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Review

Progress in the Application of Biomimetic Mineralization for Tooth Repair

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Abstract: The tooth, including enamel and dentin, is a prominent biomineral that is produced by the biomineralization of living organisms. Although the mechanical performance of the tooth is outstanding, caries easily develop in a complex oral environment. The analysis of the chemical composition and the relationship between the mechanical properties and the structure is of great importance in solving caries. In this review, the multilevel structure and mechanical properties of enamel and dentin are briefly introduced, along with caries formation and the limitations of clinical dental restoration. Furthermore, the progress of the application of a wide range of biomimetic strategies for tooth remineralization is highlighted, including the use of calcium phosphate ionic clusters to construct the mineralization front, ensuring the oriented epitaxial growth of enamel crystals and replicating the complex structure of the enamel. Moreover, compared with the current clinical treatment, in which the resin composite and glass ionomer cement are the main repair materials and the high incidence of secondary caries leads to imperfect restorations, the remineralization tactics could achieve excellent repair effectiveness in reconstructing the complicated structure, restoring mechanical strength and gaining permanent repair. A basic understanding of enamel and dentin, their potential for restoration, and hopeful prospects for tooth repair that can be applied in the clinical setting, not just in the laboratory, is provided by this review.

Keywords: biomimetic mineralization; tooth; enamel; dentin; hydroxyapatite

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1. Introduction

The tooth is a prominent biomineral, a highly mineralized tissue consisting of enamel, dentin, and pulp from the outside to the inside. With the development and application of advanced technologies, the chemical composition and complicated structure of the tooth have been investigated in depth, allowing us to grasp the relationship between the mechanical properties and the structure. The tooth, in particular the enamel, has a robust material strength that can withstand hundreds of thousands of chewing movements. However, teeth are susceptible to chemical attack when exposed to various acidic environments, leading to caries [1,2]. Dental caries is considered the oral disease with the highest prevalence rate, and dental caries is ranked by the World Health Organization (WHO) as one of the three major diseases threatening human health, along with cancer and cardiovascular diseases [3]. Oral diseases such as pulpitis and periapical periodontitis can be caused by untreated dental caries, and they can also exacerbate systemic chronic diseases such as cardiovascular and cerebrovascular disorders, posing a threat to overall health [4]. Therefore, achieving long-term stable dental restoration has become an increasingly urgent goal in this field.

Given the structure and properties of teeth, the most promising approach is to achieve a unity of materials, structure, and mechanical properties through biomimetic mineralization.

2. Structure and Properties of Teeth

The hard tissue of the tooth, a fundamental biological mineral structure, consists of dentin, enamel, and cementum. Dentin is the main structure of the tooth, and enamel, as the hardest tissue in the body, covers its surface. Enamel and dentin are discussed specifically below.

2.1. Enamel

Enamel is composed of 96% minerals (mainly hydroxyapatite (HAP)), 3% water, and 1% organic matter [5]. Because of its highly organized structure and superior material performance [6], enamel serves as a protective layer to protect the tooth from the harsh conditions of the oral environment, including mechanical stress [7], temperature changes [8], and acid attacks [9].

2.1.1. Hierarchical Structure of Enamel

Enamel has a complex hierarchical structure from the nanoscale to the micron scale (Figure 1A), which is currently divided into seven levels [10,11]. At the nanoscale, the basic unit is highly ordered, with parallel HAP nanowire crystals that are 50–70 nm in width, 20–35 nm in thickness, and very long, with the longest ones spanning the entire thickness of the enamel.

At the micron scale, HAP nanowire crystals (on the order of 10^4) bind tightly together to self-assemble into an enamel rod with a diameter of approximately 4–6 μm. These enamel rods are further arranged in an orderly manner and woven together with the internal enamel rods (interrods) to form the basic assembly unit of the enamel. Both the enamel rods and the interrods are primarily composed of HAP crystals, but the orientation of the c-axis of the crystals varies, with an angle of approximately 60 degrees [12].

Organic matter, mainly the product of protein hydrolysis [13], exists between the enamel rods and the interrods, forming the enamel rod sheath, which plays a critical role in the mechanical properties [14]. Under a scanning electron microscope (SEM), the enamel shows a lock-hole-like structure [11] and a fish-scale-like structure [12] after acid etching (using acids such as 30%–37% phosphoric acid, citric acid, lactic acid, and 3% nitric acid [15]). The formation of these unique structures is mainly due to the different acid resistance levels of the crystals in the enamel rods and the interrods, which is mainly because the gaps between the enamel rods contain higher protein content [11].

In addition, the orientation of the enamel rods varies within the enamel layer. For example, in the outer one-third of the surface layer of the enamel, the enamel rods are parallel to each other, arranged in a radiating manner, and perpendicular to the occlusal surface of the tooth [16]; in the remaining two-thirds of the inner layer, the enamel rods are arranged in a curved and intersecting pattern.

2.1.2. Mechanical Properties of Enamel

Enamel, as a representative biomineral in nature, has a unique multilevel structure that gives it excellent mechanical strength and wear resistance, and it is the hardest biocomposite found thus far in biominerals to date [13,17,18]. The hardness of enamel ranges from 3 to 6 GPa, and the elastic modulus is between 70 and 115 GPa [19]. The hardness and elastic modulus of the enamel vary at different locations, with the enamel closest to the surface having the highest hardness and elastic modulus (Figure 1B,C). From the outer surface to the junction between the enamel and dentin (dentin-enamel junction, DEJ), the hardness and elastic modulus gradually decrease [20].

This variation occurs because the chemical composition of the enamel affects both the structure and the mechanical properties of the enamel. Differences in the levels of proteins [21–23] and inorganic ions (such as magnesium ions [24], carbonate ions [25],

and fluoride ions [26]) can lead to differences in the mechanical properties of the enamel. Hoffman et al. found that hypomineralized enamel has significantly lower hardness and elastic modulus than normal enamel due to the higher protein content in hypomineralized enamel [22]. Because the enamel rod sheath contains a higher protein content than that of the enamel rod, the enamel rod has a higher hardness and elastic modulus [23].

