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Review

Relationship between dentifrices based on hydroxyapatites and human enamel remineralization

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Abstract

Enamel demineralization is an intricate process that holds significant clinical consequences, being a central part of the emergence and progression of various dental problems, most notably dental caries. This demineralization of tooth enamel is linked to a variety of factors, including the composition of oral microbiota and that of saliva, the prevalence of sugar consumption or acidic soft drinks, and, of course, oral hygiene practices. The oral microbiome plays a role in generating organic acids that foster an environment conducive to enamel breakdown. Additionally, a decrease in saliva production can lower the oral environment's ability to neutralize acids and support the remineralization process, thereby intensifying demineralization. Dentifrices enriched with biomimetic hydroxyapatites are important in preventing demineralization as they not only help with oral hygiene but also provide key ions that help strengthen enamel. Considering their similarity to the natural component in enamel, synthetic hydroxyapatites have recently emerged as potent remineralizing agents. Hence, this work aims to illustrate in a simple and concise manner, some of the aspects involved in enamel demineralization and its subsequent remineralization, namely in the relationship between enriched toothpastes containing biomimetic hydroxyapatites and their remineralization efficacy.

Keywords

demineralization, remineralization, tooth enamel, hydroxyapatite, biomimetic hydroxyapatites, tooth-paste

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Introduction

Tooth enamel is arguably the strongest tissue within the human body, a necessary attribute considering the endless masticatory cycles throughout a human lifespan. The process by which enamel is formed is called amelogenesis, which involves extracellular mineralization. While the intricacies of this process may appear convoluted and complex, for the purpose of this paper, it remains necessary to convey certain aspects in a more concise manner. Hence, remineralization is comprised of two primary stages – the secretory stage and that of maturation [1]. The extracellular matrix is secreted by ameloblasts, specialized cells that secrete unique matrix proteins (that have little in common with other known proteins) [2] and proteinases (matrix metalloproteinase-20 and kallikrein-related peptidase-4) at the dentin surface [3]. The main component in the development of enamel is amelogenin, a protein highly hydrophobic in nature, and possessing a hydrophilic C-terminus region [4]. For further clarification, one work [5] employed NMR spectroscopy to reveal four primary amelogenin structural elements, namely a N-terminus grouping of four α -helical fragments (S9-V19, T21-P33, Y39-W45, V53-Q56), an elongated random coil section interrupted by two 310 helices (P60-Q117), an extended proline-rich PPII-helical region (P118-L165), and a charged hydrophilic C-terminus (L165-D180). C-terminus is reported to be a determining factor for the parallel alignment of hydroxyapatite crystals [4]. The adsorption of amelogenin onto calcium phosphate, which encourages the alignment of amorphous calcium phosphate particles into ribbons, is critical for enamel formation [6]. The other two major structural proteins are enamelin and ameloblastin. The second most abundant protein next to amelogenin is ameloblastin and it is located between enamel rods [7]. Ameloblastin is reported to be integral in maintaining the differentiation state of ameloblasts, a crucial part of enamel formation [8]. It is also involved in cell-matrix adhesion as well as in mineral formation [9]. While the largest, enamelin is also the least abundant (around 1-5%) of all matrix proteins and its domains are mostly found near the surface of the enamel [10]. It is suggested that the presence of enamelin is necessary to control crystal growth, achieve prism structural organization, and attain the ideal thickness of enamel [11]. All these primary matrix proteins are hydrolyzed by matrix metalloproteinase 20 (MMP20). During tooth development, the majority of the organic enamel matrix, which is made up of amelogenins and enamelins, is resorbed away, leaving behind a calcified tissue that is primarily made up of minerals and a sparse organic matrix. Enamel prisms or rods, which are keyhole-shaped structures with a diameter of roughly 5

μm , are created by the structural arrangement of the material [12]. Therefore, enamel is capable of withstanding daily damage from countless masticatory cycles within the oral environment and remain intact for decades without issues. This is due to the high content of calcified tissue which bestows a high level of hardness and great resistance to wear [12]. However, while very durable, mature enamel lacks cells, thus is unable to regenerate itself. Any damage caused is irrevocable because there is no biological process capable of restoration. Classically, several processes that lead to the wear of enamel have been classified: abrasion (mechanical process involving external matter); demastication (food-teeth mechanical interplay); attrition (tooth-tooth interaction); abfraction (Pathological tooth material loss brought on by biomechanical loading pressures); erosion (chemical etching followed by dissolution); resorption (degradation of biological origin) [13].

