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Bioactive agents with biomimmetic remineralizing action

Agentes bioativos com ação biomimética remineralizante

Agentes bioactivos con acción remineralizante biomimética

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Abstract
Dental caries is considered by the WHO to be the second most common disease in the world. Considering that its action is due to enamel demineralization carried out through the metabolism of carbohydrates by dental biofilm bacteria, the use of fluoride toothpastes was established as an effective method of control. Fluoride toothpastes are an integral part of individual care to prevent caries. However, even with its proven effectiveness, there is still a prevalence of the disease. Therefore, different bioactive agents are being studied, looking for an action that is fluoride auxiliary, equivalent or even better. This review aimed to carry out a bibliographic survey of the last five years on the different bioactive agents used in the biomimetic remineralization process of different dental tissues and in the modulation of caries disease, both *in vitro* and *in vivo* studies. Different bioactive agents used for enamel remineralization were addressed in this review, including various forms of calcium phosphate as well as self-assembling peptides. Of the 275 studies found, 45 studies met the inclusion criteria and were used for this review. Published studies demonstrate the potential of different bioactive agents in the biomimetic remineralization of enamel. However, further studies are needed to further substantiate existing results and to extend and refine the application of these agents in modern oral hygiene.
Keywords: Biomimetic; Tooth remineralization; Dental caries.

Resumo
A cárie dentária é considerada pela Organização Mundial de Saúde a segunda doença mais comum no mundo. Considerando que sua ação se deve à desmineralização do esmalte realizada através do metabolismo de carboidratos pelas bactérias do biofilme dental, o uso de dentifrícios fluoretados se estabeleceu como um método eficaz de controle. Os cremes dentais com flúor são parte integrante do cuidado individual para prevenir a cárie. No entanto, mesmo com sua eficácia comprovada, há prevalência da doença. Por isso, diferentes agentes bioativos estão sendo estudados, buscando uma ação que seja auxiliar do flúor, equivalente ou até melhor. Esta revisão teve como objetivo realizar um levantamento bibliográfico dos últimos cinco anos sobre os diferentes agentes bioativos utilizados no processo de remineralização biomimética de diferentes tecidos dentários e na modulação da doença cárie, tanto em estudos in vitro como in vivo. Diferentes agentes bioativos usados para remineralização do esmalte foram abordados nesta revisão, incluindo várias formas de fosfato de cálcio, bem como peptídeos de automontagem. Dos 275 estudos encontrados, 45 estudos preencheram os critérios de inclusão e foram utilizados para esta revisão. Estudos publicados demonstram o potencial de diferentes agentes bioativos na remineralização biomimética do esmalte. No entanto, mais estudos são necessários para fundamentar os resultados existentes e para ampliar e refinar a aplicação desses agentes na higiene bucal moderna.
Palavras-chave: Biomimética; Remineralização dentária; Cárie dentaria.

Resumen
La caries dental es considerada por la OMS como la segunda enfermedad más común en el mundo. Considerando que su acción se debe a la desmineralización del esmalte realizada a través del metabolismo de los carbohidratos por las bacterias del biofilm dental, se estableció el uso de dentífricos fluorados como un método efectivo de control. Las pastas dentales con flúor son una parte integral del cuidado individual para prevenir la caries. Sin embargo, incluso con su eficacia comprobada, todavía existe una prevalencia de la enfermedad. Por lo tanto, se están estudiando diferentes agentes bioactivos, buscando una acción que sea auxiliar del fluoruro, equivalente o incluso mejor. Esta revisión tuvo

como objetivo realizar un levantamiento bibliográfico de los últimos cinco años sobre los diferentes agentes bioactivos utilizados en el proceso de remineralización biomimética de diferentes tejidos dentarios y en la modulación de la enfermedad de caries, tanto en estudios *in vitro* como in vivo. En esta revisión se abordaron diferentes agentes bioactivos utilizados para la remineralización del esmalte, incluidas varias formas de fosfato de calcio, así como péptidos de autoensamblaje. De los 275 estudios encontrados, 45 cumplieron con los criterios de inclusión y se utilizaron para esta revisión. Los estudios publicados demuestran el potencial de diferentes agentes bioactivos en la remineralización biomimética del esmalte. Sin embargo, se necesitan más estudios para fundamentar aún más los resultados existentes y ampliar y refinar la aplicación de estos agentes en la higiene oral moderna.
Palabras clave: Biomimética; Remineralización dental; Caries dental.