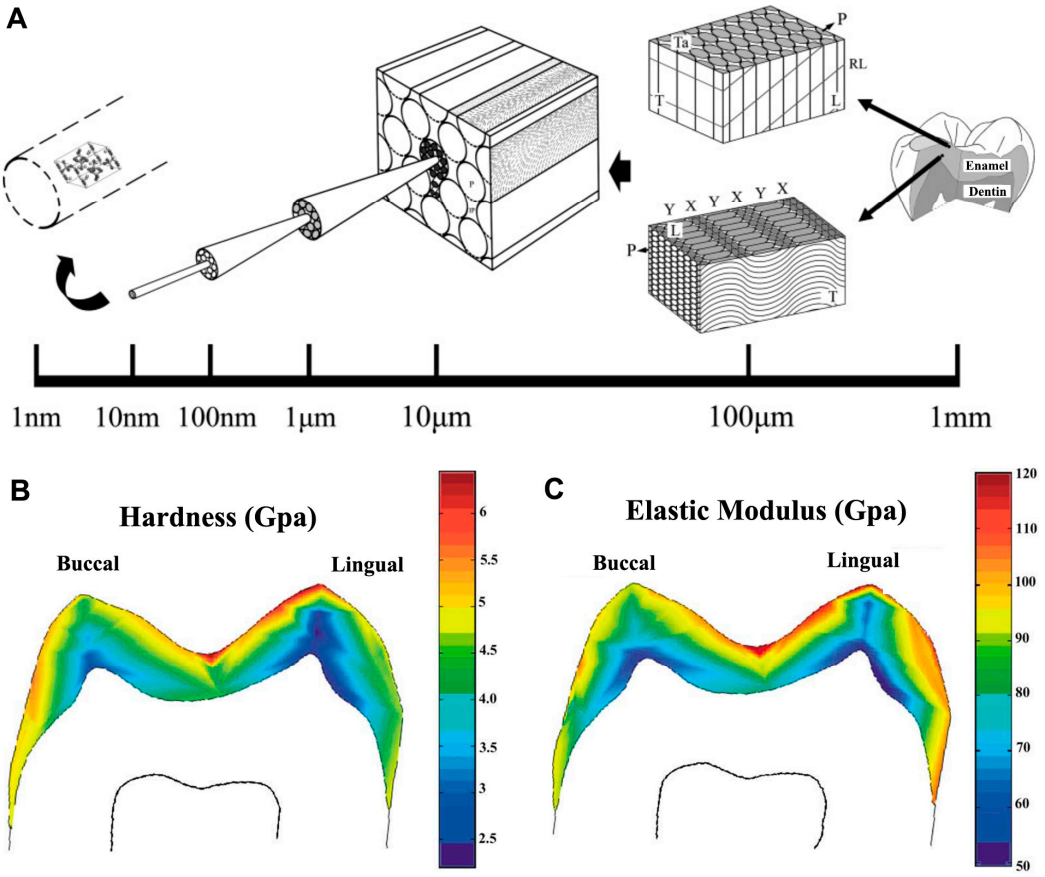

Figure 1. (**A**) Schematic illustration of the hierarchical assembly of the enamel structure, from the millimeter to the nanometer scale, reprinted with permission from Ref. [11]. 2007, John Wiley and Sons. Hardness (**B**) and elastic modulus (**C**) of the enamel as determined by nanoindentation, reprinted with permission from Ref. [19]. 2002, Elsevier.

2.2. Dentin

2.2.1. The Structure of Dentin

Dentin, the main hard tissue of the teeth, is covered by enamel in the crown and cementum in the root. Dentin is mainly composed of dentin tubules, odontoblastic processes, and intercellular substance [27,28]; it consists of approximately 70% inorganic substance, primarily HAP, 20% organic substance, and 10% water by weight. The dentin tubules and odontoblastic processes traverse the entire dentin, while the mineralized collagen fibers within the intercellular substance intertwine to form a network that constructs the dentin scaffold (Figure 2A–E). Non-collagenous proteins (NCPs) such as dentin phosphoprotein (DPP), dentin sialoprotein (DSP), and dentin matrix protein-1 (DMP-1) play a crucial role in dentin formation [29–33]. Under the regulation of NCPs, the multi-level ordered organic–inorganic composite structure is formed as HAP crystals grow along the c-axis of the collagen fibers [34,35].

2.2.2. Mechanical Properties of Dentin

Enamel and dentin have different mechanical properties and complement each other in maintaining the mechanical stability of teeth. The hardness of dentin is lower than that

of enamel due to its high organic content and the presence of water in the dentin tubules. This endows dentin with a certain degree of elasticity that provides a favorable buffering environment for enamel [36,37].

Dentin has an average hardness of approximately 0.8 GPa and an elastic modulus of nearly 20 GPa [38]. The mechanical properties of dentin are anisotropic and are influenced by several factors, including the location, density, and direction of the dentin tubules, the orientation of the collagen fibers, and the average density of the mineral phases [39]. Under evaluation with atomic force microscope (AFM), the microstructural mechanical properties of different parts of dentin vary (Figure 2F,G); the elastic modulus of highly mineralized peritubular dentin reached 40 GPa, while that of weakly mineralized intertubular dentin was lower [40]. Near the pulp, the hardness of peritubular dentin exhibited a noticeable gradient as the mineral content changed.

The mechanical properties of dentin vary depending on its mineral content. Microscopically, the mineralization of dentin can be categorized into intrafibrillar mineralization and extrafibrillar mineralization based on the relative position of HAP and the collagen matrix. Intrafibrillar mineralization plays a crucial role in determining the mechanical properties of dentin at the nanoscale [41,42]. It also helps to prevent the degeneration and degradation of collagen when exposed to external stimuli, which is essential for maintaining the integrity of dentin.

