Review

Synthetic Proteins in Dental Applications

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Abstract: Biotechnology and artificial intelligence have sparked a revolution in dentistry, with a focus on restoring natural tissue functions. This transformation has given rise to bioactive materials, inspired by biomimetics, aimed at replicating the processes found in nature. As synthetic biology advances, there is a heightened focus on signaling systems crucial for bio-based diagnostics and therapeutics. Dentistry now harnesses synthetic proteins for tissue regeneration and dental material enhancement. A current research priority is bacterial biofilm inhibition, vital for dental health. Given the role of *Streptococcus mutans* in dental caries, the development of synthetic antimicrobial peptides targeting this bacterium is underway. The balance of dental enamel between demineralization and remineralization impacts caries formation. Factors such as the presence of hydroxyapatite and salivary peptides influence enamel health. Recent studies have spotlighted salivary protein-inspired peptides for enhanced remineralization. In the realm of bone regeneration, synthetic proteins like bone morphogenetic proteins (BMP) have been spotlighted, earning FDA approval. Research is currently delving into peptides such as cementum protein 1 peptide (CEMP-1-p1) and parathyroid hormone variants like PTH (1-34), underscoring their potential in advancing dental and bone health.

Keywords: protein; peptides; bacteria; demineralization; pulp; bone; dentistry

1. Introduction

The development of biotechnology and artificial intelligence within the dental area has evolved in recent years [1]; many of the new materials currently being developed are primarily aimed at restoring the biological functions of tissues, and with this, the formulation of bioactive materials based on biomimetics [2,3]. The purpose is to mimic the biological approaches and strategies of nature [4] and this has been booming. More recently with the knowledge and advances in synthetic biology [5], the construction of signaling systems has ranged from proteins, pathways, networks, and organisms, landing in diagnostics and therapeutics, and focusing on the behavior of biological processes [6]. One of the sources of inspiration for these engineering processes involves proteins that trigger molecular and cellular functions [7].

With this, synthetic proteins arise in different fields of medicine [8] and in dentistry [9]; their development is mainly related to tissue regeneration and improvement of dental materials [10–12]. Synthetic proteins are created and modified with biotechnology and chemical synthesis techniques, by means of which synthetic amino acids can be produced, or the modification of existing proteins can be performed to carry out specific functions according to a need [13]. Their design has specific purposes; in bone and tissue regeneration, proteins related to growth factors stimulate, promote, and accelerate tissue regeneration, and healing processes, as well as stimulating cell differentiation to specific oral tissues [14,15]. Within dental materials, they are used to improve adhesion and biocompatibility with the surrounding tissues [16–20].

Research within the dental field is in a state of constant advancement, continually giving rise to novel applications and therapies rooted in synthetic proteins. These breakthroughs are dedicated to improving dental care. Consequently, the principal aim of this review is to delve into the progression of synthetic proteins and their ever-expanding significance within the realm of dentistry. This work accentuates their pivotal role in bacterial biofilm inhibition, facilitating enamel remineralization, stimulating the dentin-pulp complex, and promoting bone regeneration. It serves as a summary of innovative approaches that have emerged in recent years.

2. Bacterial Biofilm Inhibition

Dental caries remains a global public health concern [21]. The beginning of its evolution is related to the accumulation of organized biofilm and the presence of sugars from the diet, which are metabolized by bacteria producing acids, which decrease the pH of the oral cavity to a critical point (below 5.5 pH) which causes a loss of ions in the mineralized tissues of the teeth [22]. To contrast this effect, it is important, among other preventive actions, to have mineralized dental structures and adequate control of bacterial biofilms [23].

The oral cavity harbors a great variety of microorganisms; it is estimated that more than 700 different species of bacteria can coexist, although this figure may vary according to each individual and his or her oral health condition [24]. Among the most numerous species are *S treptococcus mutans* (*S. mutans*), *Streptococcus salivarius* (*S. salivarius*), *Porphyromonas gingivalis* (*P. gingivalis*), among others [25], according to their quantity, these bacteria can be found in a balance within the oral microbiota or can develop a dysbiosis [26].

Biofilms are microbial communities organized three-dimensionally in an extracellular matrix adhered to surfaces [27]. In their development, *S. mutans* are among the first colonizers and the main microorganisms responsible for the dental caries process, derived from the production of extracellular polysaccharides, such as glucan, which favors the colonization of various species of bacteria that contribute to the formation of highly structured bacterial groups [28].

Given this role played by *S. mutans*, recently, researchers have been analyzing the use of both natural and synthetic antimicrobial peptides, which provide a potential effect against this microorganism and thus in decreasing bacterial adhesion [23]. The proposal of synthetic peptides focuses on mimicry of natural peptides [29,30], fusion of functional sequences [31], modifications, and new designs.

Antimicrobial peptides (AMPs) are a vital component of the body's innate immune response, safeguarding the host against microbial infections. They are typically short proteins, usually consisting of fewer than 100 amino acids (aa). They are characterized by a positive charge and a significant proportion of hydrophobic amino acids [32]. According to their three-dimensional structure, which is essential to determine their biological function, selective interaction with other molecules, and catalysis in chemical reactions as well as molecular recognition (antigen-antibody), there are four families of AMPs: Alpha family (α)—α-helix structure, length of 30 to 40 aa, amphipathic, rich in lysine and arginine; Beta family (β)—beta-sheet structure, disulfide-rich peptides; Alpha-beta family (αβ)—structure with α-helices and beta-sheets; Non-alpha-beta family (non-αβ)—structure containing neither helices nor sheets, present abundance of proline, glycine, histidine, arginine, and tryptophan. The three-dimensional structure is essential in the design of pharmaceuticals, to interact in a specific way.

More than 2000 AMPs with immunomodulatory, antimicrobial, and antibiofilm activities have been identified, within them peptide 1018 (peptide 478963: VRLIVAVRIWRR) has demonstrated antimicrobial activity and reduction in biofilm formation on hydroxyapatite structures [33]. Polyfemusin I (PI) peptide grafting a diphosphoserine (Ser(p)-Ser(p)-tooth-binding domain to create Ser(p)-Ser(p)-polyfemusin I peptide (DPS-PI) demonstrated in in vitro study a binding to the tooth surface that prevented *S. mutans* adhesion and inhibited the development of oral biofilms in vivo [34].