Tooth decay, alternatively referred to as dental caries or cavities, represents a prevalent concern within oral health. This condition is characterized by the gradual deterioration and demineralization of the tough structures of teeth. The primary instigator is the interaction between specific bacteria residing in the oral cavity and sugars and starches derived from dietary sources. There are a large number of species within the oral cavity that can be associated with carries. Among them, *Streptococcus mutans* (*S. mutans*) is considered the specific pathogen, followed by lactobacilli, members of the *Bifidobacterium*, *Propionibacterium*, and *Scardovia* genera [14, 15]. This interaction initiates the creation of acid. Conjoined with the bacteria and food particles, this acid culminates in the formation of a sticky layer known as dental plaque, adhering to the tooth surface [16]. As time progresses, the acid generated by these bacteria starts to erode the enamel resulting in its gradual weakening and eventual formation of cavities. Furthermore, it is imperative to acknowledge that enamel demineralization isn't solely confined to the acids generated by the oral microbiota. A paramount contributing factor to this phenomenon is external acidity, principally attributed to the overconsumption of acidic soft drinks. These beverages are laden with corrosive agents, exemplified by citric and phosphoric acid that wield the power to instantly and substantially plummet the pH levels within the oral environment. To better understand this aspect, a clinical review [17] presents the case of a 25 year old man with poor oral hygiene and a 7 year cola-drinking history. Advanced decay affected the incisors and canines, whereas the premolars and molars exhibited milder lesions. Another relevant example would be a cross-sectional study with 400 middle-income adults (age 18-25) in Chennai, India [18]. Overall, a weekly consumption of carbonated

drinks was linked to less erosion when compared to daily consumption. A higher consumption was associated with a higher Erosion Index. Carbonated soft drinks showed higher erosion than non-carbonated regardless of the gender of participants.

Dental caries represents a disease continuum that commences with the depletion of ions from apatite crystals during its initial phase, ultimately resulting in the formation of cavities within lesions [19]. Here, the primary objective should be to halt or reverse the progression of demineralized lesions at an early stage in order to avert the potential for cavity formation and the consequent necessity for invasive interventions. Demineralization is the removal of mineral ions from crystals of hydroxyapatite that make up hard tissues like enamel, which, if unregulated, may culminate in dental cavities [20]. This demineralization of enamel leads to nanoscale level changes in topography, disintegration and reduced mechanical properties, and, if not properly managed, leads to hypersensitivity in the dentin and pain [21, 22]. The imperative to develop more effective tooth enamel remineralization aids is paramount in addressing the escalating challenges posed by enamel erosion and demineralization. Traditional approaches to oral care often fall short in reversing the damage caused by acidic attacks and bacterial activity. The easiest way to prevent such problems is the use of daily oral care products that are enriched with different compounds with remineralizing and antibacterial properties.

Various agents that aid in remineralization have been researched over time, with fluoride emerging as the favored option due to its ability to hinder inherent demineralization by promoting the creation of fluorapatite, a less soluble compound when compared to regular hydroxyapatite (HAP) [23]. A few decades ago, scientists started to observe a correlation: individuals residing in regions where fluoride occurred naturally in water sources exhibited fewer cavities and more resilient teeth. This finding prompted a keen interest in comprehending the potential advantages of fluoride for promoting dental well-being. Later (1930-1940) researchers carried out investigations to examine the impact of fluoride on tooth enamel [24]. Their findings revealed that fluoride played a role in fortifying enamel by facilitating the creation of fluorapatite. This compound, compared to the hydroxyapatite constituting enamel, exhibited increased resilience and decreased solubility. During the 1940-1950 period, extensive research endeavors were undertaken to investigate the concept of water fluoridation on a grand scale. These studies provided conclusive evidence that the deliberate addition of carefully regulated quantities of fluoride to communal water sources led to a noteworthy decrease in the occurrence of

cavities, all while avoiding any detrimental health impacts [25]. This pivotal discovery heralded the commencement of community water fluoridation initiatives—an enduring public health strategy that persists in numerous countries up to the present day. In the 1950s, scientists initiated efforts to integrate fluoride into formulations for toothpaste, this endeavor culminating in the introduction of fluoride-infused toothpaste. As such, Crest toothpaste, the first cavity-prevention dentifrice recognized by the American Dental Association, debuted in 1956 [26]. This innovation significantly facilitated the access of individuals to the enamel-strengthening properties of fluoride on a daily basis. Dental researchers have honed their comprehension of fluoride's mechanisms in thwarting cavities and facilitating enamel remineralization. Furthermore, they have devised a range of fluoride-infused products like mouth rinses and dental gels, tailored to accommodate diverse preferences and requirements.

