ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(2): 3908-3920
doi: 10.25083/rbl/28.2/3908.3920

Received for publication, October 01, 2023
Accepted, October, 09, 2023

Review

Relationship between dentifrices based on hydroxyapatites and human enamel remineralization

ALEXANDRA AVRAM[1], AURORA MOCANU[1], SORIN RIGA[1,2], MARIA TOMOAIA-COTISEL[1,2,*]

[1] Babes-Bolyai University of Cluj-Napoca, Faculty of Chemistry and Chemical Engineering, Research Centre of Physical Chemistry, 11 Arany Janos Str., RO-400028, Cluj-Napoca, Romania
[2] Academy of Romanian Scientists, 3 Ilfov Street, RO-050044, Bucharest, Romania

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, toothpaste

*Corresponding author: Maria Tomoaia-Cotisel. e-mail: mcotisel@gmail.com

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 classifies: 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]. Owning 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]

Nnano-Zn-Mg-hydroxyapatite (nZnMgHAP)	**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	A notable reduction in tooth sensitivity was observed after a 2-week period among both the nZnMgHAP followed by a reduction after 4 weeks.	[66]
Nnano-fluoroapatite (nFAP)	**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	The toothpaste containing nZnMgHAP exhibited notably superior effective- ness 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.	[66]
Fluoro-hydroxyapatite magnesium-strontium-carbonate hydroxyapatite conjugated with chitosan	**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, xantham gum, aroma, sodium monofluorophosphate, potassium acesulfame, ethylhexylglicerin, phenoxyethanol, sodium benzoate, citric acid.	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.	[67]

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.

Acknowledgment

The authors acknowledge their sincere thanks to the Ministry of Research, Innovation and Digitization, CNCS/CCCDI-UEFISCDI, for funding through project number 186, within PNCDI III.

Conflict of Interest

No competing interest was found during this work.

Funding/Support

This work was supported by grants from the Ministry of Research, Innovation and Digitization, CNCS/CCCDI-UEFISCDI, project number 186, within PNCDI III.

References

1. Prajapati S, Tao J, Ruan Q, De Yoreo JJ, Moradian-Oldak J. Matrix metalloproteinase-20 mediates dental enamel biomineralization by preventing protein occlusion inside apatite crystals. Biomaterials. 2016; 75: 260-270. doi: 10.1016/j.biomaterials.2015.10.031
2. Habelitz S, Bai Y. Mechanisms of enamel mineralization guided by Amelogenin Nanoribbons. J Dent Res. 2021; 100: 1434–14443. doi: 10.1177/00220345211012925
3. Neel EAA, Aljabo A, Strange A, Ibrahim S, Coathup M, Young AM, Bozec L, Mudera V. Demineralization–remineralization dynamics in teeth and bone. Int J Nanomed. 2016; 11: 4743-4763. doi: 10.2147/IJN.S107624