The structure of cationic, short, amphipathic, α-helical AMPs suggests that they are more potent against cariogenic bacteria. Some peptides designed following this structure, such as GH12, GLLWHLHLLHHLLLH-NH2, caused cell lysis and pore formation in cell membranes, suggesting that it probably works through the mechanism of microbial activity of amphipathic alpha-helical peptides, where peptides accumulate in the bacterial membrane by electrostatic interactions of negatively charged groups on the cell surface, so that subsequently, the hydrophobic parts are inserted into lipid bilayers causing pores and membrane disruption. Thus, it can be considered as a potential antimicrobial agent in the inhibition of biofilm of species such as *S. mutans* [35].

Currently, there is a growing interest in the inhibition of biofilm formation using peptide therapeutics due to their high specificity. The construction of the bioactive molecule phosphoserine-histatin 5 (Sp-H5) creates electrostatic attractive forces between positively charged amino acids (Lys12, Arg13, Lys14, Lys18, and Arg23) and the negatively charged phosphate anion of hydroxyapatite. These forces inhibit the adhesion of *S. mutans* and promote enamel remineralization. Specifically, Arg13, Lys14, Lys18, and Arg23 interact with the negatively charged phosphate anion of hydroxyapatite to inhibit *S. mutans* adhesion and facilitate enamel remineralization [33] (Figure 1).
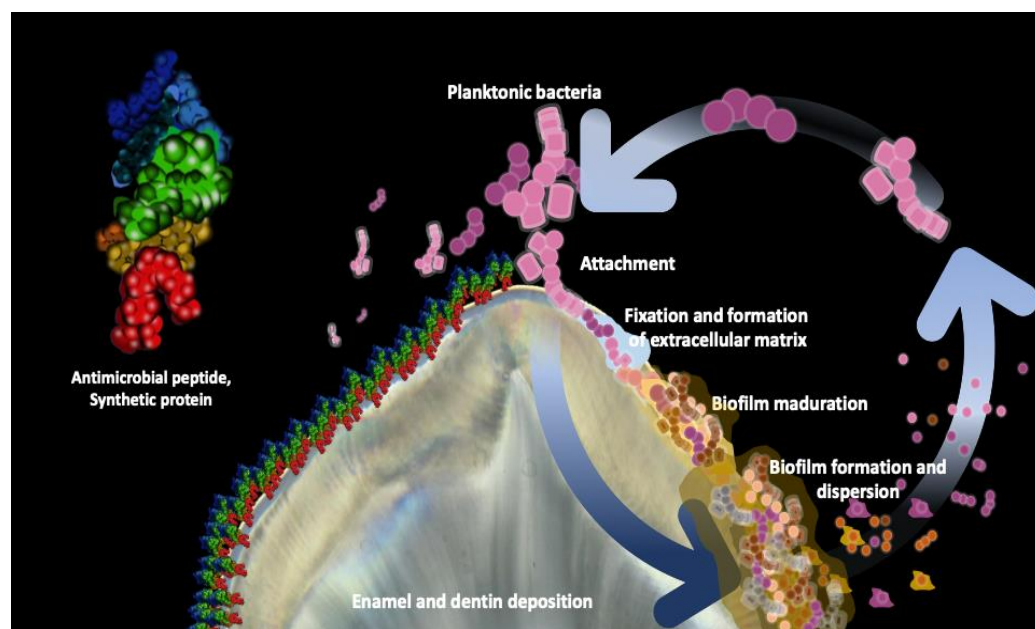

Figure 1. Enamel biofilm formation and antimicrobial synthetic protein bioactivity. The right side shows the development by which planktonic bacteria adhere to the tooth surface and begin to form an extracellular matrix, subsequently forming communities co-aggregated with other bacteria giving rise to a more complex and dense structure called bacterial biofilm, which matures and disperses. On the left is a representative image of synthetic proteins. Their highly specific amphipathic α-helical structure interacts with hydroxyapatite through electrostatic forces, which inhibit bacterial adhesion and biofilm formation. This, in turn, promotes the exchange of mineralized ions, facilitating the remineralization process.

The characteristics of the studies included in the bacterial biofilm inhibition section are summarized in Table 1.

Table 1. AMPs related to bacterial biofilm inhibition.

Study and Year [Reference]	Pepetide	Sequence	Structure	Type of Study	Antimicrobial Activity	Bacteria
da Silva 2017 [29]	[W^7]*KR12*-KAEK	KRIVQRWKDFLRKAEK-NH$_2$	α-helical	In vitro	Antimicrobial effect by determining the minimum inhibitory concentration (MIC) ranging from 7.8 to 31.25 μg mL^{-1} and minimum bactericidal concentration (MBC), 15.6 to 62.5 μg mL^{-1} On biofilm the cell viability decreased of biofilms from all strains evaluated, with biomass reduction ranging from 48 to 96%.	*S. mutans* ATCC 25175, UA 159, UA 130.
da Silva 2013 [30]	LYS-[TRP^6]-Hy-A1 (Lys-a1)	KIFGAIWPLALGALKNLIK-NH$_2$	α-helical	In vitro	Antimicrobial activity on the planktonic and biofilm growth. The MIC values ranged from 3.9 to 125 μg mL^{-1}. The MBC values ranged from 3.9 to 500 μg mL^{-1}. *S. mutans* was more resistant to the biofilm inhibiting activity of the peptide. At concentrations from 7.8 to 62 μg mL^{-1}, interference in biofilm formation, with biomass reductions ranging from 10 to 88%.	*S. oralis* ATCC 10557, *S. sanguinis* ATCC 10556, *S. parasanguinis* ATCC 903, *S. salivarius* ATCC 7073, *S. mutans* ATCC 25175 and *S. sobrinus* ATCC 6715.
Zhou 2019 [33]	Sp−H5	Phosphoserine-DSHAKRHHGYKRKF HEKHHSHRGY	Not specified	In vitro	The MIC was 2 μmol/mL. Sp−H5 kills *S. mutans* biofilm from 16× MIC. After coating on the enamel surface, Sp−H5 inhibits *S. mutans* adhesion from 2× MIC.	*S. mutans* ATCC 35668
Zhang 2019 [34]	Poly-phemusin I (PI) and tooth-binding AMP (DPS-PI)	PI (Arg-Arg-Trp-Cys-Phe-Arg-Val-Cys-Tyr-Arg-Gly-Phe-Cys-Tyr-Arg-Lys-Cys-Arg) and DPS-PI (Ser(p)-Ser(p)-Arg-Arg-Trp-Cys-Phe-Arg-Val-Cys-Tyr-Arg-Gly-Phe-Cys-Tyr-Arg-Lys-Cys-Arg)	α-helical	In vitro and in vivo (biofilm formation on rabbit incisor surfaces)	The MIC was PI = 40 μg/mL, DPS-PI = 80 μg/mL. Antibiofilm: delay in the formation of biofilm and maintaining the inhibitory effect after a diet rich in sucrose only with DPS-PI at 2× MIC.	*S. mutans* ATCC 35668