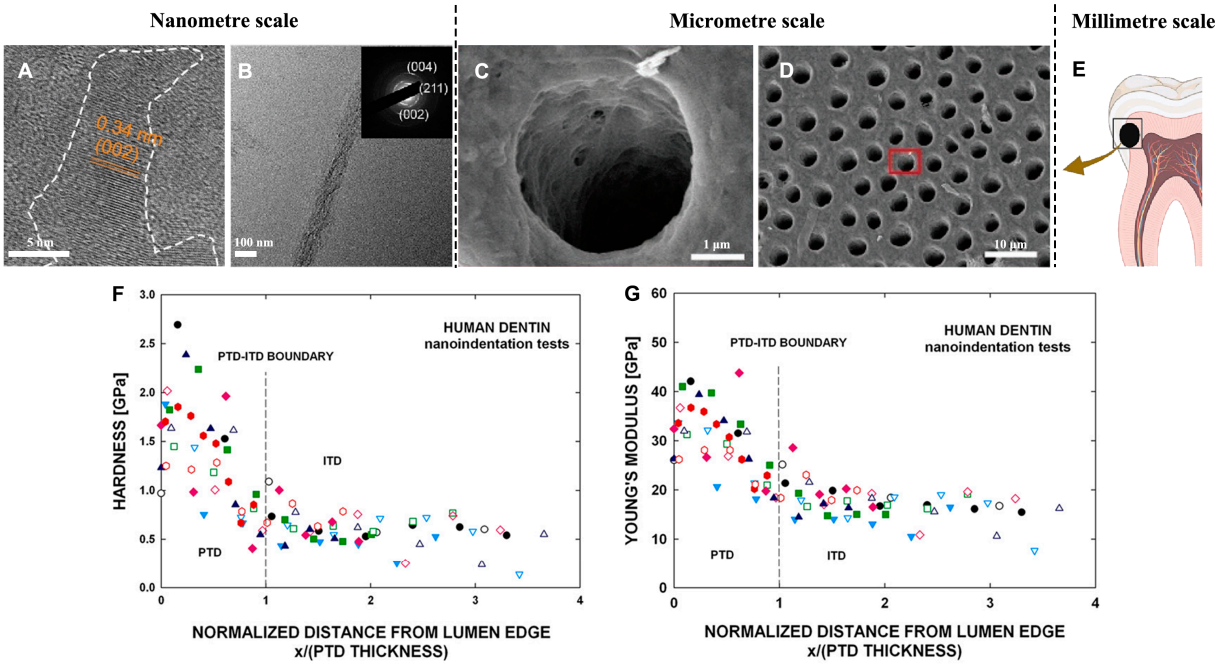

Figure 2. (**A**) High-resolution transmission electron microscopy (HRTEM) image of mineralized collagen fibrils. (**B**) TEM image of mineralized collagen fibrils. Inset: selected area electron diffraction (SAED) pattern, reprinted with permission from Ref. [43]. 2023, Elsevier. (C) High-magnification SEM images of the regions marked with red squares in (**D**). (**D**) SEM image of dentin surface and dentin tubules, reprinted with permission from Ref. [44]. 2022, Elsevier. (**E**) Schematic image showing the anatomy of the tooth. Hardness (**F**) and modulus (**G**) results for dentin plotted vs. distance from lumen (PTD, peritubular dentin; ITD, intertubular dentin), where the different colors and shapes represent different dental tubules, reprinted with permission from Ref. [40]. 2011, Elsevier.

3. Tooth Restoration

3.1. Dental Caries

Dental caries is mainly caused by an imbalance between the mineralization and demineralization of tooth minerals [1]. Normally, the gain and loss of minerals are balanced. However, acid-producing bacteria form dental plaque on the tooth surface and produce

large amounts of organic acids. Since the acids are not easily diluted by saliva, the pH value of localized areas of the enamel surface drops significantly. When the pH falls to a certain level (below 5.5, according to in vitro experiments [45]), the calcium phosphate in the dental enamel dissolves, leading to demineralization.

$$Ca_{10}(PO_4)_6(OH)_2(s) + 8H^+ (aq) \rightleftharpoons 10Ca^{2+}(aq) + 6HPO4^{2-}(aq) + 2H_2O \quad (1)$$

According to the equilibrium chemical equation for the dissolution of HAP, the dissolution of HAP crystals is related not only to the pH but also to the concentrations of calcium and phosphate ions. As acidic substances dissolve the enamel, these ions are gradually depleted, causing the pH to rise. Saliva is oversaturated with calcium and phosphate ions and then forms calcium phosphate crystals on the enamel surface, a process known as remineralization. If the amount of demineralization is equal to the amount of remineralization, dental caries will not form in the enamel. However, if the amount of demineralization exceeds the amount of remineralization, dental caries will slowly begin to form.

3.2. Clinical Therapeutic Strategies and Their Limitations

The main materials in clinical practice for the direct restoration of dental hard tissues are glass ionomer cement and composite resin. Glass ionomer cement is primarily composed of aluminosilicate glass, calcium fluoride, polyacrylic acid, and others, and has excellent adhesion and biocompatibility. It is commonly used in clinics for the repair of root caries and deciduous caries defects, and its fluoride content allows for the slow release of fluoride ions, which helps to prevent secondary caries [46]. However, its mechanical properties are not suitable, and it lacks relative aesthetics. Therefore, it is less commonly used on teeth that are subject to masticatory pressure or require high aesthetics [47]. Composite resin consists of two phases: an organic resin matrix and an inorganic/organic filler. The organic resin matrix phase is made from a mixture of multifunctional monomers and light-sensitive initiators, while the inorganic/organic filler phase contains micro/nano-sized fillers, which are mainly used as reinforcement [48]. Due to its excellent aesthetics, mechanical properties, and wear resistance, it has become the material of choice for the direct restoration of dental hard tissue defects in clinical practice, but the issue of mechanical properties remains [49].