1. Introduction

Human teeth are composed of a hard layer of tooth enamel over a softer dentin core. At the root, dentin is covered by cement, where periodontal ligaments are in the alveolar bone. The dentin is innervated and the tooth, a living organ, is connected to vascularization through the dental pulp, located in the pulp cavity. Dental enamel is the harshest substance in our body, without being brittle. It is a structure with exceptional properties, extremely resistant to chewing forces and extreme chemical and temperature variations, in addition to protecting the dental structure from external damage (Thesleff, 2003).

Enamel is composed of 92 to 96% of inorganic matter or mineral phase, and 4% of organic matter and plasma by weight. The mineral phase consists mainly of calcium phosphate in the form of hydroxyapatite crystals (Gwinnett, 1992; Ten Cate, 1989). The physical-chemical properties of enamel are due to its high hydroxyapatite content, which has the parallel arrangement of individual elongated apatite crystals and the interlacing of perpendicular prisms forming a three-dimensional arrangement. These characteristics result in a biomaterial of high physical hardness and resilience (Pandya & Diekwisch, 2019).

Calcium and Phosphate are the main ions in the constitution of hydroxyapatite crystals that make up the structure of dental enamel. Its ions present in saliva play a fundamental role in the dynamics of the process of demineralization and remineralization of the tooth, (Li et al., 2014). When they are in oversaturation, there is increased remineralization, which makes Calcium Phosphate a bioactive agent of interest. Studies involving the use of calcium phosphate in different compositions or morphologies are the object of studies in the search for toothpastes with better efficiency of dental remineralization. Demineralization consists of the loss of tooth minerals after exposure to acids, while the restoration of these minerals in the tooth structure is called remineralization (Abou Neel et al., 2016). Caries disease can be characterized as the localized destruction of susceptible dental tissues caused by a demineralization performed by the acid by-products of bacterial fermentation of carbohydrates from the diet of its host. Signs of carious demineralization can be seen in hard dental tissues, but the onset of the disease process occurs within the bacterial biofilm covering the surfaces of the teeth (Fejerskov, 2003).

Dental caries is a disease classified as multifactorial, which begins with microbiological changes within the bacterial biofilm. It can be modulated by saliva flow and composition, fluoride exposure, dietary sugar consumption and preventive behaviors, such as mechanical removal of biofilm by tooth brushing. The disease is in its early stages reversible and can be discontinued at any time, even when part of the dentin or enamel are destroyed. However, this interruption will only be effective if we remove or disorganize this biofilm efficiently (Selwitz, et al., 2007). The main objective of modern conservative dentistry is to manage non-cavitated caries lesions in a noninvasive manner through the remineralization of this affected enamel. This attempt to prevent the progression of the disease improves aesthetics, maintains the resistance and function of dental enamel. Remineralization can occur as a natural repair process, where salivary calcium (Ca^{2+}) and phosphate (PO_4^{3-}) plaque/ions are deposited in the crystalline cavities of the demineralized dental structure, resulting in liquid gain of minerals. The presence of free fluoride (F^-) ions in the oral environment can lead to the incorporation of Ca^{2+} and PO_4^{3-} ions into the crystalline network, with the resulting fluorapatite mineral significantly more resistant to acid exposures (Cate, 1999).