4. Beniash E, Simmer JP, Margolis HC. The effect of recombinant mouse amelogenins on the formation and organization of hydroxyapatite crystals in vitro. J Struct Biol. 2005; 149: 182-190. doi: 10.1016/j.jsb.2004.11.001
5. Zhang X, Ramirez BE, Liao S, Diekwisch TGH. Amelogenin supramolecular assembly in nanospheres defined by a complex helix-coil-PPII helix 3D-structure. Plos One. 2011; 6(10): e24952. doi: 10.1371/journal.pone.0024952
6. Tao J, Hanson E, Dohnalkova AC, Buchko GW, Jin B, Shaw WJ, Tarasevich BJ. Changes in the C-terminal, N-terminal, and histidine regions of amelogenin reveal the role of oligomer quaternary structure on adsorption and hydroxyapatite mineralization. Front Physiol. 2022; 13: 1034662. doi: 10.3389/fphys.2022.1034662
7. Bartlett JD. Dental enamel development: proteinases and their enamel matrix substrates. ISRN Dent. 2013; 2013: 684607. doi: 10.1155/2013/684607
8. 8. Fukumoto S, Yamada A, Nokana K, Yamada Y. Essential roles of ameloblastin in maintaining ameloblast differentiation and enamel formation. Cells Tissues Organs. 2005; 181(3-4): 189–195. doi: 10.1159/000091380
9. Kegulian NC, Langen R, Moradian-Oldak J. The dynamic interactions of a multitargeting domain in ameloblastin protein with amelogenin and membrane. Int J Molec Sci. 2023; 24: 3484. doi: 10.3390/ijms24043484
10. Masuya H, Shimizu K, Sezuku H, Sakuraba Y, Nagano J, Shimizu A, Fujimoto N, Kawai A, Miura I, Kaneda H, Kobayashi K, Ishijima J, Maeda T, Gondo Y, Noda T, Wakana S, Shiroishi T. Enamelin (Enam) is essential for amelogenesis: ENU-induced mouse mutants as models for different clinical subtypes of human amelogenesis imperfecta (AI). Hum Mol Genet. 2005; 14(5): 575-583. doi: 10.1093/hmg/ddi054
11. Hu JCC, Hu Y, Lu Y, Smith CE, Lertlam R, Wright JT, Suggs C, MacKee MD, Beniah E, Kabir ME, Simmer JP. Enamelin is critical for ameloblast integrity and enamel ultrastructure formation. Plos One. 2014; 9(3): e89303. doi: 10.1371/journal.pone.0089303
12. Sakaguchi RL, Powers JM, Eds. Chapter 2 - The oral environment, Craig's Restorative Dental Materials, 13th Edition, 2012; pag 5-23. doi: 10.1016/B978-0-323-08108-5.10002-7
13. Infeld T. Dental erosion. Definition, classification and links. Eur J Oral Sci. 1996; 104(2(2)): 151-155. doi: 10.1111/j.1600-0722.1996.tb00063.x
14. Wade WG. The oral microbiome in health and disease. Pharm Res. 2013; 69: 137-143. doi: 10.1016/j.phrs.2012.11.006
15. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner ACR, YU W-H, Lakshmanan A, Wade WG. The human oral microbiome. J Bacteriol. 2010; 192(19): 5002-5017. doi:10.1128/JB.00542-10
16. Boisen G, Davies JR, Neilands J. Acid tolerance in early colonizers of oral biofilm. BMC Microbiol. 2021; 21: 45. doi: 10.1186/s12866-021-020892