Despite the widespread use of composite resin materials for the direct restoration of dental hard tissue defects, flaws such as microleakage still pose challenges for the permanent restoration of dental hard tissues in clinical practice. Evidence-based medical studies show that the success rate of direct composite resin restorations after 10 years is only 68.7% [50], with the main reasons for failure being secondary caries and fractures of the restoration caused by microleakage [51]. This is mainly because these materials differ greatly from teeth (whose main component is calcium phosphate minerals) in terms of their chemical composition and structure, resulting in significant differences in physical and chemical properties (such as the coefficient of expansion, hardness, and elastic modulus). As a result, current materials are unable to meet the clinical demand for the long-term stable restoration of dental hard tissue defects, necessitating the development of new restorative materials that have no microleakage and tightly bonded interfaces.

4. Biomimetic Mineralization for the Remineralization of Enamel and Dentin

In clinical dentistry, traditional remineralization is achieved by using fluoride to enhance the deposition of calcium phosphate on the enamel surface, which is called an acid-resistant layer of fluorapatite. Despite this method having been successful in hardening enamel, the structure of this mineral layer is disordered and loose, and distinct from the natural enamel [52]. Inspired by the process of natural enamel formation, strategies of biomimetic remineralization have been proposed and developed for several decades [53]. These strategies include simulating in-body mineralization conditions, such as the enamel disks immersed in simulated body fluid, to mimic the function of the proteins involved in the biomineralization of the tooth [54,55], and constructing a mineralization front similar to that observed during the formation of calcified tissue [56]. These methods allow the

regrowth of HAP crystals on the enamel surface in an attempt to replicate the complex structure of the tooth and restore its mechanical properties.

During enamel remineralization, controlling the nucleation and growth of enamel crystals is of great importance and full of challenges. In this process, the crystallization of HAP follows the rules of the classical nucleation theory (CNT), which are well described in ref. [57]. Normally, researchers could add some active molecules or inorganic ions to regulate the kinetic pathway, creating a condition to ensure the oriented crystallization and obtain a layer of minerals with a well-organized structure on the enamel surface. This would involve the heterogeneous nucleation of HAP, where the calcium and phosphate ions nucleate on an existing crystal and have a lower nucleation barrier than homogeneous nucleation. An important factor, the interfacial interaction parameter, m, which is strongly dependent on the supersaturation, is crucial for the formation of ordered crystals [58,59]. Based on these mechanistic understandings, many remineralization approaches have been developed and some have achieved great feats.

4.1. Enamel Remineralization

4.1.1. Amelogenin and Amelogenin-Inspired Peptides

Amelogenin, the major protein associated with enamel formation, is secreted by the ameloblasts and accounts for 80%–90% of the proteins in the developing enamel matrix. It consists of 178 amino acids and is rich in proline, glutamic acid, histidine, and leucine. Amelogenin is a hydrophobic macromolecule, but it contains a series of hydrophilic amino acid residues at its C-terminus. In an aqueous solution, the adjustment of the pH and ion strength of the solution can induce amelogenin molecules to assemble into nanospheres [60,61], with a hydrophobic center and a hydrophilic surface. These nanospheres can further self-assemble to form microfibers [62]. These amelogenin assemblies play a key role in controlling crystal orientation and crystal-oriented growth during the enamel formation [63].

Ruan et al. used an amelogenin–chitosan hydrogel to form a mineral layer approximately 15 μm thick on the surface of the enamel (Figure 3A,B). This mineral layer was tightly bonded to the tooth surface and arranged in a bundle-like structure [55]. However, the growth of enamel crystals does not follow the c-axis of natural enamel (Figure 3C). The elastic modulus and hardness of the repair layer differed significantly from those of naturally healthy enamel. Prajapati et al. found that by adding matrix metalloproteinase-20 (MMP-20), the newly grown crystals in the repair layer could have a more uniform orientation and higher crystallinity, significantly improving the elastic modulus and hardness of the repaired enamel [64].

Since the synthesis process of amelogenin and MMP-20 is complex and costly, amelogenin-based peptides are utilized in the biomimetic mineralization of enamel. Ren et al. added hydrophilic segments (TKREEVD) to five Gln-Pro-X repeat sequences (QPYQPVQPHQPMQPQ) to form an enamel protein-derived peptide (QP5). QP5 can promote the long-term remineralization of early dental caries [54]. Ding et al. reported that the combined use of fluoride and QP5 has a potential synergistic effect on the remineralization of dental caries, and the microhardness of the repair layer is significantly improved [65]. However, the shape, size, and orientation of the mineralized layer crystals exhibit notable differences compared to natural enamel. Further research into the logic and efficacy of peptide design is of considerable importance. Inspired by the biocompatible amelogenin-inspired matrix, Wang et al. developed a phase-transited lysozyme film that mimics an N-terminal amelogenin with a central domain combined with a synthetic peptide based on the functional domains of the C-terminal amelogenin. This technology can achieve the growth of well-ordered HAP crystals both in vitro and in vivo, yielding an aligned structure that is nearly identical to that of natural enamel (Figure 3D–F) [66].

Due to the difficulty of obtaining amelogenin, there are significant limitations to its research and application. Currently, a synthetic protein polyamidoamine-amine-type dendrimer (PAMAM) can be successfully prepared and applied to the repair of the enamel. This

kind of dendritic molecule can self-assemble into spherical macromolecules in solution and then convert into linear macromolecules, a behavior similar to that of amelogenin [67,68]. Based on this, Chen et al. used carboxyl-terminated PAMAM dendritic polymers (PAMAM-COOH) as templates and incorporated fluoride ions to obtain relatively ordered rod-shaped HAP crystals on the enamel surface (Figure 3G), parallel to the long axis of the enamel rods [69]. Similarly, Wu et al. used alendronate-PAMAM-carboxyl polymer (ALN-PAMAM-COOH) to repair the enamel and conducted experiments in mice. Although a large amount of rod-shaped HAP grows on the surface of the enamel, it does not have an ordered structure (Figure 3H,I) [70]. These artificial proteins are adsorbed on the enamel surface and further self-assembled, and then induce the deposition of calcium and phosphorus in the solution to achieve ordered growth. However, there is still a significant gap compared to natural enamel, and in these experiments, the repair effect is better with the synergistic participation of fluoride ions than with PAMAM polymers alone.