In relation to the prevention of caries lesions, fluoride remains the gold standard, and there are systematic reviews confirm the role of fluoride products in the prevention of dental caries (Marinho, et al., 2003). However, recent epidemiological data suggest a concern about the stagnation of caries experience or even an increase in some population groups, despite the regular use of fluoridated toothpastes (Agustsdottir et al., 2010; AIHW, 2018; Dye, et al., 2017). Improper use of fluoride can also cause chronic and acute consequences. Chronic is called fluorosis and can be characterized as hypomineralization of dental enamel due to inadequate exposure to fluoride during the stages of dental germ formation. Acute is related to the ingestion of a large amount of fluoride, which can cause since intestinal pain to death, specifically in children (Paiva & Cury, 2001). In recent years, biomimetic concepts in this subject have been developed in oral hygiene with the objective of addressing alternatives for fluoride (Enax & Epple, 2018; Lukbe et al., 2016). Design biologically inspired or adapted from nature is referred to as "biomimetic". Especially in the field of enamel remineralization, calcium phosphates have been identified as promising biomimetic alternatives due to their similarity with natural enamel. It is assumed that calcium phosphate works by infiltrating the micropores in the initial lesions of caries, where it acts as a crystalline nucleus in the remineralization process, continuously attracting large amounts of calcium and phosphate ions from the oral fluids to the lesion, thus promoting natural remineralization processes (Van Loveren et al., 2013; Najibfard, et al., 2011; Amaechi, & Van Loveren, 2013). In addition to the remineralizing properties, in situ studies with hydroxyapatite showed anti-adhesive properties with potential to be used in biomimetic biofilm control (Kensche et al., 2017; Lelli et al., 2014). Advances in tissue engineering methods have produced biomimetic methods that have demonstrated a strong potential to regenerate enamel microstructure. Thus, this review aims to make a bibliographic survey of the last five years about different bioactive agents obtained and used in the process of biomimetic remineralization of different dental tissues and in the modulation of caries disease, both *in vitro* and *in vivo* studies, as well as their physical-chemical characteristics.

2. Methodology

The search of this narrative review was carried out in the electronic databases Periodic CAPES and PUBMED according to da Silva et al. (2020). The search terms and operators (AND and OR) used in the bases were: (biomimetic) AND (dentifrice)) OR (biomimetic)) AND (enamel)) AND (remineralization). The searches for the articles were carried out from March/2021 to June/2021. Studies published between the years 2016 and 2021 were selected. Only articles in the English language, clinical trials that were peer reviewed were included. Relevant articles and review studies were prioritized that evidence the evaluation of the impact of different bioactive agents in the face of the cariogenic challenge. The articles were read in detail, including exploration of the references of articles identified in step 2, seeking complementary studies that could report the impact of commercial toothpastes containing biomimetic agents in the face of the cariogenic challenge. As steps 2 and 3 will be repeated until no new studies are found. The titles, abstracts and complete articles of all identified studies were analyzed to determine whether they meet the inclusion criteria. Relevant references identified during the full reading of the articles were included. A companion search was conducted using Google Scholar (all available dates) to find new studies and relevant articles.

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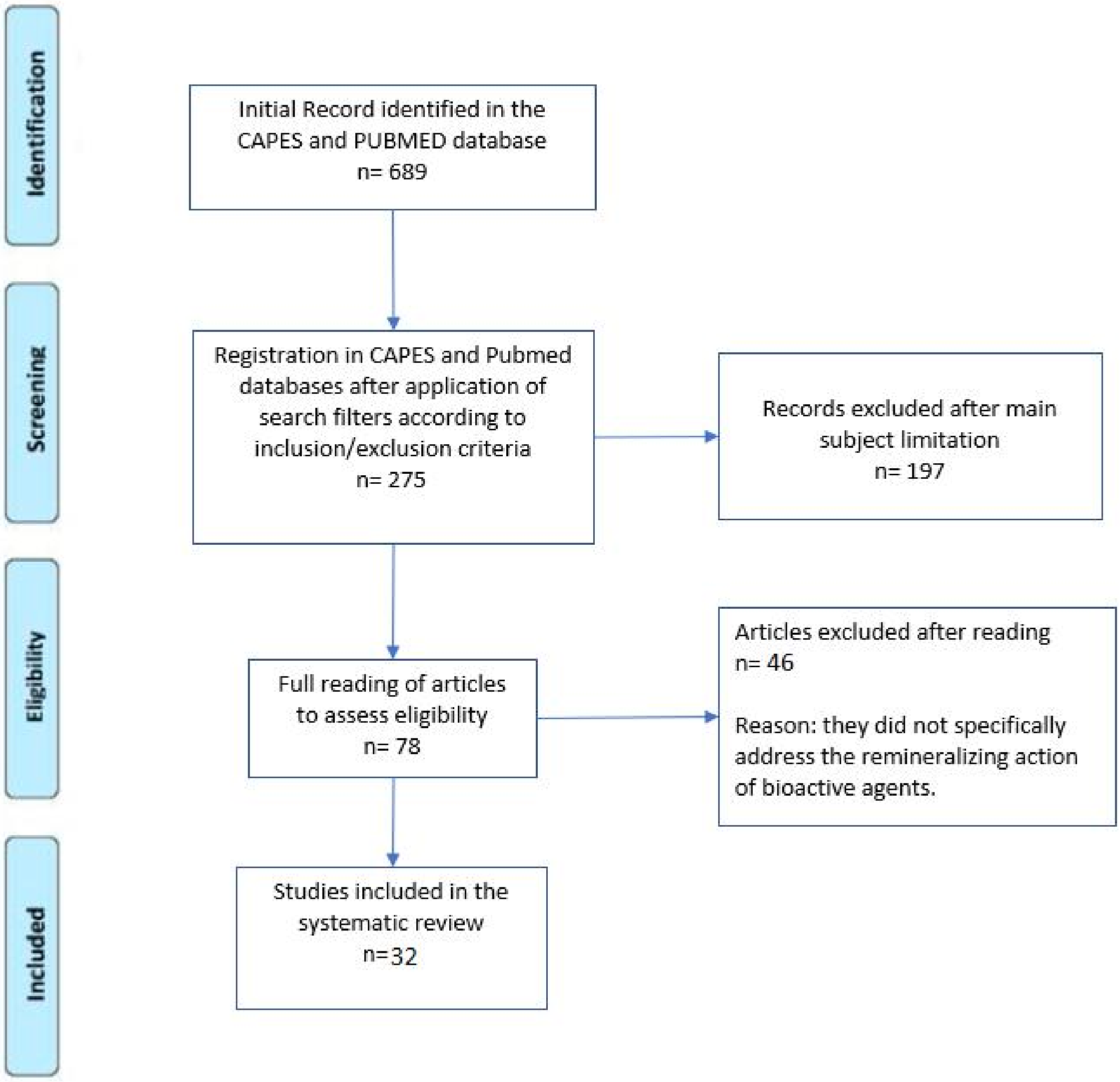
Figure 1. Flow chart of the literature search and screening process.