17. Cheng R, Yang H, Shao M-Y, Hu T, Zhou X-D. Dental erosion and severe tooth decay related to soft drinks: a case report and literature review. J Zhejiang Univ Sci B. 2009; 10(5): 395-399.
18. Kannan A, Ahmed MAA, Duraisamy P, Manipal S, Adusumillil P. Dental hard tissue erosion rates and soft drinks – A gender based analysis in Chennai city, India. Saudi J Oral Dent. 2014; 5: 21-27. doi: 10.1016/j.ksujds.2013.08.003
19. Hamba H, Nakamura K, Nikaido T, Tagami J, Muramatsu T. Remineralization of enamel subsurface lesions using toothpaste containing tricalcium phosphate and fluoride: an in vitro μCT analysis. BMC Oral Health. 2020; 20(1): 292. doi: 10.1186/s12903-020-01286-1
20. Anil A, Ibraheem WI, Meshni AA, Preethanath R, Anil S. Demineralization and Remineralization Dynamics and Dental Caries, In Dental Caries - The Selection of Restoration Methods and Restorative Materials, Rusu LC, Ardelean LC Eds., IntechOpen 2022. doi: 10.5772/intechopen.105847
21. Hayashi O, Chiba T, Shimoda S, Momoi Y. Demineralization and remineralization phenomena of human enamel in acid erosion model. J Hard Tissue Biol. 2016; 25(1): 27-34. doi: 10.2485/jhtb.25.27
22. Inchingolo AM, Malcangi G, Ferrante L. Del Vecchio G, Viapiano F, Mancini A, Inchingolo F, Inchingolo AD, Di Venere D, Dipalma G, Patano A. Damage from carbonated soft drinks on enamel: a systematic review. Nutrients. 2023; 15:1785. doi: 10.3390/nu15071785
23. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: a review of literature. Int J Clin Pediatr Dent. 2019; 12(2): 139–144. doi: 10.5005%2Fjp-journals-10005-1603
24. Carstairs C. Debating Water fluoridation before Dr. Strangelove. Am J Public Health. 2015; 105(8):1559–1569. doi:10.2105/ajph.2015.302660
25. Unde MP, Patil RU, Dastoor PP. The untold story of fluoridation: revisiting the changing perspectives. Indian J Occup Environ Med. 2018; 22: 121-127. doi: 10.4103/ijoem.ijoem_124_18
26. Miskell P. Cavity protection or cosmetic perfection? Innovation and marketing of toothpaste brands in the United States and Western Europe, 1955–1985. Bus Hist Rev. 2004; 78(1): 29–60. doi: 10.2307/25096828
27. Nassar Y, Brizuela M. The Role of Fluoride on Caries Prevention. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK587342/
28. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol. 1999; 27(1): 31-40. doi: 10.1111/j.1600-0528.1999.tb01989.x
29. Gleisner, H, Einax JW, Moes S, Welz, B, Carasek, E. A fast and accurate method for the determination of total and soluble fluorine in toothpaste using high-resolution graphite furnace molecular absorption spectrometry and its comparison with established techniques. J Phrm Biomed Anal. 2011; 54(5): 1040-1046. doi: 10.1016/j.jpba.2010.12.013
30. Tomoaia G, Tomoaia-Cotisel M, Pop LB, Pop A, Horovitz O, Mocanu A, Jumate N, Bobos LD. Synthesis and characterization of some composites based on nanostructured phosphates, collagen and chitosan. Rev. Roum. Chim. 2011; 56(10-11): 1039-1046.
31. Prejmerean C, Tomoaia-Cotisel M, Vasile E, Furtos G, Pop LB, Moldovan M, Sarosi C, Petean I. Characterisation of surface organisation and morphology of some new experimental dental resin-based composites. Int J Nano Biomater. 2011; 3(4):344-359. doi: 10.1504/IJNBM.2011.045886
32. Tomoaia G, Soritau O, Tomoaia-Cotisel M, Pop LB, Pop A, Mocanu A, Horovitz O, Bobos LD. Scaffolds made of nanostructured phosphates, collagen and chitosan for cell culture. Powder. Technol. 2013; 238: 99-107. doi: 10.1016/j.powtec.2012.05.023
33. Furtos G, Tomoaia-Cotisel M, Garbo C, Senila M, Jumate N, Vida-Simiti I, Prejmerean C. New composite bone cement based on hydroxyapatite and nanosilver. Particul Sci Technol. 2013; 31(4): 392-398. doi: 10.1080/02726351.2013.767293
34. Forizs E, Goga F, Avram A, Mocanu A, Petean I, Horovitz O, Tomoaia-Cotisel M. Thermal analysis of pure and multisubstituted hydroxyapatite pastes. Stud UBB Chem. 2017; 62(4(1)): 173-180. doi: 10.24193/subbchem.2017.4.14
35. Tomoaia G, Mocanu A, Vida-Simiti I, Jumate N, Bobos LD, Soritau O, Tomoaia-Cotisel M. Silicon effect on the composition and structure of the composition and structure of nanocalcium phosphates in vitro biocompatibility to human osteoblasts. Mater Sci Eng C. 2014; 37: 37-47. doi: 10.1016/j.msec.2013.12.027
36. Mocanu A, Furtos G, Rapuntean S, Horovitz O, Flore C, Garbo C, Danisteanu A, Rapuntean G, Prejmerean C, Tomoaia-Cotisel M. Synthesis characterization and antimicrobial effects of composites based on multisubstituted hydroxyapatite and silver nanoparticles. Appl Surf Sci. 2014; 298: 225-235. doi: 0.1016/j.apsusc.2014.01.166
37. Frangopol PT, Mocanu A, Almasan V, Garb C, Balint R, Borodi G, Bratu I, Horovitz O, Tomoaia-Cotisel M.