4.1.2. Calcium Phosphate Particle-Based Systems

Investigations into nature have shown that the growth of the zebrafish skeleton involves an integral compound known as amorphous calcium phosphate (ACP) [71]. This intermediate, amorphous substance plays a pivotal role in the formation of these integral minerals [72]. In addition, ACP is the initial deposition mineral in the early stages of enamel biogenesis [73,74].

In an attempt to mimic the biomineralization process, a variety of composite materials containing ACP nanoparticles have been extensively used in enamel mineralization efforts [75–80]. Notably, these ACP nanoparticles significantly stimulate enamel remineralization, while their instability limits their practical applicability in clinical treatment remarkably [80]. ACP has a higher solubility than calcium phosphate crystals and is easily transformed into HAP in solution. Several composites capable of stabilizing ACP have found applications in enamel restoration procedures. Phosphorylated chitosan-stabilized ACP can achieve the remineralization of enamel [76]. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) can deposit a layer of calcium phosphate mineral on the surface, which has no structure and poor mechanical properties.

Further regulation of the mineralization process is required to obtain an ordered structure. Li et al. demonstrated that the biologically inspired cooperative effect between glutamic acid and nano-apatite particles can result in the regeneration of enamel-like structures under physiological conditions. Importantly, this feasible method of enamel remodeling ensures that the mechanical properties of the repaired enamel are well preserved [81]. Wang et al. utilized carboxymethyl chitosan (CMC) and ALN to stabilize ACP into nanoparticles. Sodium hypochlorite degraded the CMC-ALN matrix into HAP@ACP core–shell nanoparticles. Finally, these nanoparticles evolved into rod-like ordered apatite crystals under the guidance of 10 mM glycine [82]. However, both oriented and ordered mineral crystals perpendicular to the enamel surface and those parallel to the enamel surface, indicated by red arrows, were found.

Shao et al. introduced an innovative calcium phosphate ion cluster (CPIC) to construct a biomimetic mineralization front and successfully replicated the complex hierarchical structure of enamel [56]. Triethanolamine can stabilize CPICs of approximately 1.5 nm in size in ethanol. It facilitates the construction of a continuous crystalline-amorphous mineralization front on the enamel surface, thus prompting the epitaxial growth of enamel crystals along the c-axis of natural enamel (Figure 3J–L). Remarkably, the repaired enamel exhibits mechanical properties similar to those of natural enamel, with a hardness value of 3.84 $\pm$ 0.20 GPa and an elastic modulus of 87.26 $\pm$ 3.73 GPa. However, this process requires strict non-aqueous conditions to allow the formation of the crystalline-amorphous mineralization front, which limits its practical application in the complex oral environment.