According to scientific literature [27, 28], fluoride primarily operates via two key pathways: stimulating remineralization and impeding demineralization. Upon fluoride exposure, teeth assimilate it into the enamel structure, fostering the creation of fluorapatite crystals. These crystals exhibit heightened resistance to acid assaults from bacteria, thereby aiding in the prevention of cavity development. Additionally, fluoride can disrupt the metabolic processes of acid-producing oral bacteria, thereby further diminishing the likelihood of enamel erosion.

However, toothpaste formulations contain different other ingredients, among which abrasives have been shown to negatively impact the added fluorine compounds. When utilizing abrasives containing aluminum and calcium, the depletion of supplemented fluorides from sodium fluoride (NaF) tends to range from 60% to 90% following one week of storage under ambient conditions. Substances such as sodium bicarbonate and sodium metaphosphate are comparatively milder in their impact, yet they still result in the deactivation of approximately 20% to 25% of the introduced fluoride content within a span of nine months of storage [29]. Also, given the narrow margin between toxic and therapeutic concentrations of fluoride, the scientific community searched for alternatives that even in higher doses would not be harmful.

The use of synthetic hydroxyapatite for enamel remineralization

Innovative remineralization aids hold the potential to replenish essential minerals like calcium and phosphate, fortifying the enamel's structural integrity and resilience. These aids could encompass advanced formulations incorporating hydroxyapatite or other enamel-strengthening compounds, tailored to facilitate efficient remineralization.

Hydroxyapatite has garnered significant research interest for its diverse potential in biomedical applications due to its unique properties and biocompatibility [30-49]. Owing to its similarity to the hydroxyapatite component in enamel, its synthetic counterpart has also been the subject of many studies regarding the demineralization-remineralization process. This is of no surprise considering that roughly at a concentration of 10%, nanoHAP has been noted to contribute to the remineralization of the enamel surface [50]. Additionally, a crucial aspect of maintaining optimal oral health involves the tooth-desensitizing attributes of hydroxyapatite [51-53]. Furthermore, smaller nanoparticles exhibit an enhanced capacity to permeate beneath the enamel surface, enhancing their efficacy in this regard [54]. The levels of calcium and phosphate present in saliva and plaque, serving as primary constituents of hydroxyapatite (HA) crystals, exert a significant influence on the process of tooth demineralization and formation. A calcium-to-phosphate ratio of 1.6 is deemed optimal for facilitating enamel remineralization when considering similar levels of supersaturation. Notably, the calcium-to-phosphate ratio in plaque fluid measures around 0.3 [55]. Consequently, an increased availability of calcium may prove beneficial in promoting enamel remineralization. Furthermore, existing literature indicates a proportional relationship between HAP concentration and both the whitening effect and adherence to enamel [56, 57]. Scientific literature outlines various pathways through which hydroxyapatite functions within the oral environment [58]:

- **Physical Enamel Revitalization:** This involves the attachment of nanoparticles to the enamel’s surface, leading to its physical restoration.
- **Chemical Impact:** Hydroxyapatite releases calcium and phosphate ions in the acidic oral environment. This establishes a connection between tooth enamel and HAP nanoparticles by forming an interface.
- **Biological Interactions:** Hydroxyapatite nanoparticles interact with microorganisms, demonstrating a biological influence.

In theory, HAP found in oral care products can affix itself to demineralized outer layers of tooth tissues and directly aid in the remineralization processes. By adhering to enamel, HAP nanoparticles create a protective layer that has demonstrated resilience against multiple acid challenges. Consistent application of HAP through oral care products consequently results in the reinforcement and renewal of this adhered layer, thereby augmenting its protective attribute [59].

In view of these benefits, several commercial toothpaste compositions containing hydroxyapatite have been developed. One of the first studies concerning a hydroxyapatite-based toothpaste reported its results on Japanese schoolchildren in the 1980s [60]. Evidently the results are in favor of HAP addition when compared to placebo, though it was more successful in girls, with a higher reduction in tooth decay occurrence as opposed to boys (35.86% among boys and 55.93% among girls). In Europe the first dentifrice containing nano-hydroxyapatite was introduced in 2006 [61]. Some of these commercial dentifrices have been subjected to testing and comparison by various scientific papers. Some studies involving toothpaste containing different types of hydroxyapatite, as found by the authors, are presented in Table 1.

