Zn ions are released, allowing the Zn to exploit a strong antibacterial effect, which interferes with the plaque formation, thus preventing further solubilization processes of the newly deposed Zn-CHA coating. Therefore, it may be suggested that the coating formed by Zn- CHA toothpastes may exploit not only a re-mineralizing effect of the dental surface, but also a beneficial effect toward bacterial plaque attacks. The main limitation of this work is that the *in vivo* re-mineralizing effect exploited by the Zn-CHA toothpaste was morphologically demonstrated only on the enamel surfaces, since the analysed extracted teeth did not present areas of dentine exposition. Therefore, further studies will be carried out *in vivo* to analyse whether a stable biomimetic CHA deposition and a repairing mechanism can be demonstrated also in dentinal surfaces. In conclusion, the present study shows that only the use of a toothpaste containing Zn-substituted CHA nanocrystals can produce a biomimetic coating on the enamel surface, thus mimicking the composition, structure, morphology and surface reactivity of the biological enamel hydroxyapatite.

## CONCLUSION

The human tooth, though being a hard structure is subject to damage and erosion resulting from dental caries. Once the tooth enamel and dentine are eroded, it does not heal naturally. Dentine remineralization is more complex and less effective than enamel remineralization because there are residual seed mineral crystals on enamel, but these are absent in dentine lesions (7, 8). Biomineralization process is an organic, matrix particle-mediated, non-classical crystallization pathway. Biomimetic remineralization, a methodology that imitates the natural process of mineralization, represents a different approach to this problem by attempting to backfill the demineralized dentine collagen with liquid-like ACP Nano-precursor particles. This bottom-up remineralization strategy does not rely on seed crystallites. biomineralization process is usually modulated by a series of NCPs (9), it is difficult to extract and purify natural NCPs. PAA and PVPA were used as the NCP analogues in biomimetic mineralization of dentine. The phosphorylated dentine collagen matrix functions as a template-molecule to attract ACP Nano-precursors and to nucleate apatite within the collagen fibrils resulting in the formation of intrafibrillar and interfibrillar remineralization of dentine. Different concentrations (20%–37%) of PA were also used to expose the dentine collagen matrix. It is suggested that treatment of dentine with 37% PA for less than 1 min does not denature the dentine collagen matrix. In the presence of NCP analogues, these prenucleation clusters aggregate into amorphous ACP Nano-precursors. fluoride-based dentin remineralization strategies also result in hyper-mineralization of the lesion surface and prevent effective remineralization of the deeper parts of the carious lesion. the remineralisation of the enamel; Supersaturation of the saliva is generated by the abundance of phosphate and calcium; and thus, the HAP constitutes the enamel. By means of precipitating calcium phosphate, the enamel surfaces are re-mineralized. When the phosphate and calcium ions pass through the protein rich pellicle layer and reach the enamel surface; remineralization occurs. Fluorides require calcium and phosphate ions from saliva to improve the natural remineralization process. non-fluoride agents that improve remineralization. Out of all calcium phosphate phases, HAP, $Ca_5(PO_4)_3(OH)$, has the highest similarity to the natural enamel. Others are β-TCP, ACP, Cax $(PO_4)y \cdot n$ H2O, are mostly synthesized from the aqueous precipitation of calcium phosphates. The structure of ACP is mostly nano-crystalline, i.e. it has a short-range order of very small dimensions in the range of a few interatomic distances; ACP seems to be a promising candidate for re-mineralizing initial caries lesions and enamel eroded by acidic attacks, especially if applied directly before, during or after acid-intervention. CSPS as active compound in toothpastes seems to act as a calcium-reservoir that can be used for remineralization of demineralized enamel or dentine.

Many attempts at re-mineralization with fluorides have been made in this regard, but it turns out that this method requires special conditions, and the process of remineralization is too long, making it unsuitable for application in clinical settings. Ca $(OH)_2$ has also been used for the same purpose, in both *in vitro* and *in vivo* experiments, and its re-mineralizing properties have been demonstrated. However, its effect on collagen fibres has not been fully investigated. One group of researchers dealing with deep carious lesions used an

innovative approach, applying clusters of ACP, which have outstanding re-mineralizing potential for collagen (2). An analogue was made with PAC and PASC which stabilize ACP, because of their ability to chelate calcium ions (property due to their many carboxyl groups), and the composition of carboxymethyl chitosan was chosen. This derivative of chitosan also has many carboxyl groups, which may delay or inhibit the spontaneous precipitation of calcium phosphate and have chelating capabilities. The carboxymethyl chitosans recommended as an indirect pulp agent in combination with ACP. Moreover, it can be converted into a matrix (scaffold) through lyophilization for further remineralization. Furthermore, the authors focus on its biological compatibilities and degradation, antibacterial properties and lack of toxicity. These examples of the application of dentin remineralization prove that this relatively new approach can be quite promising in various areas of clinical application. This is so because biomimetic nanomaterials are used. They are completely new as a concept, and thanks to their nanostructure, they come close to natural tooth tissues. However, in order for them to be implemented clinically and for practical recommendations to be offered, fundamental research studies on dentin and enamel remineralization and regeneration are still needed.

**CONFLICTS OF INTEREST:** There are no conflicts of interest.

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