Synthesis and structural characterization of strontium substituted hydroxyapatites. Rev Roum Chim. 2016; 61(4-5): 337-344.

38. Mocanu A, Balint R, Garbo C, Timis L, Petean I, Horovitz O, Tomoaia-Cotisel M. Low crystallinity nanohydroxyapatite prepared at room temperature. Stud UBB Chem. 2017; 62(2), 95-103. doi: 10.24193/subbchem.2017.2.07
39. Goga F, Forizs E, Rotaru A, Lucian A, Petean I, Mocanu A, Tomoaia-Cotisel N. Synthesis and thermal treatment of hydroxyapatite doped with magnesium, zinc and silicon. Rev Chim. 2017; 68(6): 1193-1200. doi: 10.37358/RC.17.6.5640
40. Garbo C, Sindilaru M, Carlea A, Tomoaia G, Almasan V, Petean I, Mocanu A, Horovitz O, Tomoaia-Cotisel M. Synthesis and structural characterization of novel porous zinc substituted nanohydroxyapatite powders. Particul Sci Technol. 2017; 35(1): 29-37. doi: 10.1080/02726351.2015.1121180
41. Avram A, Gorea M, Balint R, Timis L, Jitaru S, Mocanu A, Tomoaia-Cotisel M. Portland cement enriched with hydroxyapatite for endodontic applications. Stud UBB Chem. 2017; 62(4): 81-92. doi: 10.24193/subbchem.2017.4.07
42. Goga F, Forizs E, Borodi G, Tomoaia G, Avram A, Balint R, Mocanu A, Horovitz O, Tomoaia-Cotisel M. Behavior of doped hydroxyapatites during the heat treatment. Rev. Chim. 2017; 68(12), 2907-2913. doi: 10.37358/RC.17.12.6004
43. Rapuntean S, Frangopol PT, Hodisan I, Tomoaia G, Oltean-Dan D, Mocanu A, Prejmerean C, Soritau O, Racz LZ, Tomoaia-Cotisel M. In vitro response of human osteoblasts cultured on strontium substituted hydroxyapatites. Rev. Chim. (Bucharest). 2018; 69(12): 3537-3544. doi: 10.37358/RC.18.12.6787
44. Oltean-Dan D, Dogaru GB, Tomoaia-Cotisel M, Apostu D, Mester A, Benea HRC, Paiusan MG, Jianu EM, Mocanu A, Balint R, Popa CO, Berce C, Bodizs GI, Toader AM, Tomoaia G. Enhancement of bone consolidation using high-frequency pulsed electromagnetic short-waves and titanium implants coated with biomimetic composite embedded into PLA matrix: in vivo evaluation. Int. J. Nanomed. 2019; 14: 5799-5816. doi: 10.2147/IJN.S205880
45. Garbo C, Locs J, D'Este M, Demazeau G, Mocanu A, Roman C, Horovitz O, Tomoaia-Cotisel M. Si multisubstituted hydroxyapatites for bone regeneration. Int J Nanomed. 2020; 15: 1037-1058. doi: 10.2147/IJN.S226630
46. Balint R., Petean I, Frangopol PT, Mocanu A, Arghir G, Riga S, Tomoaia G, Horovitz O, Tomoaia-Cotisel M. Biomimetic nanocomposite structures designed for coating of orthopedic implants: AFM investigation. Stud UBB Chem. 2021; 66(3): 141-160. doi: 10.24193/subbchem.2021.3.08
47. Oltean-Dan D, Dogaru GB, Jianu EM, Riga S, Tomoaia-Cotisel M, Mocanu A, Barbu-Tudoran L, Tomoaia G. Biomimetic composite coatings for activation of titanium implant surfaces: methodological approach and in vivo enhanced osseointegration. Micromachines. 2021; 12(11): 1325. doi: 10.3390/mi12111352
48. Mocanu A, Frangopol PT, Balint R, Cadar O, Vancea IM, Mantiu R, Horovitz O, Tomoaia-Cotisel M. Higuchi model applied to ions release from hydroxyapatites. Stud UBB Chem. 2021; 66(3): 195-207. doi: 10.24193/subbchem.2021.3.12
49. Mocanu A, Cadar O, Frangopol PT, Petean I, Tomoaia G, Paltinean GA, Racz CP, Horovitz O, Tomoaia-Cotisel M. Ion release from hydroxyapatite and substituted hydroxyapatites in different immersion liquids: in vitro experiments and theoretical modelling study. R Soc Open Sci. 2021; 8(1): 201785. doi: 10.1098/rsos.201785
50. Juntavee N, Juntavee A, Plongniras P. Remineralization potential of nano-hydroxyapatite on enamel and cementum surrounding margin of computer-aided design and computer-aided manufacturing ceramic restoration. Int J Nanomed. 2018; 13: 2755-2765. doi: 10.2147/IJN.S165080
51. Ionescu AC, Cazzaniga G, Ottobelli M, Garcia-Godoy F, Brambilla E. Substituted Nano-hydroxyapatite toothpastes reduce biofilm formation on enamel and resin-based composite surfaces. J Funct Biomater. 2020; 11(2):36. doi: 10.3390/jfb11020036
52. de Melo Alencar C, Freitas de Paula BL, Guanipa Ortiz MI, Barauna Magno N, Martins Silva C, Cople Maia L. Clinical efficacy of nano-hydroxyapatite in dentin hypersensitivity: A systematic review and meta-analysis. J Dent. 2019; 82: 11-21. doi: 10.1016/j.jdent.2018.12.014
53. Aykut-Yetkiner A, Attin T, Wiegard A. Prevention of dentine erosion by brushing with anti-erosive toothpastes. J Dent. 2014; 42: 856-861. doi: 10.1016/j.jdent.2014.03.011
54. Chen L, AL-Bayatree S, Khurshid Z, Shavandi A, Brunton P,. Ratnayake J. Hydroxyapatite in oral care products—a review. Materials. 2021; 14:4865. doi: 10.3390/ma14174865
55. Li X, Wang J, Joiner A, Chang J. The remineralisation of enamel: a review of the literature. J Dent. 2014;