The use of hydroxyapatite that is enriched with different ions is understandable as these ions are known to generally improve upon the properties of HAP. Most of the commercial toothpaste available employ HAP with Zn, an ion with antibacterial properties that functions by inhibiting glycolytic enzymes thereby slowing down bacterial metabolism and diminishing their capacity to thrive [68]. Literature also reports that zinc contributes to addressing dental calculus formation, although it necessitates a substantial concentration for effectiveness [69]. Some of these formulations also rely on strontium and magnesium. Sr is noted for its capacity to reduce enamel demineralization and mitigate the loss of surface hardness, particu-

Table 1. Studies involving toothpastes containing hydroxyapatite and substituted hydroxyapatite and their effect on tooth enamel

Type of HAP	Toothpaste name and composition	Effect	Ref.
Zn-carbonate substituted n-HAP	Biorepair Total Protection Plus / Coswell Funo (BO) Italy Purified water, zinc carbonate hydroxyapatite, glycerin, sorbitol, hydrated silica, silica, aroma, cellulose gum, tetrapotassium pyrophosphate, sodium myristoyl sarcosinate, sodium methyl cocoyl taurate, sodium saccharin, citric acid, phenoxyethanol, benzyl alcohol, sodium benzoate.	No differences in early colonization (EC, 12 h) and biofilm formation (BF, 24 h) when compared to control	[51]
Fluoride HAP Mg, Sr-carbonate substituted n-HAP	Biosmalto Caries Abrasion and Erosion / Curasept A.p.A. Saronno (VA) Italy Purified water, glycerin, hydrated silica, fluoride hydroxyapatite, Mg-Sr-carbonate hydroxyapatite conjugated with chitosan, cellulose gum, xylitol, cocamidopropyl betaine, xantham gum, aroma, acesulfame K, ethylhexylglycerin, phenoxyethanol, sodium benzoate, citric acid.	Lower in early colonization (EC, 12 h) and biofilm formation (BF, 24 h) compared to the control. The association of fluoride and strontium may be more effective in reducing EC and BF than zinc alone at the tested concentrations	[51]

