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Engineered Fabrication of Enamel-Mimetic Materials

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Tooth enamel, which is a biological tissue mainly composed of well-aligned hydroxyapatite nanocrystals and an interlaced protein matrix, has remarkable mechanical and aesthetic behaviors. Nevertheless, it is challenging to regenerate enamel naturally, and potential pulp involvement and tooth loss may occur. As the hardest biogenic composite material, enamel has long been regarded as a promising load-bearing material. Thus, understanding the enamel formation process and enamel structural motif mechanisms is important for the design and engineering of high-performance biomimetic composites with high strength and physical resilience. Extensive studies have been conducted on mimicking the microstructure and mechanical properties of tooth enamel, and various enamel-like material synthesis protocols have been developed. In light of the engineering fabrication of enamel-like materials, this review focuses on recent progress in synthetic strategies for enamel-mimetic materials and provides a discussion of the potential applications of these materials.

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1. Introduction

As the tooth’s exterior layer, tooth enamel is the hardest biogenic composite material and exhibits an impressive Young's modulus (1.1–4.9 GPa and 62–108 GPa, respectively, with 85 vol% inorganic content) in all mammals [1,2], while maintaining high wear and fatigue resistance [3]. These superior characteristics enable tooth enamel to bear a wide range of imposed loads and contact stress (0.45–2.5 GPa), in addition to other shear forces under oral conditions, thereby protecting the inner dentin from damage and deformity [4,5].

However, once tooth enamel is damaged, this non-vital structure cannot self-regenerate due to the loss of stem cells after maturation. As a result, vulnerable dentin and pulp are likely to be affected soon after an enamel defect develops if there are no appropriate interventions, resulting in tooth loss [6]. For decades, due to the unsatisfactory aesthetic effects, excessive damage, and microleakage of existing conventional dental restoration materials, including metals, ceramics, and composite resins, their use in clinical practices remains unsatisfactory. Thus, there is a pressing need for enamel-like materials to achieve ideal treatment outcomes.

In addition to dental applications, these attractive enamel-like materials have long been considered for various applications in transportation [7], consumer electronics, and architecture. Many existing synthetic materials have quickly reached their performance ceilings in terms of enhanced toughness while maintaining high stiffness and hardness [8,9]. In nature, the evolution of hierarchical stratifications in living organisms offers a wealth of possibilities for bioinspired materials [10], including a solution for the strength–toughness conflict [11]. Thus, materials that are manufactured with properties similar to those of tooth enamel are expected to be highly utilized in future medical and other applications.

To date, numerous methods have been proposed for enamel mimetics to replicate the multifunctionality of tooth enamel for applications in various fields. In this review, we address the formation process and structural motifs of mimic strategies and discuss the advantages, limitations, and future perspectives of these selected design concepts and manufacturing methods. The synthetic strategies of this review are illustrated schematically in Fig. 1.

2. Tooth enamel: Structure and function

The formation of natural enamel includes secretion and maturation stages [6], spanning multiple length scales from an atomic...
scale to a macroscopic scale. During the secretion stage, ameloblasts elongate and extend a single process, Tomes’ process, at their surface. Once aligned with each other, mature ameloblasts start to secrete proteins, in particular amelogenin, through Tomes’ process [12]. This protein matrix is able to induce the nucleation of calcium phosphate and the organization of hydroxyapatite crystals, as well as direct its own replacement by minerals [13]. Each ameloblast will finally lay down an enamel prism (~5 μm in diameter), extending from the dentin–enamel junction (DEJ) to the enamel surface [14,15]. During the maturation stage, there is a steep increase in the replacement of most of the matrix and in the mineral volume fraction (from 10%–20% to 80%–90%) [16]. The hierarchical structure of tooth enamel is illustrated in Fig. 2 [3,17].

When examined on a micrometer scale, enamel is mainly composed of enamel prisms and interprisms containing thousands of nanocrystals, in parallel alignment to the crystallographic c-axis of the apatite lattice [18]. Between the enamel prism and the interprism structures, there is a narrow space occupied by a protein-rich organic layer named the “rod sheath.” The arrangement and orientation of the enamel prisms vary from the enamel surface to the DEJ (Figs. 2(c) and (d)) [19]. In almost one-third of the outer enamel, the alignment of the enamel prisms is largely parallel, and a cross-section of this region demonstrates an 3–5 μm diameter “keyhole” arrangement (Figs. 2(e) and (f)). In the deeper portion of the enamel, the enamel prism groups cross and wind together, forming a decussation. Near the decussating enamel beyond the surface, the enamel prisms align in a more perpendicular arrangement toward the DEJ. In addition to prismatic enamel, there is a thin aprismatic enamel layer at the surface of the enamel, which contains unaligned HAP crystallites.