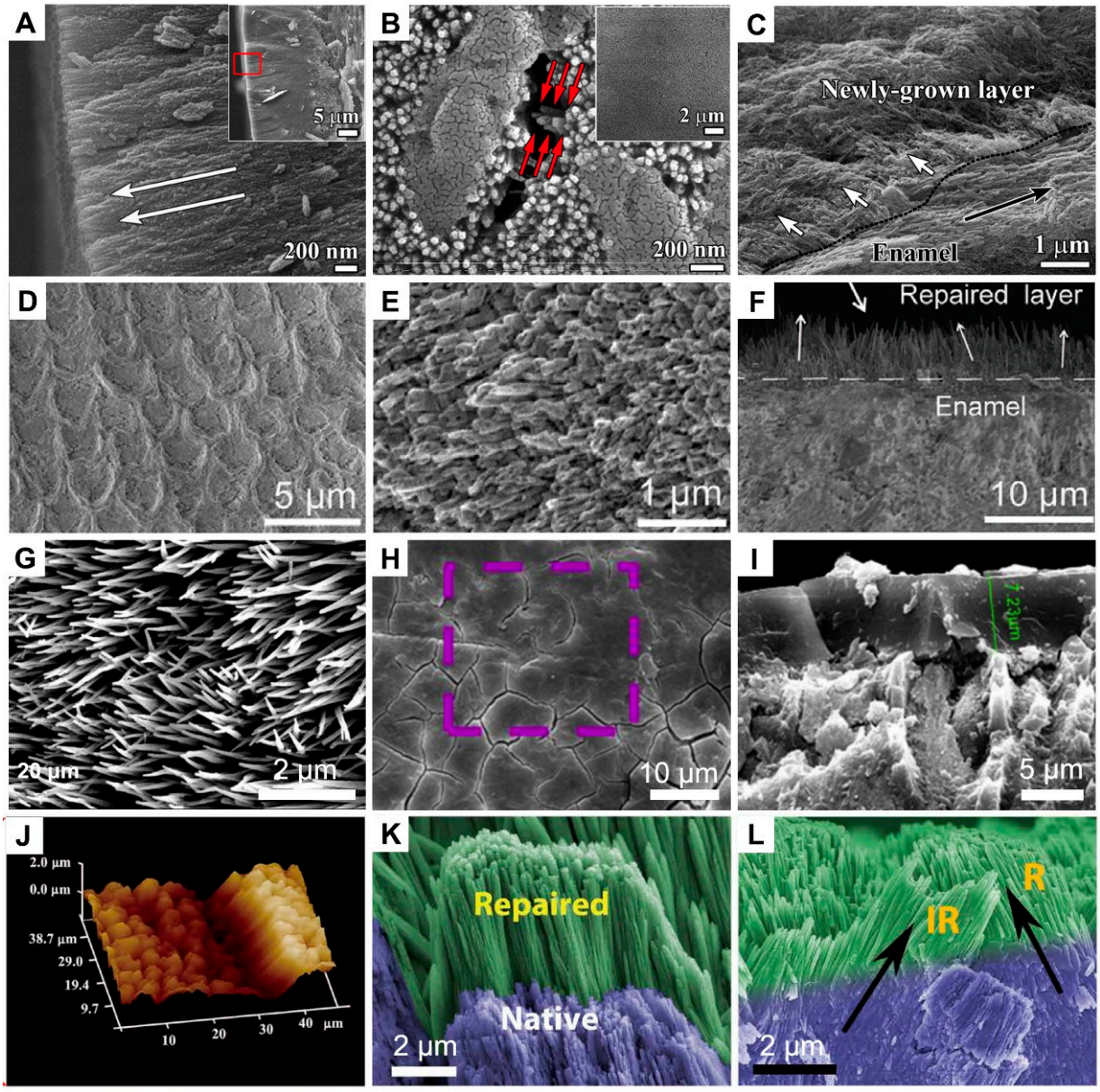

Figure 3. (**A**,**B**) SEM images of an enamel-like layer formed with amelogenin-chitosan hydrogel showed the cross-section and surface of the repaired enamel. White arrows in (**A**) indicate the HAP orientations in the newly grown layer, and the red square rectangle in inset of (**A**) corresponding to (**A**). The red arrows in (**B**) indicate a typical bundle of parallel crystals inside the newly grown layer. (**C**) A newly-grown layer that are marked with white arrows fused to the surface of the natural enamel, reprinted with permission from Ref. [55]. 2014, Elsevier. (**D**,**E**) SEM images of enamel regeneration via PTL/C-AMG and (**F**) the corresponding cross-section, in which the white arrows indicate the orientation of the newly formed minerals, reprinted with permission from Ref. [66]. 2020, John Wiley and Sons. (**G**) SEM images of crystals grown on acid-etched enamel with the PAMAM-COOH and (**H**,**I**) the ALN-PAMAM-COOH, reprinted with permission from Refs. [69,70]. 2013, Elsevier. (**J**) A three-dimensional AFM image and (**K**) an SEM image showing both acid-etched enamel and enamel repaired with CPIC. (**L**) Cross-section of the final repaired enamel, where the black arrows mark the orientation of the enamel crystals, reprinted with permission from Ref. [56]. 2019, AAAS.

4.2. Dentin Remineralization

4.2.1. Proteins or Peptides for Dentin Remineralization

According to previous studies, the deposition of HAP within and between collagen fibers is regulated by NCPs, sharing a common characteristic of high serine and aspartic acid content. These amino acids are rich in carboxyl groups and contribute to the overall negative charge of proteins, which enables them to bind to calcium phosphate via interaction with calcium ions, thereby stabilizing amorphous precursors [33,83]. Consequently, proteins play a crucial role in maintaining the stability of the solution and providing a suitable environment for the subsequent crystallization of the apatite.

The use of natural NCPs is limited by the challenges associated with their extraction, storage, and high cost. However, importantly, the specific sequences present in NCPs play a crucial role in their functionality. Inspired by the structure of NCPs, many biomimetic polypeptide molecules have been designed and synthesized.

The regulatory role of DPP in HAP nucleation and growth is believed to be primarily due to its abundant Asp-Ser-Ser (DSS) repeats. Li et al. used the 8DSS peptide to achieve the remineralization of demineralized dentin [84]. 8DSS can interact with dentin collagen, resulting in the formation of mineral precipitates on the surface and within the dentin tubules. The application of 8DSS has a significant positive effect on the elastic modulus and hardness of demineralized dentin and reduces dentin permeability [85].

DMP-1 can stabilize calcium and phosphorus ions in solution and generate nucleation precursors. Previous research has shown that the amino acid sequence responsible for collagen adsorption in DMP-1 is linked to certain amino acid sequences responsible for HAP adsorption [86]. This association results in the formation of polypeptides that exhibit strong adsorption to type I collagen, regulating mineral deposition, stabilizing amorphous precursors formed by calcium and phosphorus ions, and promoting the nucleation and growth of HAP. Additionally, the intermolecular assembly of functional domains within a β-sheet template is essential in the process of mineral nucleation induced by DMP-1 [87]. A calcium-responsive self-assembled β-tablet peptide, ID8, has been designed to preprocess collagen [88]. The ID8 peptide not only enhances the intermolecular hydrogen bonding but also improves the hydrophilicity of collagen and aids in the retention of calcium within collagen. It has the potential to help regulate collagen mineralization and facilitate the biomimetic mineralization process of early caries [89].

During biomineralization, amelogenin in enamel and NCPs in dentin affect the organization and growth of HAP crystals. The bioactivity of amelogenin at the DEJ interface exhibits a significant level of activity. The amelogenin-inspired design of a self-assembled peptide (P26) has a unique dual remineralization function of enamel and dentin [90]. P26 assembles densely packed spherical nanoparticles along collagen fibrils, facilitating the process of remineralization in dentin defects (Figure 4A–D). The dense accumulation of HAP crystals blocked the tubules and encased the exposed collagen fibril, enhancing mineral recovery and restoring the biological and biomechanical properties of demineralized dentin.

4.2.2. NCP Surrogate

Synthetic analogs of natural NCPs are frequently employed to address the limitations associated with natural NCPs. These analogs are designed to mimic the function of natural NCPs and facilitate mineralization within collagen fibers. The theory of polymer-induced liquid precursors (PILP) is an important approach in the fabrication of mineralized collagen fibers. According to the theory of PILP, the inclusion of these charged polymers in the solution can effectively stabilize the calcium phosphate clusters during the early stages of remineralization, preventing their transformation into HAP crystals [34,91]. The PILP can infiltrate into the collagen fibers via capillary action and subsequently interact with the binding sites on the collagen fibers, and over time the occurrence of crystallization produces the well-organized mineralized collagen fibers. The use of NCPs analogs commonly includes polyaspartic acid (p-Asp), polyacrylic acid (PAA), CMC, polyglutamic acid(p-Glu), PAMAM, and poly(allylamine) hydrochloride (PAH), etc.