Source: Authors.

3. Results and Discussion

The search performed in the database using the descriptors (biomimetic) AND (dentifrice)) OR (biomimetic)) AND (enamel) AND (remineralization) and the other criteria presented a total of 275 results, of which the title and abstracts were read. Of these 275 results, 197 were excluded because they were not related to the objective of the review, that is, they did not specifically address the remineralizing action of bioactive agents.

Following the full reading of the articles considered eligible, 46 were excluded. Thus, 32 articles were selected. Of the 32 articles that passed the evaluation: 6 involved the use of polypeptides and 11 associated in their hydroxyapatite research. The other articles used different bioactive agents in their approach (from remineralizing agents such as calcium phosphate to silica and agarose hydrogels).

Table 1. Summary of the studies included.

Author	Year	Title and Magazine	Type of Study	Bioactive Agent
Muller et al	2017	Polymers	In vitro	Amorphous calcium polyphosphate
Wang et al	2018	RSC Advances	In vitro	Amorphous calcium polyphosphate
Xiao et al	2017	Dental Materials	In vitro	Amorphous calcium polyphosphate
El Moshy et al	2018	F1000 Research	In vitro	Agarose gel associated with calcium phosphate
Han et al	2017	Scientific Reports	In vitro	Agarose gel associated with calcium phosphate
Zhou et al	2020	Biomedical engineering online	In vitro	CPP-ACP
Dashper et al	2019	Journal of dental research	In vitro	CPP-ACP
Sirin Karaarslan et al	2018	Journal of International Society of Preventive & Community Dentistry	In vitro	CPP-ACP
Geeta et al	2020	Journal of Conservative Dentistry	In vitro	Nano HA
Sharma et al	2017	Journal of Orofacial Sciences	In vitro	Nano HA
Scribante et al	2017	BioMed Research	In vitro	Nano HA
Zaharia et al	2017	Ceramics International	In vitro	Nano HA
Khandelwal et al	2020	Advances in Human Biology	In vitro	Nano HA
Amaechi et al	2019	BDJ Open	In vitro	Nano HA
Hemalatha et al	2020	Journal of Conservative Dentistry	In vitro	Nano HA
Manchery et al	2019	Dental research journal	In vitro	Nano HA
Kamath et al	2017	Dental research Journal	In vitro	Nano HA
Bossu et al	2019	Journal of Nanobiotechnology	In vitro	Nano HA
Ionescu et al	2020	Journal of Funciotional Biomaterials	In vitro	Nano HA
Üstün and Aktören	2019	Microscopy Research and Technique	In vitro	P11-4
Sezici et al	2021	Progress in Orthodontics	In vitro	P11-4
Sindhura et al	2018	Journal of Indian Society of Pedodontics and Preventive Dentistry	In vitro	P11-4
Alkilzy et al	2018	Jorunal of Dental Research	In vivo	P11-4
Doberdoli et al	2020	Scientific Reports	In vivo	P11-4
Wierichs et al	2017	Carues Research	In vivo	P11-4
Liang et al	2019	International Journal of Oral Science	In vitro	PAMAM
Gao et al	2019	Journal of Dentistry	In vitro	PAMAM
Hou et al	2020	International Journal of Oral Science	In vitro	PASP-PEG
Taneja et al	2019	Journal of International Society of Preventive & Community Dentistry	In vitro	Theobromine
Anthoney et al	2020	European Journal of Dentistry	In vitro	Bioactive Glass
Saffarpour et al	2017	Journal of Dentistry	In vitro	Bioactive Glass