It can be concluded that the predominant mineral phase, crystal texture, and biological organic component of tooth enamel are the primary contributors to the evolutionary mechanical properties of enamel that correspond to its functional demands [20]. It is generally recognized that the predominant mineral phase (95 wt%–96 wt%) is critical for the strength of enamel. Demineralized enamel exhibits both a lower hardness (~1 GPa) and a lower elastic modulus (~70 GPa) [21]. In regard to the crystal texture, it has been reported that both the hardness and the elastic modulus of dental enamel increase from the DEJ (hardness < 3 GPa and elastic modulus < 70 GPa) to the enamel surface (hardness > 6 GPa and elastic modulus > 115 GPa) [20]. Furthermore, it has been recognized that the crystal texture of tooth enamel is generally more ordered along the way from the DEJ to the tooth cusps, which enables the external occlusal surface to withstand the greatest masticatory forces [22]. In addition, the functional cusps, where the crystal texture is observed to be more intense and ordered perpendicular to the surface, possess a higher load-resisting capacity. Crystal structural anisotropy and the organic component are also distributed throughout the high toughness of the enamel. Structural anisotropy guides the stresses to the dentin, thus resisting damage through cracks [23], and viscoelastic protein films between the HAP crystallites distract the load to avoid fractures [6].

In general, the hierarchical structural motif, which merges hard and relatively soft components across multiple length scales, is crucial for tooth enamel’s mechanical properties and functional behaviors. Inspired by these structural mimetic principles and functional bases, an increasing number of researchers have reported various synthetic methods for enamel-like materials in recent years.

3. Synthetic strategies for enamel-mimetic materials

The replication of enamel-matching functions can be achieved through two main strategies. First, the replication of complex enamel architectures determines the functions of synthetic composites that are similar to natural enamel, through techniques including whole-tooth engineering, physicochemical enamel replication, and biochemical enamel engineering. Second, enamel-matching functions can be replicated indirectly by adjusting the properties of different synthetic composites, such as nacre-like composites and epidermis-like structures. In this section, we present recent synthetic methods for enamel-like materials and their components, techniques, achievements, and limitations based on selected studies.

3.1. Replication of enamel-like structures

3.1.1. Whole-tooth engineering

Tissue engineering based on the collaboration of tissue-specific cell sources, appropriate scaffold materials, and inductive growth and differentiation factors has been successfully demonstrated in several organs, leading to clinical applications [24]. In regard to tooth enamel, ameloblasts, as crucial cells secreting mineralization-related proteins to manipulate the enamel mineralization process mentioned above, are one of the preferred cell sources. Therefore, studies have focused on the exploration of available and feasible ameloblast-like cell lines to induce the nucleation and growth of enamel-like tissue directly. Although these studies have achieved several aspects of ameloblast characteristics, none have successfully formed enamel structures in vitro. Thus, researchers have turned to inductive environments initiated by odontogenic embryonic cells.

The natural tooth-formation process is initiated by epithelial–mesenchymal inductive interactions during the early stage and continues to develop and differentiate through the subsequent stages [25]. To replicate the development of embryonic teeth, mimicking the inductive environments appears to be a logical approach. For decades, a serumless, chemically defined medium was widely used for cap stage mouse tooth culture in vitro, but thin layers of enamel were barely obtained [26]. In 2007, Nakao et al. [27] recombined the epithelial and mesenchymal cells from embryonic mouse tooth germs following dissociation and generated complete and correct bioengineered teeth both in vitro and in vivo. Their article suggests a basis for tooth bioengineering and provides appropriate non-embryonic cell sources.
Utilizing the natural signaling cascade in the early stages of tooth formation, studies have demonstrated that adult epithelial and mesenchymal cells can respond to inductive odontogenic signals and form teeth [28,29]. In 2004, Ohazama et al. [28] were the first to recombine inductive-stage embryonic dental epithelium and adult bone marrow stromal cells to stimulate an odontogenic response. After being transferred into an adult environment (the renal capsule and maxilla of adult mice), the tooth rudiments developed into an identifiable ectopic crown similar in size to the first molar. Regarding potential adult epithelial cells, human gingival epithelial cells have shown the capability of expanding in vitro and responding to mouse embryonic tooth mesenchymal cells [29]. The artificial tooth germ formed multiple tooth-like structures after being transferred into renal capsules. Although the bioengineered teeth demonstrated well-developed roots and crowns containing well-distinguished enamel, the characteristic complex microstructure of the whole tooth from the enamel prisms to the periodontal ligament fiber remains difficult to achieve.

