Dental Enamel Formation and Its Impact on Clinical Dentistry

James P. Simmer, D.D.S., Ph.D.; Jan C-C. Hu, B.D.S., Ph.D.

Abstract: The nature of tooth enamel is of inherent interest to dental professionals. The current-day clinical practice of dentistry involves the prevention of enamel demineralization, the promotion of enamel remineralization, the restoration of cavitated enamel where demineralization has become irreversible, the vital bleaching of dental enamel that has become discolored, and the diagnosis and treatment of developmental enamel malformations, which can be caused by environmental or genetic factors. On a daily basis, dental health providers make diagnostic and treatment decisions that are influenced by their understanding of tooth formation. A systemic condition during tooth development, such as high fever, can produce a pattern of enamel defects in the dentition. Knowing the timing of tooth development permits estimates about the timing of the disturbance. The process of enamel maturation continues following tooth eruption, so that erupted teeth can become less susceptible to decay over time. Mutations in the genes encoding enamel proteins lead to amelogenesis imperfecta, a collection of inherited diseases having enamel malformations as the predominant phenotype. Defects in the amelogenin gene cause X-linked amelogenesis imperfecta, and genes encoding other enamel proteins are candidates for autosomal forms. Here we review our current understanding of dental enamel formation, and relate this information to clinical circumstances where this understanding may be particularly relevant.

Drs. Simmer and Hu are Associate Professors in the Department of Pediatric Dentistry at the University of Texas Health Science Center at San Antonio. Direct reprint requests and correspondence to Dr. James P. Simmer, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900; 210-567-3543 phone; 210-567-6603 fax; simmer@uthscsa.edu.

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Tooth enamel is unique among mineralized tissues because of its high mineral content. Enamel is made up of highly organized, tightly packed crystallites that comprise 87 percent of its volume and 95 percent of its weight. Whereas other mineralized tissues are about 20 percent organic material, mature enamel has less than 1 percent organic matter. Enamel crystallites contain more than one thousand times the volume of corresponding crystals in bone, dentin, and cementum. Enamel crystals are extremely long relative to their thickness and are highly oriented. They generally extend from the underlying dentin toward the surface of the tooth and are organized into bundles, called prisms. Superior organization and mineralization give dental enamel its outstanding physical properties, making it the hardest tissue in the vertebrate body. Despite its hardness, tooth enamel can be destroyed fairly rapidly by dental caries, an infectious disease that affects approximately 95 percent of the population of the United States. Additionally, about one in 14,000 are afflicted with inherited enamel malformations collectively known as amelogenesis imperfecta, or AI.

Mineralization involves the net movement of ions out of solution, where their charges are dissipated by interactions with water molecules, and into a solid structure stabilized by covalent interactions between oppositely charged ions. The mineral structure that forms in teeth is closely related to calcium hydroxyapatite [Ca_{10}(PO_4)_{6}(OH)_2], but contains impurities, such as carbonate substituting for phosphate in the crystal lattice. Calcium hydroxyapatite can be synthesized from chemicals in the lab, but the shape, size, and organization of the crystals are always radically different from those of dental enamel. Acid is generated by the precipitation of enamel mineral, and acidity reverses the mineralization reaction, promoting the dissolution of enamel crystals. In this review, we will describe how dental enamel forms and discuss how dental practice is affected by the nature of dental enamel and the mechanism of its genesis. For more comprehensive analyses of tooth enamel formation, the reader is referred to any of the large number of detailed reviews.1-11
Genetic Control of Dental Enamel Formation

It is easy to deduce that dental enamel formation is under genetic control. The process of enamel formation, or amelogenesis, occurs predictably in tooth after tooth, generation after generation. The size, shape, shade, and even caries susceptibility of dental enamel can be passed from parent to offspring. Genetic diseases are associated with enamel malformations that range from total enamel agenesis to localized defects. Therefore, the formation of dental enamel is somehow encoded in our genes, or DNA. But how can a gene encode a mineral? The answer is that it can’t, at least not directly. DNA can only encode RNA, and most of the RNA it encodes is used to make proteins. Dental enamel formation is highly specialized, and the proteins most directly involved in enamel biomineralization are specific for it. As a consequence, defects in the genes encoding enamel proteins generally cause enamel malformations without affecting other parts of the body. There are, however, numerous genetic syndromes associated with dental defects of all types.12