Table 1. *Cont.*

Study and Year [Reference]	Pepetide	Sequence	Structure	Type of Study	Antimicrobial Activity	Bacteria
Wang 2017 [35]	GH8 GH12 GH16	GH8, GLLWHLLH-NH$_2$; GH12, GLLWHLLHHLLH-NH$_2$; and GH16, GLLWHLLHHLLHLLHH-NH$_2$	α-helical	In vitro	GH12 showed the most potent inhibiting with MIC of 4.0–8.0 μg/mL and MBC of 8.0–32.0 μg/mL. GH8 were 16 to 32 times higher than GH12, and GH16 showed antimicrobial activity only against Lactobacillus species. GH12 exhibited an inhibitory effect on biofilm formation of *S. mutans*, with MBIC50 values of 8.0 μg/mL.	*S. mutans* UA159, *S. gordonii* DL1, *S. sanguinis* ATCC10556, *L. acidophilus* ATCC14931, *L. casei* ATCC393, *L. fermentium* ATCC9338, *A. viscosus* ATCC15987, and *A. naeslundii* ATCC12104

3. Enamel Remineralization

Dental enamel is composed of 96% inorganic material, 3% water, and 1% organic matrix [36], and is constituted of basic units currently referred to as enamel rods [37] arranged in interprismatic zones [38]. Mineralization is carried out by ameloblasts through the elaboration and secretion of protein matrix [38] throughout life. This mineralized tissue is exposed to a dynamic process of demineralization and remineralization. When teeth are exposed to acidic or sugary foods and beverages, these acids can erode tooth enamel and cause demineralization, because in this condition, the pH decreases below 5.5 (which is considered the critical pH) and consequently hydroxyapatite (HA) begins to dissolve. Therefore, demineralization of the inorganic enamel matrix takes place when this process occurs in a prolonged and repeated manner [21]. On the other hand, the buffering capacity of the saliva counteracts this condition by depositing mineral ions in the molecular structure of the enamel with the help of specific proteins such as amelogenins and HA interaction, but when this balance is lost, the demineralization process predominates. In this imbalance, together with constant salivary pH fluctuations, the presence of cariogenic bacterial biofilms, and the consumption of fermentable carbohydrates, the pathophysiology of dental caries is established [39,40].

One of the key components of tooth enamel strength that aids the remineralization process is HA, a chemical compound with a crystalline form of calcium phosphate, found naturally in teeth and bones, but is nowadays also available synthetically [41–43]. In teeth, 96% of the enamel is HA, with the remaining 3% consisting of water, collagen, and other proteins. The presence of inorganic ions in human saliva indicates a state of supersaturation with respect to HA crystals; inorganic ions in high concentrations in human saliva indicate that HA crystals are supersaturated and that salivary proteins regulate remineralization [41,42,44,45].

In recent years research has been developed to make more efficient remineralization of enamel using synthetic peptides in physiological conditions. When applied to the teeth, HA binds to the enamel and releases crucial calcium and phosphate ions, which are integral for the creation of apatite crystals [42]. This helps to restore the mineral structure of the enamel and strengthen the enamel [38]. In general, self-assembly peptide is recognized as a good template for HA initiation of nucleation or growth.

Various research studies have demonstrated that salivary proteins, such as histatin and statherin, exhibit a significant attraction to HA [43]. The adsorption of specific salivary proteins and peptides onto dental surfaces precedes the formation of dental biofilm, resulting in the creation of a film known as the acquired enamel pellicle (AEP). The composition of the AEP plays a vital role in determining its functions and understanding this can be valuable in designing strategies to combat dental caries. Marin et al., investigated how engineered peptides DR9-DR9 and DR-RR14 within the AEP control dental caries. They employed a validated in vitro biofilm model of *S. mutans* and found that the DR9-RR14 peptide significantly reduced enamel demineralization ($p < 0.0001$) in both experimental conditions. These findings suggest that DR9-RR14 may primarily control caries development through a physicochemical mechanism [46].

Synthetic peptides offer the advantage of being programmable for assembly into specific conformations and nanostructural patterns, making them highly applicable in biomimetic bone and tooth mineralization. Cheng Li et al., conducted a study to design and synthesize self-assembling β-sheet peptides ID4 and ID8, optimizing their short sequence patterns. They investigated the self-assembly and gelation properties of these peptides and assessed their stability under neutral physiological conditions. Furthermore, the study explored their effects on HAP nucleation and their potential for in vitro remineralization of initial caries lesions. They obtained very interesting results, in which ID8 showed better efficacy than ID4 [47].