Considering that most of these bioengineering studies utilized a mouse model, further evidence for medical application requires data from a large-animal model. In 2017, Ono et al. [30] reported successful tooth restoration using bioengineered tooth germ after autologous transplantation into a reconstituted canine model, as illustrated in Fig. 3. The bioengineered tooth demonstrated correct ultrastructure, such as enamel rods, with components (Ca/P 2.05) similar to those of natural teeth (Ca/P 1.95). In response to orthodontic force, its periodontal ligament mediated tooth movement without ankylosis, which resembles physiological tooth functionality.

Theoretically, whole-tooth engineering is an ultimate goal for enamel mimetics and holds great promise for lost tooth replacements; however, it is still far from practical application. In addition to the regulatory mechanisms for tooth germ development, which remain unclear, the low frequency of successful regeneration and the limited number and morphology of the given type of teeth are limitations with respect to medical applications. Moreover, odontogenic embryonic cells lose their odontogenic potential after in vitro expansion [31]. According to a theory that cell compaction promotes differentiation [32] and on the basis of the “community effect” in embryonic tooth germ cells [33], further development in three-dimensional (3D) culture systems may reveal the differentiation control mechanism [34].

3.1.2. Physicochemical enamel replication

The repair and remineralization of enamel using HAP nanocrystals has successfully replicated enamel-like architectures, but practical organized mineralized HAP structures remain difficult to obtain. The adoption of other, different materials has resulted in an enamel-inspired columnar hierarchical architecture containing an organic phase, dubbed “abiotic enamel,” which exhibits enamel-matching properties.

3.1.2.1. In situ growth of enamel-like structures. (1) Induced by calcium phosphate ion clusters. Early studies adopted traditional physical methods to synthesize HAP—such as surfactant docusate sodium salt-assisted mineralization, hydrothermal mineralization adopting chelators, and mineralization utilizing simulated body fluid—to grow enamel-like structures. However, limited by extreme conditions and/or toxic additives, the synthetic flake-like crystals, and their poor adhesion to enamel [35], these methods are usually modified and function as a basic process for other approaches.

One of the main growth units of HAP and amorphous calcium phosphate (Ca₅(PO₄)₃·nH₂O, ACP) is the calcium phosphate ion cluster (CPIc) [36]. Attributed to the dissociation of CPIcs, Yamagishi et al. [37] and Onuma and Ito [38] developed a white fluorinated crystalline paste to grow HAP nanocrystals seamlessly in the affected site, forming a thin enamel-like layer (20–30 μm thick in 15 min). However, the acid paste and the high
concentration of hydrogen peroxide could cause gum inflammation. Inspired by the biomineralization at the growth frontier, which relies on the integrated crystalline–amorphous interface, Shao et al. [39] adopted a novel kind of CPIC that mimicked the mineralization frontier to repair affected enamel through epitaxial growth (Fig. 4(a)). The mechanical properties of the repair layer were similar to—or slightly better than—those of natural enamel (with a hardness value of \((3.84 \pm 0.20)\) GPa, an elastic modulus of \((87.26 \pm 3.73)\) GPa, and a coefficient of friction of 0.18 ± 0.008). The coefficient of friction of the repaired enamel suggested that its wear resistance was similar to that of natural enamel. However, the thickness of the repair layer was limited to 2.8 μm.