42(Suppl. 1): S12–S20. doi: 10.1016/s0300-5712-(14)50003-6

56. Fabritius-Vilpoux K, Enax J, Herbig M, Raabe D, Fabritius HO. Quantitative affinity parameters of synthetic hydroxyapatite and enamel surfaces in vitro. Bioinspired, Biomim Nanobiomaterials. 2019; 8(2): 141–153. doi: 10.1680/jbibn.18.00035
57. Niwa M, Sato T, Li W, Aoki H, Aoki H, Daisaku T. Polishing and whitening properties of toothpaste containing hydroxyapatite. J Mater Sci Mater Med. 2001; 12: 277-281. doi: 10.1023/a:1008927502523
58. Enax J, Epple M. Synthetic hydroxyapatite as a biomimetic oral care agent. Oral Health Prev Dent. 2018; 16(1): 7-19. doi: 0.3290/j.ohpd.a39690
59. Meyer F, Enax J, Amaechi BT, Limeback H, Fabritius H-O, Ganss B, Pawinska M, Paszynska E. Hydroxyapatite as remineralization agent for children's dental care. Front Dent Med. 2022; 3: 859560. doi: 10.3389/fdmed.2022.859560
60. Kani T, Kani M, Isozaki A, Shintani H, Ohashi T, Tokumoto T. Effect to apatite-containing dentifrices on dental caries in school children. J Dental Health. 1989; 39:104–109.
61. Pepla E, Besharat LK, Palaia G, Tenore G, Migliau G. Nano-hydroxyapatite and its applications in preventive, restorative and regenerative dentistry: A review of literature. Ann. Stomatol. 2014; 5: 108. doi: 10.11138/ads/2014.5.3.108
62. Guntermann L, Rohrbach A, Scafer E, Dammaschke T. Remineralization and protection from demineralization: effects of a hydroxyapatite-containing, a fluoride-containing and a fluoride and hydroxyapatite-free toothpaste on human enamel in vitro. Head & Face Med. 2022; 18: 26. doi: 10.1186/s13005-022-00330-5
63. Vlasova N, Samusenko V, Novikova I, Nikolenko D, Nikolashvili N, Gor I, Danilina A. Clinical efficacy of hydroxyapatite toothpaste containing Polyol Germanium Complex (PGC) with threonine in the treatment of dentine hypersensitivity. Saudi Denl J. 2022; 34: 310-314. doi: 10.1016/j.sdentj.2022.03.001
64. Colombo M, Mirando M, Rattalino D, Beltrami R, Chiesa M, Poggio C. Remineralizing effect of a zinc-hydroxyapatite toothpaste on enamel erosion caused by soft drinks: Ultrastructural analysis. J Clin Exp Dent. 2017; 9(7): e861-e868. doi: 10.4317/jced.53790
65. Poggio C, Mirando M, Rattalino D, Viola M, Colombo M, Beltrami R. Protective effect of zinc-hydroxyapatite toothpastes on enamel erosion: An in vitro study. J Clin Exp Dent. 2017; 9(1): e118-e122. doi: 10.4317/jced.53068