NaF/1% nHAP	Apacare / Cumdente GmbH Aqua, Hydrated Silica, Sorbitol, Propylene Glycol, Glycerin, Sodium C14-16 Olefin Sulfonate, Hydroxyapatite, Aroma, Cellulose Gum, CI 77891, Sodium Fluoride, Allantoin, Sodium Saccharin, Tetrapotassium yrophosphate, Limonene	Apacare exhibited a higher effect than Biorepair. This difference in effectiveness could be attributed to the distinct levels of abrasiveness between the two products and the presence of fluoride in Apacare toothpaste.	[53]
ZnCO ₃ -HAP	BioRepair / Dr. Kurt Wolff Forschung Aqua, Zinc Carbonate Hydroxylapatite, Hydrated Silica, Glycerin, Sorbitol, Silica, Aroma, Cellulose Gum, Sodium Myristoyl Sarcosinate, Sodium Methyl Cocoyl Taurate, Tetrapotassium Pyrophosphate, Zinc PCA, Cetraria Islandica Extract, Sodium Saccharin, Citric Acid, Phenoxyethanol, Benzyl Alcohol, Methylparaben, Propylparaben	Biorepair demonstrated the least effectiveness in the current experiment, as well as in a separate in vitro study evaluating the impact of toothpastes with specialized formulations on enamel erosion.	[53]
HAP	Mirasensitive / Hager Werken Aqua, Hydroxyapatite, Xylitol, Sorbitol, Propylene Glycol, Potassium Citrate, Tetrapotassium Pyrophosphate, Sodium C 14–16 Olefin Sulfonate, Disodium Pyrophosphate, Cellulose Gum, Aroma, Sodium Fluoride, Cocamidopropyl Betaine, Sodium Saccharin, Limonene, CI77891	Mirasensitive fell in between Apacare and Biorepair when it comes to dentine loss. Evidence suggests that HAP nanoparticles could penetrate dentine.	[53]
HAP	Karex / Dr. Kurt Wolff GmbH & Co. KG, Bielefeld, Germany 10% hydroxyapatite, aqua, hydrated silica, glycerin, xylitol, hydrogenated starch hydrolysate, silica, cellulose gum, sodium methyl cocoyl taurate, sodium sulfate, menthol, eucalyptol, 1,2-hexanediol, caprylyl glycol, sodium cocoyl glycinate, tetrapotassium pyrophosphate, phosphoric acid, zinc chloride, cetylpyridinium chloride	The HAP-containing toothpaste Karex had a highly significant lower remineralization potential compared to the fluoride-containing toothpaste Elmex This may be attributed to pH values	[62]
HAP	Garda Silk toothpaste Water, Hydrated Silica, Glycerin, Hydrogenated Starch Hydrolysate, Hydroxyapatite, Sodium Methyl Cocoyl Taurate, Xanthan Gum, Aroma, Echinacea Purpurea Extract, Polyol Germanium Complex (PGC) with the Amino Acid Threonine, Menthol, Sodium Saccharin, O-Cymen-5-Ol, Hydroxyacetophenone.	Tooth sensitivity was significantly reduced after 14 days of treatment	[63]
Zn-HAP	Microrepair® Biorepair / Coswell S.P.A., 40050 Funo, Italy Zn-HAP toothpaste without fluoride	The extent of damage observed on enamel surfaces subsequent to treatment with Zn-HAP dentifrice underscored the preservation of rod integrity. This preservation resembled a less progressed state of demineralization when contrasted with samples treated with toothpastes containing fluoride and those without fluoride	[64]
Zn-HAP	Microrepair® Biorepair / Coswell S.P.A., 40050 Funo, Italy Zn-HAP toothpaste without fluoride	Enamel hardness after four cycles of erosive challenge and toothpaste treatment was higher for Microrepair® Biorepair when compared to a non-fluoride one and one containing fluoride.	[65]
ZnHAP	Microrepair® Biorepair Plus / Coswell S.P.A., 40050 Funo, Italy Zn-HAP toothpaste without fluoride and zinc pyrrolidone carboxylic acetate (Zn-PCA) without fluoride	Enamel hardness after four cycles of erosive challenge and toothpaste treatment was higher for Microrepair® Biorepair Plus when compared to a non-fluoride one and one containing fluoride.	[65]
Nanohydroxyapatite (nHAP)	Curapox Aqua, Sorbitol, Hydrated Silica, Glycerin, Hydroxyapatite, Cellulose Gum, Sodium Myristoyl Sarcosinate, Sodium Methyl Cocoyl Taurate, Aroma, Xanthan Gum, Stevia Rebaudiana Extract, Anethole, Tetrasodium Glutamate Diacetate, Tocopheryl Acetate, Eucalyptol, o-Cymen-5-ol, Citric Acid, Vitis Vinifera (Grape) Seed Extract, Tannase, Thymol, Limonene	A notable reduction in tooth sensitivity was observed after a 2-week period among both the nHAP followed by a reduction after 4 weeks.	[66]

<p>Nnano-Zn-Mg-hydroxyapatite (nZnMgHAP)</p>	<p>Curaprox Aqua, Sorbitol, Hydrated Silica, Glycerin, Zn-Mg-hydroxyapatite, Cellulose Gum, Sodium Myristoyl Sarcosinate, Sodium Methyl Cocoyl Taurate, Aroma, Xanthan Gum, Stevia Rebaudiana Extract, Anethole, Tetrasodium Glutamate Diacetate, Tocopheryl Acetate, Eucalyptol, o-Cymen-5-ol, Citric Acid, Vitis Vinifera (Grape) Seed Extract, Tannase, Thymol, Limonene</p>	<p>A notable reduction in tooth sensitivity was observed after a 2-week period among both the nZnMgHAP followed by a reduction after 4 weeks.</p>	<p>[66]</p>
<p>Nnano-fluoroapatite (nFAP)</p>	<p>Curaprox Aqua, Sorbitol, Hydrated Silica, Glycerin, Fluorapatite, Cellulose Gum, Sodium Myristoyl Sarcosinate, Sodium Methyl Cocoyl Taurate, Aroma, Xanthan Gum, Stevia Rebaudiana Extract, Anethole, Tetrasodium Glutamate Diacetate, Tocopheryl Acetate, Eucalyptol, o-Cymen-5-ol, Citric Acid, Vitis Vinifera (Grape) Seed Extract, Tannase, Thymol, Limonene</p>	<p>The toothpaste containing nZnMgHAP exhibited notably superior effectiveness compared to the toothpaste with nFAP after the 2-week assessment and demonstrated greater efficacy compared to other tested toothpaste variants after the 4-week evaluation.</p>	<p>[66]</p>
<p>Fluoro-hydroxyapatite magnesium-strontium-carbonate hydroxyapatite conjugated with chitosan</p>	<p>Biosmalto Caries Abrasion and Erosion / Curasept S.p.A., Saronno (VA), Italy Purified water, glycerin, hydrated silica, fluorohydroxyapatite, magnesium-strontium-carbonate hydroxyapatite conjugated with chitosan, cellulose gum, xylitol, cocamidopropyl betaine, xanthan gum, aroma, sodium monofluorophosphate, potassium acesulfame, ethylhexylglycerin, phenoxyethanol, sodium benzoate, citric acid.</p>	<p>The toothpaste resulted in the formation of a mineral deposit on the specimen's surface, which led to the closure of dentinal tubules and the filling of spaces between enamel prisms. This process effectively replenished the mineral content that had been diminished due to acid etching.</p>	<p>[67]</p>