(2) Wet-chemical growth of amorphous ceramics. The challenge of current research on most in situ enamel-repair techniques is to create materials that are thick enough to be feasible for practical material production, while they usually possess a weak interfacial connection. Therefore, studies have turned to the amorphous phase between HAP nanorods [40]. Through an in situ wet-chemical growth strategy, Wei et al. [17] achieved a coating of amorphous compatible ZrO₂ ceramics (~400 nm in thickness) on the enamel (Fig. 4(b)), which demonstrated a mechanical performance comparable to that of natural enamel (Young’s modulus of ~82.5 GPa and hardness of ~5.2 GPa). The coated layer exhibited high resistance to bacterial adhesion and proliferation on the surface and enhanced resilience toward mastication damage through a strong chemical connection on the interface between the repair layer and the substrate enamel. However, the ZrO₂ ceramic layer only mimicked the intergranular phase between HAP nanorods instead of mimicking the entire hierarchical stratification of the enamel, and the thickness of the repaired layer remained limited.

3.1.2.2. De novo synthesis of enamel-like structures. (1) HAP assembly. In the natural formation process of tooth enamel, HAP crystals undergo a self-assembly process induced by the protein matrix and cells, resulting in a multiscale, highly aligned architecture. Inspired by this process, different driving forces for the nucleation and/or arrangement of HAP crystals have been developed. Zou et al. [41] reported the development of a layer of enamel-like HAP that was transformed directly from monetite (CaHPO₄). In an alkali solution with microwave heating assistance, the hydrolyzed CaHPO₄ lattices served as a template for HAP nucleation. The synthetic HAP bundles (~250 nm in diameter and > 1 μm in length) aligned in parallel with an enamel-like orientation.

Fig. 3. Generation and morphology of a bioengineered tooth. (a) Schematic illustration of whole-tooth regeneration; (b) photographs of the no transplantation group, the natural tooth germ transplantation group, and the bioengineered tooth germ transplantation group; (c) micro-computed tomography (CT) images and histological analysis of the natural tooth group, the natural tooth germ transplantation group, and the bioengineered tooth germ transplantation group. E: enamel; D: dentin; C: cementum; PDL: periodontal ligament; AB: alveolar bone; HE: haematoxylin-eosin. Reproduced from Ref. [30] with permission.
However, the size of the crystals and the thickness of the enamel-like layer were still distinct from those of enamel apatite.

To synthesize a large highly aligned HAP nanowire bulk, researchers recently developed self-assembling ultralong HAP nanowires with high aspect ratios (> 10 000) [42]. After a controlled injection process, these highly orientated HAP nanowires built a 3D prism-like structure with the assistance of sodium oleate [43]. Nevertheless, the synthetic HAP structure exhibited

Fig. 4. In situ fabrication and morphology of enamel-like structures using (a) CPICs and (b) amorphous ZrO$_2$. TEM: transmission electron microscopy; R: enamel rod; IR: enamel inter-rod; HA: hydroxyapatite. (a) Reproduced from Ref. [39] with permission; (b) reproduced from Ref. [17] with permission.
mechanical properties (e.g., a Young's modulus of ∼13.6 GPa) far from those of enamel, even when enhanced by the infiltration of dental resin. Although this recent approach illustrates the self-assembly capability of synthetic HAPs and their potential for printing techniques, the disappointing outcomes to date indicate that synthetic HAPs may not be the best choice for de novo synthetic abiotic enamel.

(2) Layer-by-layer (LBL) deposition. The LBL technique is a repeated deposition process that starts with the formation of a charged monolayer on a substrate. The first layer attracts an oppositely charged material to produce a layer on its surface; then, a new layer can be absorbed, which eventually results in a multilayered architecture [44]. Through the LBL technique, a clay/polymer multi-layer nanocomposite was prepared from abundant, inexpensive components. The nanocomposite exhibited extraordinary properties, making this technique consistently interesting to researchers. Yeom et al. [2] reported a (ZnO/LBL)n abiologic enamel utilizing a hydrothermal LBL deposition of ZnO nanowires and a polyelectrolyte matrix. This composite demonstrated enamel-matching viscoelastic figures of merit (VFOM; 0.7–0.9) and a weight-adjusted VFOM (> 0.8), thereby exceeding the limits of traditional materials. In addition, the abundant and inexpensive components this technique requires are obvious advantages for its practical applications.