Currently, new remineralization proposals are based on proteins such as amelogenin, dentin phosphoprotein, or synthetic peptides with physiological properties like salivary proteins; their interaction is specific in demineralized areas promoting a supersaturated

state of calcium and phosphate ions [48]. Amelogenin is a protein of the extracellular matrix, of which 90% presents three domains, an amelogenin that has a tyrosine peptide (TRAP), a central dominium proline, and a terminal glutamine. Yaur Li et al., studied the effect of TRAP. They synthesized this peptide in vitro to study its remineralization effect of dental grinding in bovine; results showed decreased lesion depth and production of HA crystals. The mechanism of acting as a calcium ion transporter enhances the efficacy of the recombinant amelogenin peptide TRAP in the regeneration of enamel tissue [45].

Another important finding related to amelogenin, is that it is a leucine-rich enamel matrix protein that promotes mineralization [49]. In this sense, some research has been conducted on the Gln-Pro-X gene sequence synthesizing the amelogenin-derived QP5 peptide (QPYQPVQPHQPMQQQQPQTKREEVD) composed of five Gln-Pro-X repeats (QPYQPVQPHQQPMQPQ) and the C-terminal end of amelogenin (TKREEVD). The results of the studies identify the ability of the QP5 peptide to bind to demineralized enamel and HA, increasing the surface microhardness of dental enamel and favoring a lower loss of minerals [50]. Another leucine-rich amelogenin peptide (LRAP) has been proposed for remineralization in vitro of the deeper layers of the enamel. It was found that the concentration of 120 µg mL^{-1} LRAP (commercial porcine LRAP) microhardness and mineral distribution were improved by promoting the remineralization pattern [51].

The continuous research on amelogenin's physiological significance has driven the development of peptides derived from it. It was discovered that certain structural elements such as polyproline, N- and C-terminal domains, and C-terminal orientation play crucial roles in promoting biomimetic remineralization processes [52,53].

Amelogenins assemble into nanospheres, globular aggregates, and nanochains during enamel formation, which favors the formation of HA crystals. In recent years different studies have been conducted to characterize these primary structures of native amelogenin from different species, such as human, bovine, porcine, and murine [52], highlighting that the N-terminal domain is a unique site with the ability to bind calcium. The highly charged C-terminal domain adopts a favorable structure to promote remineralization of the HA crystals, porcine and murine [52], highlighting that the N-terminal domain is a single site with the ability to bind calcium. The highly charged C-terminal domain adopts a favorable structure to promote remineralization; in the development of peptides, eleven or more polyproline repeats are suggested to allow the formation of HA crystals [53,54]. It has also been reported that peptides with eight repetitive aspartate–serine–serine (8DSS) sequences exhibit biostimulatory effects in the mineralization of murine enamel [55].

Efforts to maintain an adequate balance in the de-remineralization processes and new proposals with the modification or creation of peptides are focused on promoting an increase in the microhardness of the enamel to avoid the gradual loss of mineral ions and the formation of microscopic pores that can cause the penetration of bacteria into the dentin-pulp complex (Figure 2).

The characteristics of the studies included in the enamel remineralization section are summarized in Table 2.

Table 2. AMPs related to enamel remineralization.

Study and Year [Reference]	Peptide	Sequence	Structure	Type of Study	Remineralization effect
Valente 2018 [43]	DR9-DR9, DR9-DR14	DSpSpEEKFLRDSpSpEEKFLR (DR9-DR9) DSpSpEEKFLRRKFHEKHHSHRGYR (DR9-RR14)	Not specified	In vitro	The effect on hydroxyapatite (HA) crystal growth inhibition that promotes enamel remineralization. The presence of phosphoserine at positions 2 and 3 in DR9 resulted in a higher degree of HA inhibition. The presence of 4 phosphorylated sites in the DR9-DR9 suggests a more stable and strong binding conformation to the HA crystal.
Yarbrough 2010 [44]	2DSS, 6 DSS, 8DSS, 4ESS, 4NSS, 4DTT, 4ETT, 4NTT, 8DAA, 8NAA, 8ASS.	2DSS (DSSDSS), 4DSS (DSSDSSDSSDSS), 6DSS (DSSDSSDSSDSSDSSDSS), 8DSS (DSSDSSDSSDSSDSSDSSDSSDSS), 4ESS (ESSESSESSESS), 4NSS (NSSNSSNSSN SS), 4DTT (DTTDTTDTTDTT), 4ETT (ETTETTETTETT), 4NTT (NTTNTTNTTNTT), 8DAA (DAADAADA-ADAADAADAADAADAA), 8NAA (NAANAANAANAA NAANAANAANAA), 8ASS (ASSASSASSASSASSASSASSASS)	Not specified	In vitro	Binding of DSS-containing peptides to defined HA substrates depends strongly on the length of the peptides, additional increase in affinity seen in peptides with eight repeats; the HA binding affinity of the 8DSS peptide was 290,000 M-1, It compares favorably with measured values for histatins (K = 353,000–1,903,000 M-1), a class of small antimicrobial peptides that are known to bind HA with high affinity. With these high affinities the peptides can bind to mineralized tissues and recruit calcium phosphate to demineralized surfaces.
Li 2023 [45]	Peptide 1. N- and C-termini of porcine amelogenin. Peptide 2. TRAP	Peptide 1. MPLPPHPGHPGYINF(p-S)YEVLTPLK-WYONMIRHPYTSYGYEPMGGWATDKTKREEVD Peptide 2. MPLPPHPGHPGYINF(p-S)YEVLTPLK-WYONMIRHPYTSYGYEPMGGW	Not specified	In vitro	The results of this study indicated the potential of the recombinant amelogenin peptide TRAP to promote the remineralization of incipient enamel caries.
Marin 2022 [46]	DR9-DR9 and DR9-RR14	Not specified	Not specified	In vitro	DR9-RR14 peptide displayed a potential protective effect against enamel demineralization but did not have a significant effect on *S. mutans* biofilm biomass.