Source: Authors.

Calcium phosphate

Most of the biomimetic materials found in the literature are composed of calcium phosphate in its different structures, enriched or not by biomaterials. Muller et al. (2017) developed new toothpaste containing amorphous calcium polyphosphate micro particles enriched with retinyl acetate ("a-polyP/RA-MP") containing Zn2+ ions, applied as Zn-polyP micro particles ("Zn-a-polyP-MP"). This new material showed an inhibiting effect of Streptococcus mutans. In addition, toothpaste containing "a-polyP/RA-MP" showed repairing cracks in the regions of dental enamel and reseal dentinal tubules.

The work of Wang et al. (2018) consisted of the incorpotraction of 1 wt% of sodium fluorescein, 25% of amorphous polyacrylic acid (PAA-ACP) and nano particles of amorphous calcium phosphate stabilized in a self-conditioning adhesive (Clearfil S3 Bond) as a fluorescent mineralizing adhesive. In this work it was observed that the adhesive material penetrated approximately 20-30 μm of demineralized enamel and approximately 50-60 μm of demineralized dentin after 24 hours and 4 weeks of storage in artificial saliva, indicating that the material acted beyond the superficial tissue, penetrating adjacent layers.

Xiao et al. (2017) proposed the development of a dental remineralizer using carboxymethylchicansan peptide-mediated nanocomplexes and amorphous calcium phosphate (CMC/PCA). The results showed that with the use of chimeric peptide, ACP nanoparticles modified by Sodium Hypochlorite (NaClO) can be arranged to form enameled apatite crystals and bind to demineralized enamel specifically to achieve rapid biomimetic remineralization.

The studies analyzed here point to a remineralizing activity, with action on different dental tissues, such as repairing structure in caries lesions, going beyond the enamel surface and affecting the adjacent dentin, causing occlusion of the dentinal tubules and antimicrobial action.

Agarose

An alternative to the use of Calcium Phosphate is its association with agarose gels. The authors El Moshy, Abbass and El-Motayam (2018) and Han et al. (2017) performed this combination because they believed that the presence of the gel would potentiate the remineralization of collagen fibers of dentin.

In the article by El Moshy et al., an agarose gel associated with calcium phosphate was used as a model to evaluate the degree of remineralization of healthy human enamel samples submitted to pH cycling. The results showed that there was a decrease in surface roughness according to the follow-up time and increased surface microhardness, indicating that the hydrogel was able to boost the remineralization of the affected enamel.

In a second *in vitro* study by Han et al., conducted in rabbit incisor teeth, an agarose hydrogel loaded with calcium and phosphate was used to remineralize dentin and induce the formation of a layer of Hydroxyapatite (HA).

The physical analyses of the samples (scanning electron microscopy, X-ray diffraction and nanoindentation) showed that HA particles were deposited inside the collagen matrix and on the surface of the collagen matrix. The cavity of the dentinal tubules was also deposited by HA particles, some being partially occluded, and the tissue regenerated on the surface of the dentin composed of highly organized HA crystals. Research evaluating the potential of agarose hydrogel has shown positive results for remineralization and combating acid challenge in different dental tissues.

Amorphous calcium phosphate and casein phosphopeptide phosphate complex (CPP-ACP)

The CPP-ACP complex is formed when amorphous calcium phosphate is binding to casein phosphopeptide. This combination is of interest for research of dental materials since amorphous calcium phosphate could release calcium and phosphate ions, which maintains a supersaturated state in these ions, optimizing the remineralization process (Reynolds, 1998).