(3) 3D printing. The 3D printing of graded ceramic–polymer composites has paved the way for the use of ceramics with a parallel rod alignment that mimics the prisms in tooth enamel [45]. Feilden et al. [46] manipulated alumina platelet (∼5 μm diameter) alignment during extrusion through a computer-controlled nozzle to build hierarchical ceramic scaffolds infiltrated by a soft phase (Fig. 5). Although this research was not inspired by tooth enamel, the alignment of the nanoplatelets in the composites was substantially consistent with the structural design basis of enamel. The mechanical properties varied according to the alignment of the nanoplatelets. A trans-filament orientation showed the highest flexural strength ((202 ± 10) MPa), compressive strength (452 MPa), and fracture toughness ((3.0 ± 0.3) MPa·m1/2). Bioinspired Bouligand-type structures, which are similar to decussation enamel prisms, have provided guidance for crack propagation by demonstrating enhanced toughness and resistance (resistance curves). The resultant composites also revealed a high Young's modulus ((99.1 ± 0.6) GPa). The enhanced toughness of these composites, in combination with their high strength, opens up avenues for various applications [47], such as in the aerospace and automotive fields. For dental applications, it is crucial to be able to print higher resolution enamel-matching microstructures, which is impractical to date.

3.1.3. Biochemical enamel engineering

Proteins play a critical role in inorganic precipitation during enamel mineralization [13]. Enamel proteins, which consist of amelogenin, enamelin, and ameloblastin, are crucial for the natural nucleation of calcium phosphate and the organization of HAP crystals. As the predominant protein (> 90% in volume), amelogenin acts as an inducer for apatite crystals and as a stabilizing factor for ACP [48]. Therefore, numerous methods assisted by amelogenin or its analogues to control the biomineralization process have been used to regenerate enamel-like structures.

The gel-like amelogenin-rich matrix offers unique organic–inorganic interactions during the initial enamel formation stage. To mimic the gel-like matrix for mimetic enamel, various hydrogel mineralization models have been developed and modified. However, without amelogenin and its analogues, the resultant crystal layers are very dissimilar to natural enamel. An amelogenin-containing chitosan (CS-AMEL) hydrogel system was reported, in which Ca–P clusters were stabilized and guided by amelogenin, and the composite chains were fused and evolved into aligned apatite nanocrystals (15–30 μm in thickness) directly on the enamel surface [49]. With the incorporation of matrix metalloproteinsase-20 (MMP-20), the amelogenin protein gradually degraded, and the synthetic enamel layer demonstrated a more uniform orientation, increased modulus, and increased hardness (a 1.8-fold increase in elastic modulus, and a 2.4-fold increase in hardness compared with the same hydrogel without MMP-20), which prevented protein occlusion.

Considering the difficulties presented by the expression and purification of amelogenin, studies have explored alternative molecules that can substitute for this expensive protein. The C-terminal domain of full-length amelogenin has been recognized as essential for proper enamel mineral formation, and its analogues, such as leucine-rich alternative splicing amelogenin peptide [50] and synthetic oligopeptide amphiphiles containing the C-terminus of amelogenins [51], promote the remineralization of enamel. In 2017, leucine-rich alternative splicing amelogenin peptide (LRAP) was reported to selectively promote needle-like crystal alignment along the c-axis; however, this method was still restricted by the thickness of the synthetic bundles (∼2 μm) [50].
as were the methods in other studies using alternative molecules containing the C-terminus of amelogenins.

It has also been reported that amelogenin and other acidic enamel proteins fold into ordered β-sheets from disordered random coils upon interaction with ACP [52]. Therefore, alternative molecules were exploited to synthesize artificial dental enamel by replicating the disorder–order interplay during enamel formation, containing self-assembling β-sheet peptides [53], elastin-like polypeptide [54], and intrinsically disordered proteins (IDPs) [55]. Most of these studies were impacted by the thickness (~1 μm) of the synthetic enamel layers. However, with the exploration of IDPs under near-physiological conditions, Elsharkawy et al. [55] synthesized tunable hierarchically ordered fluorapatite (Ca₅(PO₄)₃F, FAP) crystal structures (tens of micrometers in length and height) exhibiting enamel-matching acid and protease resistance (Fig. 6). However, the hardness (a hardness value of (1.1 ± 0.8) GPa and an elastic modulus of (33.0 ± 20.1) GPa) determined for the artificial crystals was only half of that of natural enamel.