66. Polyakova M, Sokhova I, Doroshina V, Arakelyan M, Novozhilova N, Babina K. The effect of toothpastes containing hydroxyapatite, fluoroapatite, and Zn-Mg-hydroxyapatite nanocrystals on dentin hypersensitivity: a randomized clinical trial. J Int Soc Prev Community Dent. 2022; 12: 252-259. doi: 10.4103/jispcd.jispcd_333_21
67. Degli Esposti L, Ionescu AC, Brambilla E, Tampieri A, Iafisco M. Characterization of a toothpaste containing bioactive hydroxyapatites and in vitro evaluation of its efficacy to remineralize enamel and to occlude dentinal tubules. Materials. 2020; 13(13): 2928. doi: 10.3390/ma13132928
68. Enax J, Amaechi BT, Schulze zur Wiesche E, Meyer F. Overview on adjunct ingredients used in hydroxyapatite-based oral care products. Biomimetics. 2022; 7:250. doi: 10.3390/biomimetics7040250
69. Lynch RJM. Zinc in the mouth, its interactions with dental enamel and possible effects on caries; a review of the literature. Int Dent J. 2011; 61(Suppl. 3): 46–54. doi: 10.1111/j.1875-595X.2011.00049.x
70. Wang YL, Chang HH, Chiang YC, Lin CH, Lin CP. Strontium ion can significantly decrease enamel demineralization and prevent the enamel surface hardness loss in acidic environment. J Formos Med Assoc. 2019; 118(1(1)): 39-49. doi: 10.1016/j.jfma.2018.01.001
71. Dai LL, Mei ML, Chu CH, Zhao IS, Lo ECM. Effect of Strontium-doped bioactive glass on preventing formation of demineralized lesion. Materials. 2021; 14(16): 4645 doi: 10.3390/ma14164645
72. Jawed M, Al Abdulmonem W, Alkhamiss A, Alghsham R, Alsaeed T, Alhumaydhi FA, Hershan AA, Shahid SM. Role of serum magnesium in dental caries. Bahrain Medical Bull. 2021; 43(1): 327-330.
73. Uwitonze AM, Rahman S, Ojeh N, Grant WB, Kaur H, Haq A, Razzaque MS. Oral manifestations of magnesium and vitamin D inadequacy. J Steroid Biochem Molec Biol. 2020; 200: 105636. doi: 10.1016/j.jsbmb.2020.105636
74. Florea DA, Mocanu A, Pop LC, Tomoaia G, Dobrota C-T, Varhely Jr C, Tomoaia-Cotisel M. Remineralization of tooth enamel with hydroxyapatite nanoparticles: an in vitro study. Stud UBB Chem. 2023; 68(2): 99-113. doi:10.24193/subbchem.2023.2.07
75. Du M, Chen J, Liu K, Xing H, Song C. Recent advances in biomedical engineering of nano-hydroxyapatite including dentistry, cancer treatment and bone repair. Compos B. 2021; 215: 108790. doi: 10.1016/j.compositesb.2021.108790
76. Li L, H Pan, J Tao, X Xu, C Mao, X Gu, R Tang. Repair of enamel by using hydroxyapatite nanoparticles as the building blocks. J Mater Chem. 2008; 18: 4079–4084.