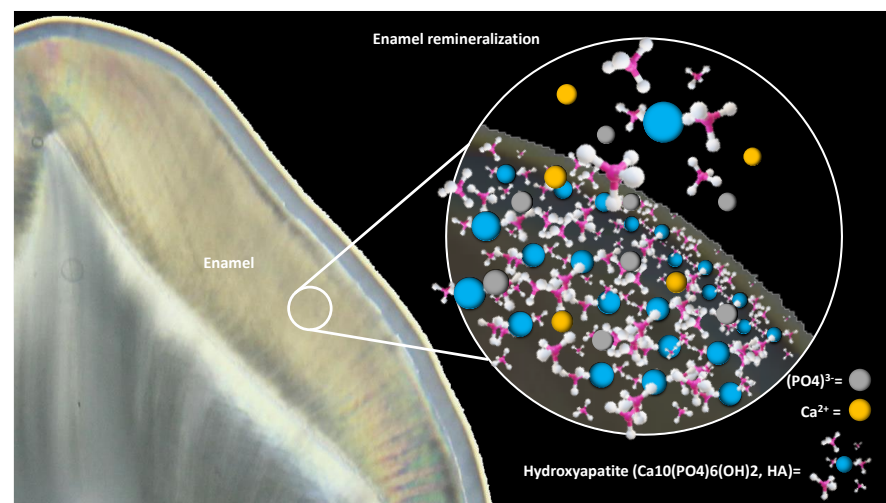| Study and Year [Reference]	Peptide	Sequence	Structure	Type of Study	Remineralization effect
Li 2020 [47]	ID4 and ID8	ID4 (Ac-Ile-Asp-Ile-Asp) ID8 (Ac-Ile-Asp-Ile-Asp-Ile-Asp-Ile-Asp)	β-sheet	In vitro	ID8 showed better potential than ID4 for remineralization of initial caries lesions.
Kwak 2017 [49]	Leucine-rich amelogenin peptide (LRAP)	Not specified	Not specified	In vitro	LRAP has the capacity to promote the linear growth of mature enamel crystals along the c-axis and regulate the size, shape, and orientation, demonstrating a potential for the development of a new approach to regenerate enamel structure.
Ding 2020 [50]	QP5	QPYQPVQPHQPMQPQTKREEVD	Not specified	In vitro	QP5 peptide binds to demineralized enamel and HA, increasing the surface microhardness of dental enamel and favoring a lower loss of minerals.
Wang 2020 [53]	C-AMG	Not specified	β-sheet	In vitro and in vivo	C-AMG facilitated the oriented arrangement of amorphous calcium phosphate (ACP) nanoparticles and their transformation to ordered enamel-like HA crystals and recovered the highly oriented structure and mechanical properties to levels close to natural enamel.
Zheng 2019 [55]	8DSS	DSSDSSDSSDSSDSSDSSDSSDSS	Not specified	In vivo	8DSS demonstrates the regression of enamel demineralization and boosts enamel remineralization in a rat model with a potential comparable to NaF effects.

Figure 2. Understanding the significance of hydroxyapatite in ion exchange of calcium and phosphate for biomimetic remineralization. Its structure is mainly composed of repeating units of calcium phosphate; the presence of hydroxyl ions gives it unique biochemical properties, which makes it biocompatible and bioactive. The release of calcium and phosphate ions favors remineralization of tooth enamel, which is why many synthesized peptides are based on mimicking the function of this compound.

4. Stimulation of the Dentin-Pulp Complex

One of the current challenges in dentistry is the development of biomaterials that present adequate adhesion to collagen fibers and in turn promote the proliferation and differentiation of the cells of the dentin-pulp complex [56]. Collagen is a fibrous protein that constitutes a major part of the extracellular matrix of dentinogenesis providing strength, stability, and protection to this highly structured complex [57,58]. Collagen is a protein found in various tissues of the human body, including skin, bones, tendons, and teeth.

Regarding the function of collagen as a dental remineralizer, it is important to understand its role in tooth structure and dental mineralization in general. Collagen is pivotal in maintaining structural integrity and creating a vital biological setting for cellular interactions. It contributes to the formation and architecture of connective tissues and is a component of the organic matrix in tooth enamel. Although collagen itself is not a dental remineralizer, it is an essential component of the organic matrix of enamel that provides a supporting structure for hydroxyapatite crystals [59–61].

The proposed synthetic β-sheet peptide ID8 (Ile-Asp-Ile-Asp-Ile-Asp-Ile-Asp-Ile-Asp) promotes a calcium response and self-assembles to induce hydroxyapatite nucleation and intrafibrillar mineralization of collagen, contributing to calcium retention within collagen increasing its hydrophilicity. These peptides have similar regulatory effects or other macromolecules, so it is expected that they can be used as potential candidates in tissue mineralization [62].

Intrafibrillar mineralization of collagen in the "hybrid layer" is a key point in addressing collagen demineralization itself, which interferes with improving the adhesion of current restorations [62,63]. Cloyd et al., proposed a multifunctional peptide that combines a hydroxyapatite-binding peptide (HABP1) with a collagen-binding peptide (TKKLTLRT), evaluated its use for potential direct mineralization within the hybrid layer in dentin and identified intermolecular interactions that enhance the nanomechanical properties and intrafibrillar remineralization in collagen, which could promote sealing of the restorative material–tooth structure interface [63].

Peptides derived from the sequence of dentin phosphoprotein, containing multiple repetitions of the tripeptide aspartate–serine–serine (DSS), exhibit strong binding to calcium phosphate compounds. When immobilized, these peptides can effectively attract calcium

phosphate to polystyrene beads modified with the peptide or to human dentin surfaces that have been demineralized. The affinity on the hydroxyapatite surface is defined by the following sequence: (DSS)n repeats, and although similar repeated sequences-(NTT)n, (DTT)n, (ETT)n, (NSS)n, (ESS)n, (DAA)n, (ASS)n, and (NAA)n [44].

Cell-based tissue engineering promotes microenvironments that facilitate odontogenic differentiation for the regeneration of the dentin-pulp complex. Some proposals in the literature focus on simulating the extracellular matrix or creating peptides that promote improvements in adhesion and rapid vascularization of the pulp tissue [60,61].