In pursuit of thicker HAP repair layers, another analogue of amelogenins—namely, dendritic polymers—has been reported to guide the nucleation and provide a template for the mineralization of HAP [56]. In particular, poly(amido amine) (PAMAM) dendrimers, which are similar to the supramolecular assembly of amelogenins, are capable of regulating the size and shape of HAP. Through the modification of different functional groups and several generations of dendrimers, Chen et al. [57] synthesized phosphate-terminated PAMAM (PAMAM-PO₃H₂) and a regenerated enamel prism-like HAP layer (11.23 μm in thickness in three weeks) showing 97% recovered microhardness and an adhesive force of 50 N. Moreover, in vivo regeneration (6.63 μm in thickness) experiments demonstrated the great potential of PAMAM-PO₃H₂ for human enamel restoration compared with other in vitro biochemical enamel regeneration methods.

In addition to amelogenin, a few analogues of other mineralization-related molecules have been developed to promote enamel remineralization, such as aspartate–serine–serine peptides [58] and polydopamine [59]; nevertheless, the thickness of the repair layer has not been addressed.

3.2. Generation of enamel-matching mechanical properties

3.2.1. Freeze-casting technique

In terms of mechanical properties, ideal enamel-replacement materials must possess damage tolerance and good durability based on sufficient hardness, stiffness, and strength. Among all sorts of materials, ceramics and ceramic-based composites are some of the most promising choices [60–64]. However, they are much harder and stiffer than natural enamel and dentin (e.g., the hardness of yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) is approximately 12 GPa, which is ~4 times harder than natural enamel) and exhibit poor damage tolerance and a high tendency toward flaws [1,65]. Tan et al. [66] reported a bidirectional freeze-casting technique to fabricate porous scaffolds and then infiltrate the scaffolds with dental resin (Fig. 7). Freeze-casting is a promising technique for fabricating nacre-like composites. The resultant nacre-mimetic composites with brick-and-mortar architectures resembled human enamel in terms of their Young’s modulus ((42 ± 4) GPa), hardness, stiffness, and strength; they also exhibited a high level of toughness (a J-integral fracture toughness of ~1.7 kJ·m⁻² and an American Society for Testing and Materials (ASTM) valid crack-growth toughness of ~9.6 MPa·m¹/₂), making them meaningful for dental applications.

As reported earlier, the freeze-casting technique can serve as a basis for other techniques, such as magnetically assisted slip casting to generate nacre-like composites [67]; however, the mechanical properties of the composites made in this way remain distinct from the properties of tooth enamel thus far.

3.2.2. Hybrid LBL assembly technique

Without the ability to self-repair, natural enamel can hardly heal once it is damaged because of the distinct dynamic characteristics between inorganic crystals and surrounding proteins. To avoid sacrificing the mechanical properties of natural enamel,
when synthesizing self-repair materials, the target technique should seamlessly integrate different, well-matched compositions. Inspired by human skin, researchers utilized graphene oxide (GO) and polymers in a hybrid LBL assembly technique [68]. The high content of the GO nanosheets in the linear-LBL film layer equipped the synthetic composites with excellent barrier and mechanical properties. This hard barrier layer deposited on top of a relatively soft exponential-LBL layer resulted in epidermis-like hierarchical composites. The controlled polymer dynamics promoted the stimulated interlayer diffusion through the soft cushion provided by the polymers, and the hard layer served as a sealing barrier to achieve self-repair. This smart coating decreased the catastrophic failure rate and maintenance expenses, while maintaining a high level of hardness approaching that of tooth enamel (a hardness value of \((2.27 \pm 0.09) \text{ GPa}\) and an elastic modulus of \((31.4 \pm 1.8) \text{ GPa}\)) and higher than any other self-healable synthetic films to date. This material would be practical in diverse applications, such as healthcare and consumer electronics. Selected studies on each strategy are listed in Table 1 [2,19,30,39,43,46,50,55,57,66,68,69].