77. He LH, Swain MV. Enamel—A ''metallic-like'' deformable biocomposite. J Dent. 2007; 35: 431-437. doi: 10.1016/j.jdent.2006.12.002
78. Babayevska N, Woźniak-Budych M, Litowczenko J, Peplińska B, Jarek M, Florczak P, Bartkowiak G, Czarnecka B, Jurga S. Novel nanosystems to enhance biological activity of hydroxyapatite against dental caries. Mater Sci Eng C Mater Biol Appl. 2021; 124: 112062. doi: 10.1016/j.msec.2021.112062
79. Cagetti MG, Cocco F, Wierichs RJ, Wolf TG, Salerno C, Arghittu A, Campus G, Efficacy of HAF toothpastes in primary and permanent dentitions. A 2-years triple-blind RCT. J Dent. 2022; 121: 104049. doi: 10.1016/j.jdent.2022.104049
80. de Rossi A, Cunha Araujo Ferrira D, Assed Bezerra da Silva R, Mussolino de Queiroz A, Assed Bezerra da Silva L, Nelso-Filho P. Antimicrobial activity of toothpastes containing natural extracts, chlorhexidine or triclosan. Braz Dent J. 2014; 25(3): 186-190. doi: 10.1590/0103-6440201300027
81. Gallagher A, Sowinski J, Bowman J, Barrett K, Lowe S, Bosma ML, Creeth JE. The effect of brushing time and dentifrice on dental plaque removal in vivo. J Dent Hyg. 2009; 83(3): 111-116.
82. Danistean A, Gorea M, Avram A, Rapuntean S, Tomoaia G, Mocanu A, Garbo C, Horovitz O, Tomoaia-Cotisel M. Antimicrobial activity of ceramic disks loaded with silver ions and nitroxoline. Stud UBB Chem. 2016; 61(3(1)): 275-283.
83. Horovitz O, Tomoaia-Cotisel M, Racz C. Tomoaia G, Boboş LD, Mocanu A. The interaction of silver nanoparticles with lipoic acid. Stud UBB Chem. 2009; 54(3):89-96.
84. Horovitz O, Tomoaia-Cotisel M, Tomoaia G, Bobos LD, Cozar O, Barbu-Tudoran L, Mocanu A. Investigation on the self-assembled arrangement of silver nanoparticles in the presence of protein and amino acids. J Optoelectron Adv. Mater-Symposia. 2010; 2(1): 39-43.
85. Mocanu A, Pasca RD, Tomoaia G, Garbo C, Frangopol PT, Horovitz O, Tomoaia-Cotisel M. New procedure to synthesize silver nanoparticles and their interaction with local anesthetics. Int J Nanomed. 2013; 8: 3867-3874.
86. Mocanu A, Horovitz O, Racz CP, Tomoaia-Cotisel M. Green synthesis and characterization of gold and silver nanoparticles. Rev Roum Chim. 2015; 60(7-8): 721-726.
87. Rapuntean S, Balint R, Paltinean GA, Tomoaia G, Mocanu A, Racz CP, Horovitz O, Tomoaia-Cotisel M. Antibacterial activity of silver nanoparticles obtained by co-reduction with sodium citrate and tannic acid. Stud UBB Chem. 2018; 63(3): 73-85.
88. Balint R, Paltinean GA, Mocanu A, Horovitz O, Tomoaia-Cotisel M. Interaction of silver nanoparticles with vancomycin: An Uv-Vis study. Stud UBB Chem. 2019; 64(2(2)): 335-343.
89. Avram A, Gorea M, Rapuntean S, Mocanu A, Paltinean GA, Varhelyi Jr C, Petean I, Horovitz O, Tomoaia-Cotisel M. In-vitro antibacterial activity of novel nanostructured composites based on forsterite and silver nanoparticles. Rev Chim (Bucharest). 2020; 71(1):13-21.
90. Ujica MA, Paltinean GA, Mocanu A, Tomoaia-Cotisel M. Silver and gold nanoparticles: Callenges and perspectives. Annals - Series on Biological Sciences, of the Academy of Romanian Scientists. 2020; 9(1): 97-139.
91. Avram A, Mocanu A, Horovitz O, Tomoaia G, Tomoaia-Cotisel M. Antibacterial effect of hydroxyapatite and silver. Academy of Romanian Scientists Annals Series on Physics and Chemistry. 2022; 7(2): 7-33 doi: 10.56082/annalsarsciphyschem.2022.2.7
92. Araujo Teixeira J, Vieira Costa e Silva A, dos Santos Junior VE, Correia de Melo Junior P, Arnaud M, Goretti Lima M, Pelagio Flores MA, Montenegro Stamford TC, Dias Pereira JR, Gadelha Ribeiro Targino A, Galembeck A, Rosenblatt A. Effects of a new nano-silver fluoride-containing dentifrice on demineralization of enamel and streptococcus mutans adhesion and acidogenicity. Int J Dent. 2018; 2018:1351925. doi: 10.1155/2018/1351925
93. Zsako J, Chifu E, Tomoaia-Cotisel M. Rotating rigid-plate model of carotenoid molecules and the behaviour of their monolayers at the air/water interface. Gazz Chim Ital. 1979; 109(11-12): 663-668.
94. Chifu E, Tomoaia-Cotisel M, Andrei Z. Mixed monolayers of canthaxanthin with lipids. Stud UBB Chem. 1979; 24(2): 63-67.
95. Chifu E, Tomoaia-Cotisel M. Insoluble monolayers of lecithin and carotenoid pigments. Rev Roum Chim. 1979; 24(7): 979-986.
96. Chifu E, Tomoaia M, Ioanette A, Behaviour of canthaxanthin at the benzene/water and air/water interfaces. Gazz Chim Ital. 1975; 105(11-12): 1225-1232.
97. Chifu E, Tomoaia M, Nicoară E, Olteanu A. Isozeaxanthin films at the oil/water and air/water interfaces. Rev Roum Chim. 1978; 23(8): 1163-1169.
98. Chifu E, Tomoaia-Cotisel M, Andrei Z, Bonciu E. β-apo-8-carotenoic acid ethyl ester films at fluid interfaces. Gazz Chim Ital. 1979; 109(6-7): 365-369.