Zhou et al. (2020) investigated the remineralization of demineralized human dentin slices using CPP-ACP combined with sodium tripolyphosphate (TPP). The results showed that surface deposits of groups CPP-ACP (A) and CPP-ACP+TPP (B) gradually increased over time. After 21 days, the surface of group B dentin slices was usually covered by a new mineralization layer and the dentinal tubules were well occluded.

The use of PPT in conjunction with for the treatment of demineralized dentin slices increased the remineralization effect compared to CPP-ACP alone. The authors believe that this combination resulted in intrafibrillar dentin remineralization and restored the mechanical properties of dentin slices after remineralization.

Another study with with CPP-ACP carried out by Dashper et al. (2019) proposed to investigate the antibacterial action of the CPP-ACP. In this work they used a model of polymicrobial biofilm of caries with 6 bacterial strains present on the supragingival plaque (Streptococcus sanguinis (NCTC 7863), *S. Mutans Ingbritt*, *Actinomyces naeslundii* (NCTC 10301), *Veillonella parvula* (ATCC 17745), *Lactobacillus casei* (NCDO 161), and *Fusobacterium nucleatum* (ATCC 10953). Treatment with CPP-ACP caused a 50% inhibition of enamel demineralization a decrease in A*ctinomyces naeslundii* and *Lactobacillus casei*, however promote an increase in *Streptococcus sanguinis* (50%) and *Fusobacterium nucleatum* (36%) in polymicrobial biofilm.

The same line of study, Sirin Karaarslan et al. (2018) compared the effects of different remineralization methods on clinical and daily use on the biofilm's *S. mutans*. In this study, the antibacterial potential of CPP-ACP was compared with Fluoride and Ozone. There were no significant differences between remineralization agents. According to these studies, CPP-

ACP has ideal properties for a bioactive remineralizing agent of dental enamel, since it assists in the process of remineralization and occlusion of dentinal tubules, as well as an essential antibacterial action in the prevention of carious lesions.

Nano hydroxyapatite

Nano hydroxyapatite's morphology and crystalline structure (Nano HA) is similar to apatite crystal of dental enamel can be seen as a substitute for the natural mineral constituent of enamel, favoring biomimetic remineralization (Zhang & Deng, 2015). In this way, Geeta, Vallabhaneni and Fatima, (2020) demonstrated in their research the potential for remineralization of nano HA in a pH cycling test, comparing it with bioactive glass (SHY-NM), calcium casein-amorphosphate phosphate (CPP-ACP) and fluoride in initial enamel caries lesions. Nano HA had the highest surface hardness, demonstrating a higher enamel remineralization potential than fluoride. Similar studies with Nano HA were conducted by Sharma, Rao, Shenoy and Suprabha, (2017), Scribante et al. (2020), Zaharia et al. (2017) and Khandelwal et al. (2020). These authors also used the hardness test as a parameter of remineralizing potential of nano HA, which in their studies also demonstrated excellent remineralizing action, concluding its viability as a remineralizing agent.

When compared with fluoride, the use of Nano HA in toothpastes, according to Amaechi et al. (2019), Hemalatha et al., (2020) Khandelwal et al., (2020) and Manchery, et al., (2019), has the advantage of presenting results similar to fluoride without the risk of fluorosis. As for antimicrobial capacity, Kamath, et al. (2017), Bossu et al. (2019) and Ionescu, et al. (2020) concluded that the use of Nano HA can inhibit biofilm adds and growth in deciduous tooth samples, which may contribute to the prevention, stagnation, and control of caries disease.

Self-assembling peptides

In addition to products of enamel-like composition, synthetic self-assembling peptides are being developed as bioactive agents to be used in the remineralization of dental tissue affected by initial caries lesions. It is a regenerative approach that consists of replacing the affected enamel matrix with a biomimetic matrix to potentiate the effect of remineralization in deeper lesions.

The self-assembling peptide P11-4 could form a three-dimensional matrix in the subsurface of the enamel lesion and mimic enamel matrix proteins (Kirkham et al., 2007; Kind et al., 2017). Clinically this peptide is available as Curodont repair.