Biomaterials for dental pulp regeneration include both natural and synthetic materials. However, synthetic materials must closely mimic natural tissues to achieve biomimetic modifications, ensuring precise control over the temporal and spatial aspects of pulp-dentin complex regeneration. To achieve this objective, two primary strategies are utilized: stem cell transplantation and stem cell homing [57]. Notably, the stem cell homing strategy eliminates the need for in vitro stem cell isolation and cultivation, rendering it a promising clinical approach.

The continuous work on the biological characteristics of dental pulp stem cells offers a broad perspective in regenerating the pulp-dentin complex, maintaining histological characteristics similar to native tissue, physiological functions focused on pulp innervation, and immunity [57]. Utilizing synthetic proteins for dental pulp regeneration involves a strategic use of signaling molecules and the activation of vital signal transduction pathways, such as Wnt/β-catenin and BMP/Smad. These pathways enhance various stem cell functions, such as migration, proliferation, odontoblastic differentiation, and the promotion of nerve and blood vessel regeneration [58] (Figure 3).
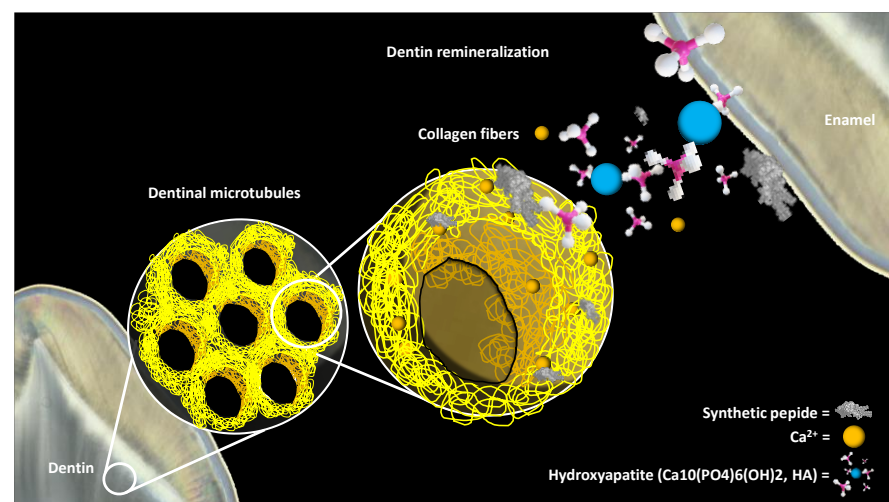

Figure 3. Dentin-pulp complex biomimetic remineralization by synthetic peptide. The yellow diagrams presented in the graph represent the dentinal tissue. Intrafibrillar collagen mineralization plays a crucial role in improving the adhesion of current dental restorations. To achieve this, the use of synthetic proteins that are incorporated into collagen was investigated. These synthetic proteins promote a calcium response and, upon self-assembly, induce the formation of hydroxyapatite, which favors the remineralization of the affected tissue, increasing the protection of the dentin-pulp complex.

The characteristics of the studies included in the stimulation of the dentin-pulp complex section are summarized in Table 3.

Table 3. AMPs related to stimulation of the dentin-pulp complex.

Study and Year [Reference]	Peptide	Sequence	Structure	Type of Study	Dentin-Pulp Complex Effect
Han 2021 [56]	TVH-19	TKRQQVVGLLWHLLHHLLH-NH$_2$	Not specified	In vivo	Amounts of 10 to 200 μg/mL of TVH-10 did not show cytotoxic features or any difference in the proliferation when compared with the untreated hDPCs, after 1, 2, and 4 days of incubation. TVH-19 induces differentiation of hDPSCs, promotes tertiary dentin formation, relieves inflammation, and reduces apoptosis, indicating the potential applications in indirect pulp capping.
Xia 2020 [60]	RGD and VEGF	Not specified	β-sheet	In vivo	The results of this study show the survival and differentiation of dental pulp stem cells (hDPSCs) in promoting regeneration of the dentin-pulp complex in partially pulpotomized rat molars over a period of 28 days. RGD and VEGF mimetic peptide epitopes provided a 3D microenvironment for hDPSCs which enhanced angiogenic and odontogenic differentiation.
Li 2022 [62]	ID8	Ile-Asp-Ile-Asp-Ile-Asp-Ile-Asp	β-sheet	In vitro	The calcium-sensitive self-assembly ability of ID8 gives it the inherent advantage of forming polyelectrolyte-calcium complexes easily, these peptides are expected to be potential tools for biomimetic mineralization of collagen.
Cloyd 2023 [63]	HABP1 CBP	TKKLTLRT	Not specified	In vitro	The engineered peptide demonstrated intermolecular interactions that enhanced nanomechanical properties and offer a promising route for collagen intrafibrillar remineralization.

5. Bone Regeneration

Bone regeneration in the field of dentistry has experienced significant advances thanks to the application of synthetic proteins [64]. These innovations have become an essential pillar that has enabled success in dental procedures that require the restoration and repair of bone tissue. In the context of bone regeneration in dentistry, various peptides have been investigated for their potential to promote bone tissue formation or accelerate healing in dental procedures [65].