larly in more acidic environments [70, 71]. The incorporation of magnesium is also pertinent as it can actively enhance dental well-being by mitigating oral inflammation and leveraging its antimicrobial properties [72]. It also holds a role in facilitating proper calcium integration within tooth structure with insufficient Mg levels resulting in weakened enamel, irrespective of calcium levels [73]. A more recent study [74] evaluated 2 experimental toothpaste formulations, one containing simple HAP and one a substituted HAP with Mg, Zn and Si. While both toothpastes exhibited an improvement when compare to the artificially demineralized enamel, the substituted HAP toothpaste demonstrated a superior performance, resulting in consistent improvements in the morphology of the dental enamel surface.

Over the course of ten treatment days, investigations through Atomic force Microscopy (AFM) revealed complete remineralization of demineralized enamel lesions, as evidenced by changes in structural morphology and surface roughness. While synthetic HAP seems like an ideal choice for remineralization purposes there are certain aspects one needs to consider. Firstly, the dimensions of the HAP particles have to be smaller than 100 nm, as it is reported that nanohydroxyapatite is much more effective towards promoting enamel remineralization [75]. For instance, Li et al. [76] revealed that 20 nm nano-hydroxyapatite particles form a better bond with enamel than

larger particles of more conventional hydroxyapatites or calcium phosphates. Here, the enamel surface, which is coated with a layer of hydroxyapatite (HAP) measuring approximately 40–50 nm in thickness, exhibits a hardness of 4.6 ± 0.4 GPa and an elastic modulus of 95.6 ± 8.4 GPa. These values are similar to the ones exhibited by natural enamel (hardness: 4.2 ± 0.2 ; elastic modulus: 94.1 ± 5.4 GPa) [77]. The nanoscale dimensions of HAP enable it to easily infiltrate enamel pores or micro cracks, potentially leading to remineralization. Moreover, nanoHAP can be utilized as a filler for minor cavities and contribute to the enhancement of tooth whiteness [78]. This underscores its role in supporting saliva's restorative functions, ensuring suitable mineral density on enamel surfaces, and addressing plaque-related issues.

The composition and physico-chemical parameters of dentifrice formulations also play a role in the effectiveness of the remineralizing agent. To put things into perspective one can discuss the addition of both HAP and fluoride within toothpaste. For instance, a study involving school children conducted a comparison between regular fluorinated toothpaste and toothpaste containing hydroxyapatite with fluoride substitution [79]. The results indicated that the hydroxyapatite with fluoride substitution demonstrated superior effectiveness in preserving and rejuvenating dental health compared to the conventional addition of sodium fluoride. Interestingly, another study [62] revealed that a commercial

toothpaste with fluoride (olaflur - 1.400 ppm; Elmex CP GABA GmbH, Hamburg, German) had significantly higher remineralization when compared to a commercial toothpaste containing hydroxyapatite (10% hydroxyapatite; Karex Dr. Kurt Wolff GmbH & Co. KG, Bielefeld, Germany). This discrepancy is explained by the authors to the fact that acidic pH (Elmex) leads to better remineralization results from neutral pH (Karex).

The remineralization and desensitizing improvements in enamel by using hydroxyapatite dentifrices are indeed promising. This can be noticed through the increasing number of commercial toothpastes present on the market, with newer compositions containing different types of substituted HAPs.