Studies aimed at demonstrating the action of peptide P11-4 were found. Üstün and Aktören (2019) performed a pH cycling in human enamel and compared the remineralizing action of P11-4 (Curodontrepair) with phosphopeptide-fluoride casein of amorphous calcium phosphate (CPP-ACFP) (MI Varnish) and Sodium Fluoride (DuraphatVerniz).

The results indicated that Curodontrepair (P11-4) had the highest efficacy in remineralization, mean hardness of 6.50 after 30 days, when compared with the other groups (CPP-ACFP: 5; NaF: 3.50; Control: 2.00), suggesting that treatment with P11-4 may lead to regression of the carious lesions of the initial enamel more effectively than the IM varnish and the varnish. Duraphat. Et al. (2021) and Sindhura, et al. (2018), achieved similar results. Curodontrepair was effective in reducing fluorescence loss and caries injury, while Sindhura et al. (2018) noted a uniform deposition of minerals when compared to CPP-ACP.

The tests with P11-4 also showed promising results when used *in vivo* tests. In their research, Alkilzy, et al. (2018) proposed a randomized controlled blind trial conducted in children who had visible early active caries. The objective was to investigate the safety and clinical efficacy of P11-4 in the treatment of early caries when compared to fluoridated varnish (control group). Compared to the control group, the test group showed clinical and statistically significant improvement in all results at 3 and 6 months. The authors concluded that the biomimetic mineralization facilitated by P11-4 in combination with fluoride application is a simple, safe and effective noninvasive treatment for early caries lesions, which is superior to the gold standard currently used fluoride alone.

Doberdoli et al. (2020) obtained data that complement the study by Alkilzy et al. (2018). In this study, a randomized, controlled trial with fluoridated varnish. Single blind was designed, comparing the combined treatment of a single application of P11-4 and fluoridated varnish (Test Group 1) and the combined treatment of a single application of P11-4 and a twice-a-week self-application of self-assembling Peptide Matrix (SAPM) (Test Group 2) against gold standard fluoridated varnish applied twice a year (Control). Data obtained in the clinical trial showed that SAP P11-4, applied in combination with fluoridated varnish or SAPM twice a week, is a superior treatment for early caries lesions compared to fluoridated varnish alone, due to its fluorescence means being higher until day 180, demonstrating that the varnish remained longer.

In contrast, Wierichs, et al., (2017) obtained conflicting data. Their study compared well stablished and new treatment methods regarding their ability to hinder demineralization. The treatments consisted in application of a self-assembly peptide (Curodont™ Repair [C]), a low viscosity resin (Icon® [I]), 2 fluoride solutions (10,000 ppm F-: Elmex fluid [E] and 43,350 ppm F-: Tiefenfluorid® [T]), and no intervention (N). Compared to the negative control, the application of fluorides and low viscosity resin significantly hampered the loss of minerals. In addition, the masking effect after application of low viscosity resin was significantly better than all other groups. In contrast, the application of the self-assembly peptide cannot significantly reduce additional mineral loss as well as colorimetric values. For samples treated with self-assembly peptide P11-4, a significantly higher mineral loss was observed compared to the respective negative control samples with brushing. The authors speculated that one of the reasons why the additional use of P11-4 even seemed to hinder the "demineralization inhibitor effect" observed with the use of fluoride toothpaste could be pH-dependent reactivity of P11-4.

Innovative materials

Apart from the biomimetics described as the most used materials in research on dental enamel remineralization, several other new materials are also being tested as described. Poly dendrines (amine starch) (PAMAM) are highly branched polymers characterized by the presence of internal cavities, a series of reactive terminal groups and a well-defined size and shape (Chen, et al., 2013).

In recent studies, PAMAM dendroros have been associated with amorphous calcium phosphate (PCA). In their review, Liang et al. (2019) concluded that PAMAM+ACP together demonstrate synergistic effects and produce triple benefits: excellent nucleation models, superior acid neutralization and ion release. Gao et al. (2019) investigated the remineralizing action of an adhesive containing PAMAM and amorphous calcium phosphate (NACP) nanoparticles in enamel lesions in a cyclic environment of saliva/artificial acid. The PAMAM or NACP adhesive alone did not induce sufficient remineralization of demineralized enamel. In contrast, PAMAM + NACP yielded the greatest remineralization of enamel lesions. The authors concluded that the method is promising to remineralize dental lesions, increase hardness, protect dental structures in the margins and improve the longevity of the tooth-restoration interface to inhibit secondary caries.