Gower et al. used p-Asp to stabilize ACP and achieve the intrafibrillar mineralization of collagen fibers and proposed the PILP mechanism by which collagen mineralization occurs [92]. By using p-Asp as an analog of NCPs, the process of the remineralization of artificial caries was successfully achieved [93], resulting in an increase in mineral content within the collagen fiber, improved crystallinity, and the restoration of mechanical properties in the damaged dentin area [94]. HAP crystals overgrew on the surface of the mineralized collagen and firmly adhered to the tubule wall, resulting in a compact occlusion of the dentin tubules (Figure 4E–H) [44].

Another polyelectrolyte, PAA, which is one of the most widely used in the study of collagen mineralization, can mimic the functions of NCPs and is relatively low in cost. Its molecular weight and concentration could affect the mineralization process; as the concentration of PAA increases and the molecular weight of PAA decreases, the crystallization rate of HAP decreases, thus affecting the mineralization degree of collagen fibers. This is mainly because of the variations in the efficiency of PAA/Ca complexation, as well as the influence of PAA molecular weight migration on precursor adsorption efficiency [95–97].

These polyelectrolytes could interact strongly with calcium phosphate minerals, stabilizing ACP and inhibiting the nucleation of HAP, thereby inducing intrafibrillar mineralization. It seems unlikely that intrafibrillar mineralized collagen fibrils with a higher degree of mineralization could be obtained in a mineralized system without NCP or its surrogates. At present, these organic additives are necessary in the field to ensure that we obtain well-mineralized collagen similar to that observed in bone.

4.2.3. Based on the Promotion of Collagen Remineralization for the Repair of Dentin

In the process of collagen mineralization, in addition to emphasizing the stabilizing effect of NCPs on ACP precursors, attention is also given to the ability of NCPs to bind calcium and collagen, attract ACP precursors, promote their penetration into the fiber, and initiate nucleation at specific collagen sites. Matrix phosphoproteins irreversibly bind to collagen in hard tissue, providing a template function by which negatively charged phosphate esters bind to positively charged interstitial regions in collagen, forming a negatively charged surface [98–100]. The increased local ion oversaturation created on this surface facilitates the nucleation and growth of HAP.

Several phosphorus-containing reagents, such as polyvinylphosphonic acid (PVPA), sodium trimetaphosphate (STMP), and sodium tripolyphosphate (STPP), are used as template analogs to anchor collagen fibers and induce intrafibrillar mineralization. Using PAA and PVPA as dual biomimetic analogues in Portland cement/phosphate-containing solution systems, the remineralization of etched-dentin to a depth of approximately 5 µm has been achieved with interfibrillar and intrafibrillar remineralization [101], as well as the remineralization of the resin–dentin interface [102]. STMP and STPP can be fixed to collagen by chemical phosphorylation and irreversible binding sites for phosphate groups on the dentin surface [103]. When dentin is treated with STMP, HAP forms in the phosphorylated collagen matrix within the fibers, inducing collagen mineralization and achieving artificial caries recrystallization [104].

Other organic biomolecules can also modify collagen fibers and regulate the mineralization of collagen fibers and dentin. Citrate can enhance the wetting effect of ACP on collagen fibers by significantly reducing the interfacial energy between them, and therefore the pretreatment with citrate effectively improved the process of collagen mineralization and dentin remineralization (Figure 4I–L) [105]. Wu et al. found that aminoglycan molecules such as hyaluronic acid can accelerate mineralization by providing more nucleation sites and shortening the induction time of ACP-mediated HAP crystallization [43]. In addition, there are many organic molecules, such as dopamine acrylamide [106], PAA [107], polydopamine (PDA) [108], and CMC [109], which contain chemical groups that can coordinate with calcium ions or have a high affinity for calcium phosphate minerals. This strategy paves the way for researchers to search for reagents that can enhance the mineralization of

collagen fibers, improve dentin remineralization, and have the potential to develop as a clinical therapeutic drug.