**4. Limitations and application perspectives**

In principle, HAP biomineralization strategies are a distinctly effective method to produce synthetic enamel-like materials [70]—yet synthetic enamel-like apatite (including HAP and/or FAP) architectures are still quite un-suitable for practical applications. Although whole-tooth regeneration has been demonstrated to be successful, it remains extremely complex and elusive, as described above. In addition, physicochemical and biochemical methods for generating enamel-repair layers still present several problems: ① The limited thickness of the repair layers makes them impractical, except for use with early caries. ② Although several methods have been developed for achieving relatively thicker repair layers, most demonstrate limited structural replications that are very dissimilar to the distinctive ordered hierarchical structure of enamel across multiple length scales. ③ The complete mechanical properties and enhanced multifunctionality of tooth enamel remain unattained. ④ Repair layers usually grow only on the surface of the affected enamel, which seems to be a practical advantage for enamel restoration. However, the majority of these methods involve slow biomineralization processes, reducing their potential for *in situ* restorative application. Combined with the limited thickness they produce, these methods cannot be applied to manufacture restorative materials *in vitro*. ⑤ Finally, the limited replication of the enamel structure is a general problem for all methods. Instead of replicating the entire hierarchical stratification of the enamel, most methods mimic only a small portion of the enamel, such as that observed with parallel prism enamel or decussation prisms. Although these methods have been used to grow highly ordered particles, capsules, films, and carbon nanostructures, the extent to which enamel can be replicated by these methods remains unclear [71]. Thus, the materials produced by these methods are not yet practical substitutes for traditional materials.

Enamel-mimetic strategies with foreign components typically utilize different highly organized structural designs (similar to or different from those of natural enamel) based on various components to achieve enamel-matching properties. The resultant abiotic composites have opened up a new avenue for ideal materials in various fields. In regard to dental materials, the biocompatibility, chemical stability, and surface properties of such composites must be taken into consideration. Therefore, utilizing biocompatible ceramics and ceramic-based composites could provide a clever shortcut. With further fabrication assisted by computer-aided design and manufacturing (CAD/CAM), these materials could be promising candidates for dental restoration. It has been recognized that these methods have satisfactory potential for industrial application, exemplified by electronics, transportation, and building. First, these methods address the problem of limited thickness and can manufacture the materials in bulk, which is a prerequisite...
## Table 1
Summary of selected studies on synthetic strategies for enamel-mimetic materials.