99. Chifu E, Tomoaia-Cotisel M, Ioanette A. Mixed insoluble monolayers of cholesterol and β-apo-8-carotenal. Gazz Chim Ital. 1979; 109(6-7): 397-398.
100. Chifu E, Zsako J, Tomoaia-Cotisel M. Xanthophyll films. I. Single-component monolayers at the air/water interface. J Colloid Interface Sci. 1983; 95(2): 346-354.
101. Tomoaia-Cotisel M, Chifu E. Carotenoid pigment films at fluid interface. Rev Chim (Bucharest). 1981; 32(11): 1063-1069.
102. Tomoaia-Cotisel M, Chifu E. Mixed insoluble monolayers with β-apo-8-carotenoic acid ethyl ester. Gazz Chim Ital. 1979; 109(6-7): 371-375.
103. Tomoaia-CotiseL M, Chifu E, Zsako J. Mixed monolayers of egg lecithin and carotenoids. Colloids Surf. 1985; 14: 239-246.
104. Tomoaia-Cotisel M, Zsako J, Chifu E. Ejection curves and miscibility of egg lecithin with some carotenoid derivatives. Rev Roum Chim. 1987; 32(7): 663-670.
105. Tomoaia-Cotisel M, Albu I, Chifu E. Adsorption of carotene and albumin at the oil/water interface. Stud UBB Chem. 1979; 24(2): 68-73.
106. Tomoaia-Cotisel M, Zsako J, Sălăjan M, Chifu E. Interaction of unimolecular films of some carotenoids with electrolytes at the air/water interface, in Water and Ions in Biological Systems (A. Pullman, V. Vasilescu, and L. Packer Eds.), Union of Societies for Medical Sciences, Bucharest, Romania, 1985; pp. 371-381.
107. Tomoaia-Cotisel M, Chifu E. Xanthophyll films. II. Two-component monolayers of some xanthophylls and egg lecithin at the air/water interface. J Colloid Interface Sci. 1983; 95(2): 355-361.
108. Rácz LZ, Paltinean GA, Petean I, Tomoaia G, Pop LC, Arghir G, Levei E, Mocanu A, Rácz CP, Tomoaia-Cotisel M. Curcumin and whey protein binding and structural characteristics of their complex evidenced by atomic force microscopy. Stud UBB Chem. 2022; 67(3): 61-74. doi: 10.24193/subbchem.2022.3.05.
109. Rácz LZ, Rácz CP, Horovitz O, Tomoaia G, Mocanu A, Kacso I, Sárközi M, Dan M, Porav S, Borodi G, Tomoaia-Cotisel M. Complexation of Curcumin using Whey Proteins to Enhance Aqueous Solubility, Stability and Antioxidant Property. Stud UBB Chem. 2022; 67(3): 75-99 doi: 10.24193/subbchem.2022.3.06.
110. Rácz LZ, Rácz CP, Pop LC, Tomoaia G, Mocanu A, Barbu I, Sárközi M. Roman I, Avram A, Tomoaia-Cotisel M,. Toma VA. Strategies for improving bioavailability, bioactivity, and physical-chemical behavior of the curcumin. Molecules. 2022; 27: 6854. doi: 10.3390/molecules27206854
111. Rácz CP, Racz LZ, Floare CG, Tomoaia G, Horovitz O, Riga S, Kacso I, Borodi G, Sarkozi M, Mocanu A, Roman C, Tomoaia-Cotisel M. Curcumin and whey protein concentrate binding: Thermodynamic and structural approach. Food Hydrocoll. 2023; 139: 1088547. doi: 10.1016/j.foodhyd.2023.108547
112. Li B, Pan T, Lin H, Zhao Y. The enhancing antibiofilm activity of curcumin on Streptococcus mutans strains from severe early childhood caries, BMC Microbiol. 2020; 20: 286. doi: 10.1186/s12866-020-01975-5
113. Hu P, P Huang, MW Chen. Curcumin reduces Streptococcus mutans biofilm formation by inhibiting sortase A activity. Arch Oral Biol. 2013; 58: 1343-1348. doi: 10.1016/j.archoralbio.2013.05.004
114. Shanmugapriya K, Kim H, Saravana PS, Chun BS, Kang HW. Astaxanthin-alpha tocopherol nanoemulsion formulation by emulsification methods: investigation on anticancer, wound healing, and antibacterial effects. Colloids Surf. B Biointerfaces. 2018; 172: 170–179. doi: 10.1016/j.colsurfb.2018.08.042