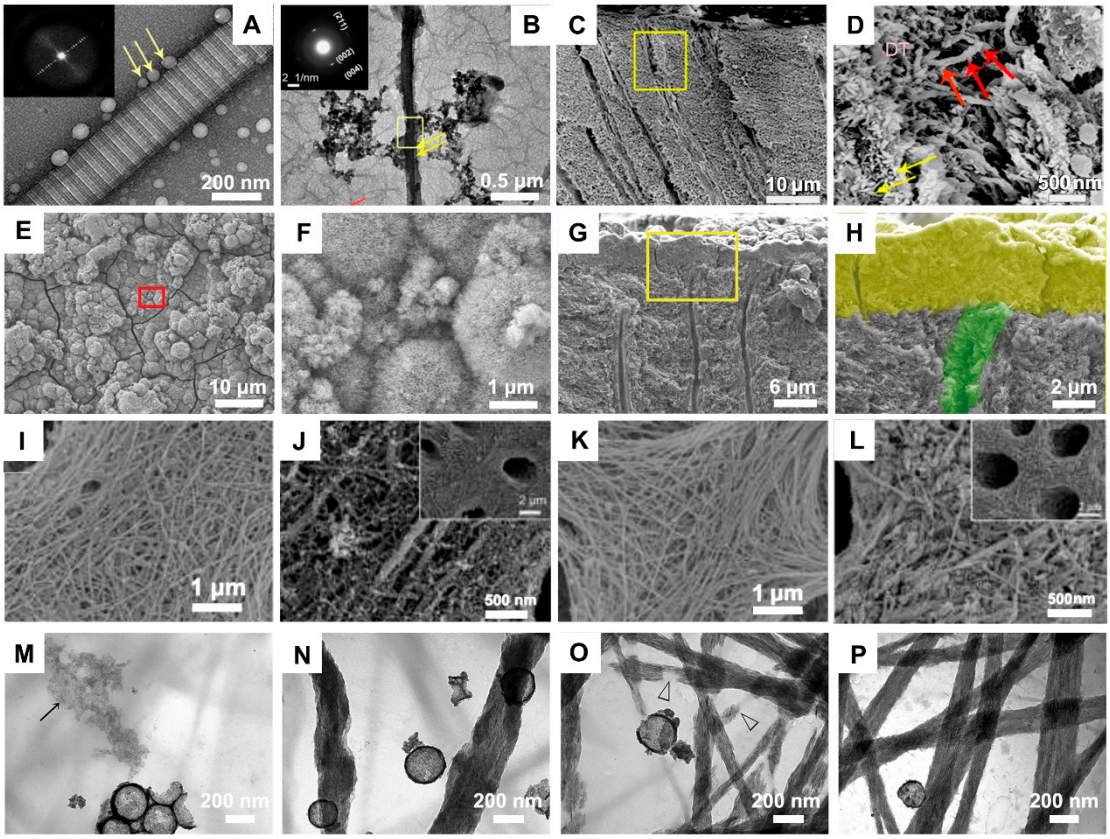

Figure 4. (**A**–**D**) Peptide P26 assembly aligned along the collagen fibril (indicated with yellow arrows in (**A**)) and induced collagen and dentin remineralization (indicated with yellow arrows in (**B**) and red arrows in (**D**)), reprinted with permission from Ref. [90]. 2020, American Chemical Society. The yellow square in (**B**) indicates the area where the SAED data were recorded. High magnification of SEM images of (**D**) corresponding to the regions marked by rectangles in (**C**), where new crystals grew along the tubule walls (yellow arrows), the diameter of the collagen increased over time, resulting in a "beaded string-like" appearance due to HAP precipitation (red arrows). (**E**–**H**) PILP-induced dentin remineralization and occlusion of dentin tubules, reprinted with permission from Ref. [44]. 2022, Royal Society of Chemistry. High magnification of SEM images of (**F**,**H**) correspond to the regions marked by rectangles in (**E**,**G**), respectively. (**I**–**L**) Remineralization of dentin without (**J**) or with (**L**) citrate pretreatment, reprinted with permission from Ref. [105]. 2018, John Wiley and Sons. (**M**–**P**) TEM images of PAH-ACP@hmZrO_2-mediated collagen mineralization, reprinted with permission from Ref. [110]. 2018, Elsevier. The open arrowheads in (**O**) indicated the partially mineralized collagen fibrils.

4.2.4. ACP Nanoparticles for Dentin Remineralization

The mineralization theory based on PILP has been widely recognized and studied. However, its application in the real oral environment is challenging due to the requirement of saturated calcium and phosphorus ion concentration. It is difficult to continuously provide a sufficient concentration of calcium and phosphate for mineralization in the oral cavity. As a precursor to mineralization, ACP is prone to phase transition in solution. Constructing a proper stabilization and transport system for ACP is an excellent strategy for remineralization, allowing for the backfilling of ACP into demineralized dentin and collagen.

The delivery system utilizes a range of materials, such as polycaprolactone, chitosan, PLGA, and mesoporous silica. Several studies have loaded ACP into mesoporous nanoparticles [111,112]. For example, amine-functionalized enlarged pore silica nanoparticles (AF-eMSN) attached to PAA-ACP successfully achieved mineralization, which is the first attempt to deliver ACP by using enlarged pore silica for mineralization in collagen fibers [113]. Using zirconia, a biocompatible ceramic commonly used in dentistry, to synthesize mesoporous nanocapsules loaded with PAH-ACP, the released PAH-ACP still retains its ability to penetrate and mineralize collagen fibers (Figure 4M–P) [110].

Considering the process of composite resin adhesion and the issues of collagen degradation and microleakage at the demineralized collagen interface, the incorporation of ACP particles into self-etching adhesives or resins is a strategy that has been extensively studied. The use of self-etching adhesives as a carrier of ACP nanoprecursors facilitates continuous biomimetic remineralization [114]. Core-shell chlorhexidine/ACP (CHX/ACP) nanoparticles have been synthesized and utilized to enhance dental resin composites, providing them with exceptional mechanical strength, antimicrobial activity, and remineralization capabilities [115]. These strategies could further release calcium and phosphate ions to create supersaturation relative to the HAP crystals, and could also infiltrate directly into the fibers, leading to intrafibrillar mineralization, which shows promise for clinical applications.

5. Conclusions and Outlook

In summary, the intricate structure of teeth endows them with superior mechanical properties, but also increases the difficulty of their repair. Existing clinical restorative materials differ significantly from natural dental tissues in terms of chemical composition, structure, and performance. This difference usually results in an inability to achieve durable, long-term restorative results. The strategy of biomimetic mineralization for repairing dental hard tissues presents a highly promising avenue. Recent studies have demonstrated the satisfactory remineralization of both enamel and dentin under specific experimental conditions. Nevertheless, further exploration is needed for this approach to be successfully implemented in clinical applications.

Given the complexity of the oral environment, the effectiveness of biomimetic mineralization technology in clinical applications may be influenced by several factors, including the oral microbial population and oral enzyme activity of each patient. Therefore, it is crucial to carry out more in-depth in vivo animal studies and clinical research, which are essential to optimize the application of this technology and to develop biomimetic mineralization materials that are more adaptable to the oral environment.

At present, the repair layers of biomimetic mineralization materials are mostly at the micron level and large-scale repairs at the millimeter level have not yet been achieved. Therefore, we can consider applying biomimetic mineralization materials in the form of oral cleaning products (such as toothpaste and mouthwash), aiming to inhibit demineralization, promote remineralization, and prevent dental caries. In addition, biomimetic mineralization materials can be used in combination with existing dental restorative materials to improve their durability and functionality.

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