Currently, various growth factors (GFs) have been assessed, such as bone morphogenetic protein (BMP), platelet-derived growth factor (PDGF), and parathyroid hormone (PTH) peptides, to promote local bone regeneration [66]. However, within the most relevant and extensively studied proteins to date, the role of BMP stands out in the field of medicine. Such proteins have received approval from the United States Food and Drug Administration (FDA) for their use in specific clinical applications [67], with the approval for maxillofacial and oral surgery in 2007 and commercially available as Infuse Bone Graft (Medtronic, Parkway, Minneapolis, MN, USA). These osteoinductive proteins constitute a set of molecules derived from a non-mineralized bone matrix. They have the potential to induce differentiation of pluripotent mesenchymal cells into osteoprogenitor cells [66]. A small number of this group of proteins have osteoinductive properties. Schwarz et al., evaluated in vitro the influence of recombinant human growth and differentiation factor 5 (rhGDF-5) and natural bone mineral (NBM) coated with recombinant human bone morphogenetic protein 2 (rhBMP-2) as potential candidates for guided tissue regeneration, concluding that both proteins exhibited potential for use in this procedure [68]. Dupoirieux et al. reported on the effect of dimeric rhGDF-5 and its monomeric form rhGDF-5C465A on the bone healing of cranial defects in rats [69]. Furthermore, an evaluation was carried out to examine the impact of rhGDF-5 on bone regeneration in the membrane surrounding titanium dental implants placed in the mandibles of beagle dogs [70]. Lately, in the realm of periodontology, an investigation was conducted to evaluate maxillary sinus augmentation using bio-implants containing bone morphogenetic protein and autogenous bone in a rabbit model [71]. In terms of viable proteins, research has also emphasized the application of composite pedicled muscle flaps and rhBMP-7 for mandibular reconstruction, resulting in positive results [72]. It is crucial to highlight that science is exploring not only proteins but also small peptides because they are easy to synthesize and manipulate while exhibiting low immunogenic activity. In both in vivo and in vitro studies, the peptide derived from cementum protein 1 (CEMP-1-p1) was examined. It has shown a significant capacity to promote the proliferation and differentiation of human periodontal ligament cells, encouraging a phenotype resembling mineralization. This finding has the potential to reshape our understanding [73].

The importance of maintaining an adequate balance of minerals such as calcium and phosphorus in the regeneration process is attributed to the critical role of the parathyroid hormone. This hormone plays an essential function in regulating homeostasis, which has a direct impact on bone health [65]. PTH is a natural protein consisting of 84 amino acids, from which a 34 amino acid peptide has been evaluated, showing activity like the full protein. PTH 1-34 stimulates the proliferation and differentiation of osteoblasts and prevents their apoptosis [74]. Aside from the PTH peptide (1-34), related proteins such as PTHrP (1-36) and the recently developed abaloparatide peptide may exhibit distinct anabolic effects in individuals with osteoporosis, making them relevant candidates for application. This is particularly relevant due to the significant correlation between osteoporosis and tooth loss [75].

One of the most recent studies in dentistry, conducted by Hsu et al., focused on evaluating three peptides: intermittent PTH (1-34), PTHrP (1-36), and abaloparatide. These peptides influenced the expression of genes related to bone formation in cementoblast cells, including Bsp, Col1a1, Opg, RANKL, and Mmp13. PTH (1-34) demonstrating an immediate effect by increasing COL1A1 protein levels after treatment, while abaloparatide showed a delayed increase in COL1A1, observed 18 h after treatment. In contrast, PTHrP had no

significant impact on the expression of COL1A1 in these cells. Moreover, microcomputed tomography revealed that PTH (1-34) injections led to heightened mineral density in the molar roots of mice. In contrast, abaloparatide injections increased mineral density in both incisors and molar roots in mice [76].

These discoveries indicate the potential for the development of treatments focused on enhancing both dental and bone health. Such advancements could carry substantial implications for dental practitioners and their patients (Figure 4). The characteristics of the studies included in the stimulation of the dentin-pulp complex section are summarized in Table 4.
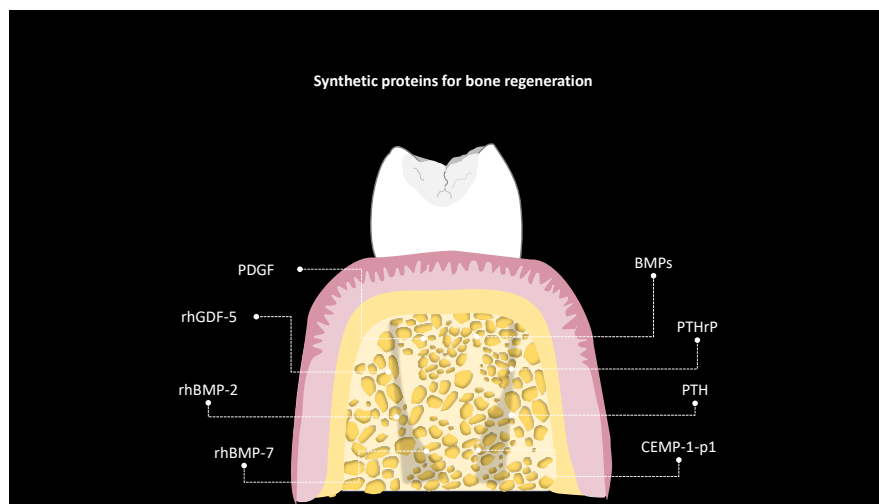

Figure 4. Synthetic proteins for bone regeneration. This illustrative diagram showcases a collection of synthetic proteins and peptides that hold notable importance in the realm of bone regeneration within the field of dentistry—synthetic proteins for bone regeneration. Among these compounds, Bone Morphogenetic Proteins (BMPs), Abaloparatide, PTHrP (Parathyroid Hormone-Related Protein), PTH (Parathyroid Hormone), the peptide CEMP-1-p1 (Cementum-Related Peptide), rhBMP-7 (Recombinant Human Bone Morphogenetic Protein-7), rhBMP-2 (Recombinant Human Bone Morphogenetic Protein-2), and rhGDF-5 (Recombinant Human Growth/Differentiation Factor-5) are featured. Additionally, Platelet-Derived Growth Factor (PDGF) is included. Collectively, these substances represent pivotal elements in the promotion and enhancement of bone regeneration within the field of dentistry.

Table 4. AMPs related to Bone regeneration.