Other bioactive agents have been considered to act in the remineralization of dental tissue, demonstrating a great diversity and availability in the market.

Hou et al. (2020) designed an innovative material, poly (aspartic acid) -polyethylene glycol (PASP-PEG), synthesized to build a mineralizing and anti-adhesive surface that could be applied to repair demineralized enamel. Microhardness, cytotoxicity, and adsorption tests were performed. The authors concluded that PASP-PEG can queen the free mineral ions in the solution to perform auto curative remineralization in situ, effectively improving the mechanical properties of acid-etched enamel and forming a non-fouling coating that significantly reduces bacterial bonding.

In their study, Taneja, et al. (2019) investigated the remineralization potential of two concentrations of theobromine (100 mg/L and 200 mg/L) with fluoridated toothpaste, NovaMin (Bioactive Glass) and nanohydroxyapatite. Theobromine is a chemical compound of the alkaloid group in the form of white crystalline powder and differs from the caffeine molecule only by a methyl group (1,3,7 dimethylxane). Decreases caries or promotes remineralization by increasing crystallinity. After 72 hours of demineralization, they noticed a loss of superficial integrity in all study groups. Pore defects could be seen, proving loss of aprismatic enamel and the presence of destroyed enamel prisms. But after a 14day remineralization treatment, the poorly defects in the samples were all filled in, restoring surface integrity. An elementary analysis comparison performed for theobromine and fluoride showed an increase in Ca/P ratio without any significant difference. Thus, the authors concluded that theobromine a promising new effective remineralizing agent alternative to the agents already available.

Bioactive Glass (BAG) is a class of bioactive material composed of calcium, sodium, phosphate, and silicate. They are reactive when exposed to body fluids and deposit calcium phosphate on the surface of particles (Andersson & Kangasniemi, 1991). *In vitro* and *in vivo* studies have shown that BAG particles can be deposited on dentin surfaces and subsequently occlude dentinal tubules inducing the formation of carbonated HA and similar materials (EARL, 2011).

Anthoney et al. (2020) and Saffarpour, et al., (2017) tested the action of Bioactive Glass in dentin. In their research, Anthoney et al. (2020) submitted dentin blocks to pH cycling, and then brushed with different experimental toothpastes (EXT) that contained bioactive glass (n-FBG: 1.5% by weight [EXT-A], 2.5% by weight [EXT-B] and 3.5% by weight [EXT-C]), nanozinc oxide (n-ZnO). All dentifrices containing nano-fluoridated bioactive glasses demonstrated uniform occlusion of the dentinal tubules; however, highly concentrated EXT toothpastes showed more occlusion. Saffarpour et al. modified bioactive glass as a remineralizing treatment. In this study three groups were analyzed: 1- BAG, 2- MODIFIED BAG with 5% strontium (Sr) and 3- MODIFIED BAG with 10% Sr. After the application of the BAG, the samples were stored in artificial saliva. The authors identified that the dentinal tubules were partially occluded by BG and BG modified with 5% sr, while these were almost completely obstructed after the use of modified BG with 10% of Mr. Also concluded that the addition of 10% of Sr in BG increases the formation of apatite, however, apatite dissolves over time. The addition of 5% Sr to BG stabilizes the apatite network and increases remineralization. These different bioactive agents demonstrated the ability to create apatite crystals within completely demineralized collagen fibers. Therefore, new experiments in this field will bring better products.

4. Conclusion

Currently, most commercially available fluoride-free remineralizing systems aim to increase efficacy and minimize the potential risks associated with fluoride. In this review, was possible to conclude that the use of different bioactive agents in a biomimetic strategy for enamel regeneration may be the next step of regenerating dentistry, where these agents serve as a framework for new formation of enriched enamel in demineralized tissues.

In addition to the remineralizing action, antibacterial action was observed, as well as occlusion of dentinous tubules, beneficial properties for the prevention and control of caries disease, and these agents can be used in toothpastes, since they constitute the measure of individual prevention in oral health more accessible, due to its low cost and wide availability.

However, it should be noted that the vast majority of the studies analyzed are *in vitro* studies, which highlights the need for more *in vivo* studies to understand the real remineralizing potential of these agents. There is also a lack of studies comparing these agents not only with fluoride, but also with each other.

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