Remineralization and dentifrices efficacy

Reduction in dental caries coupled with antimicrobial and antioxidant effect

Clearly hydroxyapatite is the “new fluorine” in toothpastes. The results are exceptional in terms of remineralization and HAP can be used in higher amounts without raising any of the concerns fluorine does, especially regarding children. However, as previously mentioned, one of the aspects regarding enamel deterioration involves bacteria. This includes microorganisms like *Streptococcus mutans* and *Streptococcus sobrinus*, the primary culprits behind dental caries; *Pseudomonas aeruginosa* and *Enterococcus faecalis*, associated with periodontal diseases; *Candida albicans*, implicated in conditions like candidiasis and various superficial or systemic infections, including dental caries [80].

While a strong remineralizing agent, HAP does not possess any intrinsic antibacterial properties. It could be said that by lattice substitution with different ions (i.e. Si, Mg) HAP could gain such properties. While this is true, the brushing time (contact of HAP with enamel) has to be taken into account. While the general consensus is that brushing time has to be 2-3 minutes, the general population only brushes for 45 seconds [81], thus limiting the contact time of active ingredients with enamel.

Therefore, there is a need to further enhance dentifrices with other antibacterial compounds that would act faster than the ions in HAP. Silver is a well-known antibacterial agent [82-91]. There are quite a few dental hygiene products containing silver in various forms and concentrations. Silver in toothpaste has been proven to be quite effective against *S. mutans* the major bacterial strain in the oral microbiota. For example, one study [92] compared an experimental toothpaste containing nano-silver fluoride with one

containing sodium fluoride. While both toothpastes proved efficient in remineralization (with the addition of fluoride), the one containing nano-silver led to a lower MIC and provided better results regarding bacterial adhesion and pH decreases. While there are some concerns within the general public regarding potential silver toxicity the concentrations are not that high and toothpaste is generally not ingested. However, to address this, other compounds of more natural origin could also be employed as antibacterial ingredients. Different plant extracts such as carotenoids [93-111] would probably be more palatable to the general public. For example, curcumin was shown to present an inhibitory effect on the biofilm viability of clinical strains of *S. mutans* [112]. Notably, the study suggested that the prolonged exposure to curcumin yields a more potent inhibitory effect compared to short-term exposure. This biofilm reduction could be explained through an inhibition in the activity of sortase A, the enzyme responsible for the covalent attachment of Pac proteins to the cell wall in *S. mutans* [113]. Conversely, a nanoemulsion of astaxanthin has also been shown to exhibit an in vitro effect on *S. mutans* with a minimum inhibitory concentration (MIC) of 0.5-2 µg/mL and a minimum bactericidal concentration (MBC) of 2-8 µg/mL [114]. The difference in concentration arises from variations in the preparation of nanoemulsions.

It can be firmly asserted that the integration of natural plant extracts and highly potent bioactive compounds, recognized for their exceptional antibacterial properties, into toothpaste formulations signifies a paramount stride towards fostering a comprehensive and ecologically responsible method for enhancing oral health.

Conclusions

Hydroxyapatite-based toothpaste formulations have surged in significance due to their remarkable potential in bolstering enamel remineralization and overall dental health. Research findings unequivocally support their efficacy in combating tooth decay, particularly when fortified with essential ions like zinc, strontium, and magnesium. Notably, the utilization of nano-sized hydroxyapatite particles exhibits superior capability in infiltrating enamel and enhancing mineral density. The composition of toothpaste emerges as a pivotal factor, with formulations encompassing both hydroxyapatite and fluoride substitution yielding notably superior outcomes. Nevertheless, it's imperative to acknowledge that these toothpastes inherently lack antibacterial properties, necessitating the integration of antibacterial agents such as silver or natural extracts to effectively counter bacterial concerns. Hence, the quest for alternative antibacterial compounds becomes

imperative to effectively combat bacterial challenges. Silver, a long-established and potent antibacterial agent, has proven its mettle within toothpaste formulations by effectively tackling notorious oral troublemakers like *S. mutans*. Furthermore, the exploration of natural antibacterial elements, exemplified by plant extracts such as curcumin and astaxanthin, presents an enticing and potentially efficacious path towards elevating oral health. In essence, the amalgamation of these antibacterial warriors into toothpaste formulations signifies a substantial leap towards embracing a more comprehensive and eco-conscious approach to oral hygiene.

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Conflict of Interest

No competing interest was found during this work.

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