<table>
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<th>Synthetic strategies</th>
<th>Methods</th>
<th>Major components</th>
<th>Achievements</th>
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<td><strong>Replication of enamel-like structures</strong></td>
<td><strong>Whole-tooth engineering</strong></td>
<td></td>
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<tr>
<td>In situ growth of enamel-like structures</td>
<td>Autologous transplantation of reconstructed tooth germ</td>
<td>HAPs</td>
<td>Correct ultrastructure such as enamel rods; components similar to natural tooth (Ca/P 2.05 and Ca/P 1.95, respectively); enamel-matching functionality, with periodontal ligament-mediated tooth movement without ankylosis in response to orthodontic force (10 gf for 30 days); successful in vivo experiment involving autologous transplantation in a large-animal model</td>
<td>Unclear regulatory mechanisms for tooth germ development; inefficient reconstructing condition; limited number and morphology of the given type of teeth; loss of odontogenic potential after in vitro expansion</td>
<td>[30]</td>
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<tr>
<td>Physicochemical enamel replication</td>
<td>Using CPCS to mimic the mineralization frontier</td>
<td>HAPs</td>
<td>Seamless repair layer; excellent mechanical properties, with a hardness of (3.84 ± 0.20) GPa, an elastic modulus of (87.26 ± 3.73) GPa, and a COF of 0.180 ± 0.008, slightly exceeding that of natural enamel</td>
<td>Limited thickness of 2.8 μm, incubation for 48 h</td>
<td>[39]</td>
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<tr>
<td>In situ growth of enamel-like structures</td>
<td>Using in situ wet-chemical growth technique</td>
<td>ZrO2 ceramics</td>
<td>Enamel-matching mechanical properties, with a Young's modulus of ~82.5 GPa and a hardness of ~5.2 GPa; enhanced resilience toward mastication damage; high resistance to bacterial adhesion and proliferation</td>
<td>Limited thickness of ~400 nm</td>
<td>[19]</td>
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<tr>
<td>De novo synthesis of enamel-like structures</td>
<td>HAP assembly</td>
<td>HAPs</td>
<td>Self-assembly synthetic HAPs; potential for printing technique</td>
<td>Limited mechanical properties, with a Young's modulus of ~13.6 GPa</td>
<td>[43]</td>
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<td></td>
<td>LBL deposition</td>
<td>ZnO nanowires + a polyelectrolyte matrix</td>
<td>Inexpensive components; enamel-matching properties, with a VFOM of 0.7–0.9, a weight-adjusted VFOM of &gt; 0.8, and a Young's modulus of (39.8 ± 0.9) GPa</td>
<td>Limited hardness of (1.65 ± 0.06) GPa</td>
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<td>3D printing</td>
<td>Alumina platelets</td>
<td>Enamel-matching mechanical properties, with high flexural strength ((202 ± 10) MPa), compressive strength (452 MPa), a high Young's modulus ((99.1 ± 0.6) GPa), and high fracture toughness ((3.0 ± 0.3) MPa)m1/2; Bouligand structures similar to the decussation enamel prisms, exhibiting R-curves behavior</td>
<td>Limited resolution of structure</td>
<td>[46]</td>
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<tr>
<td><strong>Biochemical enamel engineering</strong></td>
<td>Utilizing CS-AMEL reinforced by MMP-20</td>
<td>HAPs</td>
<td>Better uniform orientation; improved mechanical properties, with an increased modulus and hardness (a 1.8-fold increase in elastic modulus and a 2.4-fold increase in hardness compared with the same hydrogel without MMP-20); preventing protein occlusion; HAP component</td>
<td>Limited thickness of 15–30 μm</td>
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<td>Adopting LRAP</td>
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<td>Using IDPs</td>
<td>FAPs</td>
<td>Enamel-matching elastic modulus of (33.0 ± 20.1) GPa; a larger structure tens of micrometers in length and height; enamel-matching acid and protease resistance</td>
<td>Limited hardness of (1.1 ± 0.8) GPa; limited replication of structure, as it further grew into a larger circular structure; fluoride component</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td>Synthesizing PAMAM-PO3H2</td>
<td>HAPs</td>
<td>Enamel-matching mechanical properties with a regenerated enamel prism-like HAP layer showing 97% recovered microhardness; an adhesive force of 50 N; successful in vivo regeneration experiment with a thickness of 6.63 μm</td>
<td>Limited thickness of 11.23 μm in 3 weeks; exact mechanical properties unknown</td>
<td>[57]</td>
</tr>
<tr>
<td><strong>Generation of enamel-matching mechanical properties</strong></td>
<td>Freeze-casting technique</td>
<td>3Y-TZP + a light-curing methacrylate resin</td>
<td>Enamel-matching mechanical properties, with an enamel-matching Young's modulus ((42 ± 4) GPa), hardness, stiffness, and strength; high toughness (J-integral fracture toughness of ~1.7 kJ/m2, ASTM valid crack-growth toughness of ~9.6 MPa/m1/2)</td>
<td>Nacre-inspired structure</td>
<td>[66]</td>
</tr>
<tr>
<td>Hybrid LBL assembly technique</td>
<td>Depositing a hard linear-LBL film layer on top of a relatively soft exponential-LBL layer to mimic the epidermis structure</td>
<td>GO + poly (vinyl alcohol) + tannic acid</td>
<td>Enamel-matching hardness of (2.27 ± 0.09) GPa; self-healing potential</td>
<td>Limited elastic modulus of (31.4 ± 1.8) GPa; epidermis-like structure</td>
<td>[68]</td>
</tr>
</tbody>
</table>

COF: coefficient of friction; R-curves: resistance curves.

* 1 gf = 0.0098 N.
for practical application. Second, the majority of the HAP-free synthesis methods achieve enhanced hardness, stiffness, Young’s modulus, and strength without compromising the high level of toughness, and thus have properties similar to those of natural enamel and distinctly exceeding those of traditional materials. In addition, their unique characteristics—as such as their high VFOm, guidance for crack propagation, and self-healing ability—make them practical in several specific fields. Third, economic considerations are regarded as being as important as mechanical properties. Therefore, methods that involve abundant and inexpensive components are preferable.

Further developments may focus on the synthesis of enamel mimetic materials with properties exceeding those of natural tooth enamel. For example, high-temperature-tolerant materials could be applied under extreme environments [72]. From a wider perspective, the ultimate goal for enamel-like materials is to achieve complexity, multifunctionality, sustainability, and self-repair ability. Although the mineralization and related mechanical process of tooth enamel remain unclear and the reported theories are still under debate, the exploration and replication of natural enamel will provide intriguing progress for material science in this field.

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Compliance with ethics guidelines

Lingyun Zhang, Yunfan Zhang, Tingting Yu, Liying Peng, Qiannan Sun, and Bing Han declare that they have no conflict of interest or financial conflicts to disclose.

References


[8] Zhou T, Song Z, Sundmacher K. Big data creates intriguing progress for material science in this field.

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