Study and Year [Reference]	Peptide	Sequence	Structure	Type of Study	Bone Regeneration Effect
Schwarz 2009 [68]	rhBMP-2	Not specified	Not specified	In vivo	All treatment procedures investigated supported bone regeneration at 24 weeks; rhBMP-2 could have the potential to improve healing outcome, particularly during the early stages, and could therefore be considered as a potential candidate for guided tissue regeneration.
Dupoirieux 2009 [60]	rhGDF-5	Not specified	Not specified	In vivo	The data of this study show that dimeric GDF-5 and its monomeric form rhGDF-5C456A have a positive effect on membranous bone growth in vivo. The newly formed bone in the specimens was composed of trabecular bone with abundant vascularization and bone marrow.
Lee 2010 [70]	rhGDF-5	Not specified	Not specified	In vivo	Sites implanted with rHGDF-5/β-TCP exhibited greater enhanced cementum and bone formation compared with β-TCP and sham-surgery controls in one-wall intrabony defects in dogs. rHGDF-5/β-TCP has a greater potential to support periondontal regeneraton.
Ayoub 2007 [72]	rhBMP-7	Not specified	Not specified	In vivo	Histologically, the induced bone regenerate showed maturation from woven to lamellar bone. This study confirms that bone can be formed within a muscular 'scaffolding' at the site of a created defect.
Correa 2019 [73]	CEMP-1-p1	MGTSSTDSQQAGHRRCSTSN	Not specified	In vitro and in vivo	An amount of 5 μg/mL of CEMP-1p1 was an optimal concentration to promote cell proliferation. Histomorphometry evaluation indicated that the peptide promoted new bone formation at 30 and 60 days. The bone formation in vivo was demonstrated with a rat model, which is defined as the area of bone that naturally regenerates throughout the life of the animal and is <10% of the initial defect size. These results show osteoinductive properties which enhanced the physiologic formation and maturation of newly formed bone.

6. Challenges and Limitations

Synthetic biology holds great promise for addressing current clinical needs. Its focus on improving biological functions and gene expressions in diverse cellular and molecular areas has contributed to the engineering of new peptides; however, most advances within this field are still controlled in vitro and in vivo settings [77].

Each clinical area still has limitations identified by researchers within these controlled settings, with most experts finding that further studies are required to assess cytotoxic resistance before an effective commercial product can be developed [29]. To know the appropriate concentrations of peptides designed to counteract antimicrobial actions is necessary, as it has been identified that bacteria can exhibit tolerance in the production of extracellular polymeric matrix as a defense mechanism [30], suggesting the importance of monitoring bacterial resistance testing to avoid these mechanisms.

It has been recognized that the interaction of the constructed peptides and the oral environment to which they are subjected is highly complex, due to the self-regulatory mechanisms of each organism, which is why it is suggested that toxicity tests be carried out using various animal models before initiating controlled clinical trials [43].

Another limitation identified is that most in vitro models work with monospecies biofilm, and when a highly communicated and structured multispecies biofilm has been identified in the organism, the design of experiments based on this organization could suggest the effect within a more biomimic environment [35].

Although refinements of biological responses using bioengineering concepts continue to be made, there are also controversies surrounding the processing of these synthetic proteins, including waste management, alteration of the natural gene pool, and the possibility that microorganisms designed for specific functions may have unwanted side effects in the environment [78].

7. Ethical Considerations and Regulatory Aspects

The development of synthetic biology is still in an emerging form; however, its rapid development favors a more accessible and less expensive technology, which leads to the need to reinforce bioethical standards among researchers. The promotion of a continuous and renewed dialogue between the scientific community and social experts is suggested to strengthen the code of ethics. To encourage participation and accountability in this area, there are several scientific committees in Europe, the USA and Singapore; however, the implementation of global governance would allow for greater ethical commitment in the various nations developing these technologies.

The International Genetically Engineered Machine (iGEM) competition has included debate and reflection upon the safety and security needs for applications of synthetic biology. These efforts indicate a willingness by the community to engage with the social sciences; yet the next step requires more formal, permanent, and recurring collaboration between stakeholders in synthetic biology's physical and social sciences [78]. Currently, no risks related to synthetic biology have been identified; most ethical concerns are based on the need to implement regulations governing these techniques to reduce the potential abuses associated with them in the future.

8. Future Perspectives

The convergence of biotechnology and bioactive materials is reshaping the landscape of dentistry, offering an array of transformative possibilities. Strategies for the regeneration of the oral cavity like rehabilitation, and tissue reconstruction, can be simulated via bio-nanomaterials to recover the function and aesthetics of these areas. The appropriate cells for this connective tissue arrangement need to meet flexibility, mobility, and be dynamic. Improving biomimetic effects by interactions with proteins or inorganic substances, is currently a subject of extensive research.

Dentistry is no longer confined to a reactive approach but is increasingly adopting proactive, minimally invasive, and regenerative strategies that hold great potential for

enhancing oral health. Innovations in bioactive materials, particularly synthetic proteins, and peptides, are revolutionizing periodontal disease management and tissue regeneration in dentistry.

Current dentistry based on biology and supported by new technological developments and even accompanied by artificial intelligence, favors the creation of new materials that facilitate advanced therapies for biomimetic remineralization, dental pulp regeneration, specific cell differentiation, and improved dental implant procedures, promising superior clinical outcomes aspiring to a brighter future in this area.

With an emphasis on prevention and early intervention, bioactive materials are re-defining oral health maintenance, offering proactive solutions to dental pathologies. Interdisciplinary collaborations are propelling dental research and clinical practice into uncharted territories. The fusion of biochemistry, materials science, and dentistry is fostering groundbreaking research approaches and translating innovations from the laboratory to chairside applications.

Molecular and genetic profiling will enable tailored treatment strategies, optimizing regenerative and restorative interventions. Dental materials are on the cusp of a significant transformation, with bioactive and highly biocompatible materials that will redefine the standards for dental prosthetics and implantology. Dental practitioners will need to adapt to these evolving paradigms through continuous education and training, ensuring they can navigate emerging technologies and techniques. Embracing these innovations will position dentists at the forefront of delivering state-of-the-art patient care. While the scientific potential is promising, addressing the challenges of affordability and accessibility remains paramount. Ensuring that these advanced treatments and materials are economically viable and widely accessible is crucial for equitable dental healthcare.

9. Conclusions

In vitro and in vivo studies suggest that engineered peptides may have beneficial effects on decreasing bacterial biofilm formation and remineralization of incipient carious lesions, favoring cell adhesion and improving tissue engineering for the periodontium. However, it is important to control toxicity testing in animal models before proceeding to clinical trials, and this highlights the importance of addressing biosafety and ethical risks to further advance research on these technologies in dentistry.

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