Stages of Dental Enamel Formation

Amelogenesis occurs in stages in a well-delineated extracellular compartment. Dentin and enamel formation take place simultaneously, and both processes start along a line that will become the dentino-enamel junction, or DEJ. On the enamel side of the DEJ, crystal nuclei elongate into long thin ribbons. These ribbons are evenly spaced, oriented parallel to each other, and extend from the DEJ to the mineralization front just outside the membrane of ameloblasts (the cells lining the extracellular compartment on the enamel side). As ameloblasts secrete enamel proteins, the crystallites continue to grow in length, but grow very little in width and thickness. The final length of enamel crystals is determined by how long the ameloblasts continue to add enamel proteins, which also determines the final thickness of the enamel layer as a whole. Disturbances during the secretory stage of amelogenesis result in pathologically thin or hypoplastic enamel. At a certain point, which is decided by the genetic program, ameloblasts undergo a transition that greatly reduces their secretion of enamel proteins. Instead of structural proteins, proteinases are secreted, and the organic matrix is degraded and suddenly disappears from the extracellular compartment. These changes terminate the growth of enamel crystallites in length, and vastly accelerate their growth in width and thickness. Crystal elongation is arrested by curbing the secretion of enamel matrix constituents such as amelogenin, ameloblastin, and enamelin.

Mineral deposition on the sides of the crystallites accelerates, in part, because of the degradation and removal of growth-inhibiting enamel protein cleavage products. In humans, the maturation stage, during which the crystallites grow in width and thickness, takes about three to four years. This process is necessary to harden the enamel layer, and is directed by maturation-stage ameloblasts as they cycle through smooth and ruffle-ended phases. Fluoride is incorporated into crystal structure during the maturation stage. Disturbances during the maturation stage of amelogenesis result in pathologically soft (hypomutation) enamel of normal thickness.

Defining an Extracellular Space

During embryonic development, cells covering the cranial neural crest (CNC) invade the underlying connective tissue and migrate into the maxillary and mandibular prominences. These migratory cells share characteristics of epithelial and connective tissues and are commonly referred to as “ectomesenchyme.” Deciduous tooth initiation occurs at twenty different sites along the maxillary and mandibular processes. At each site, the oral epithelium thickens as the underlying CNC-derived ectomesenchyme concentrates or “condenses” beneath it.13 Interactions between these two tissue types ultimately lead to the formation of two opposing sheets of columnar cells: ameloblasts and odontoblasts. The extracellular space between the ameloblasts and odontoblasts is where each tooth develops. Dentin forms on the side of the odontoblasts, and enamel forms on the side of the ameloblasts. The cells in these sheets are connected by intercellular junctions and constitute a barrier that precludes the passage of molecules between the cells. Thus, a significant result of the early developmental program is
the generation of a well-delineated extracellular space where dentin and enamel form. The cells lining it determine the content of this space: ameloblasts on one side, and odontoblasts on the other.

The histology of tooth formation is classically divided into bud, cap, and bell stages (Figure 1). Significant advances have been made in the developmental biology of tooth formation, for which the reader is referred to recent reviews. There are a variety of genetic conditions in which teeth fail to develop. In humans, tooth agenesis occurs in assorted patterns, and appears to be caused by the early arrest of tooth formation. Defects in specific master genes encoding transcription factors that affect the expression of numerous other genes are the root cause. Mutations in $Msx_1$ and $Pax_9$ genes result in different patterns of oligodontia (Figure 2). When the master gene that is affected is involved in developmental processes in addition to tooth formation, the resulting familial tooth agenesis is a feature of a larger syndrome such as in ectodermal dysplasia or Rieger’s syndrome.

Formation of the Dentino-Enamel Junction (DEJ)

Odontoblasts initiate the secretion of an extracellular matrix. Odontoblasts secrete a predentin matrix that contains mostly type I collagen. The collagen molecules assemble into cables that are primarily oriented so that they extend outward—toward the ameloblasts. An assortment of noncollagenous proteins are also secreted, the most abundant being dentin sialophosphoprotein or DSPP. During the formation of the DEJ, DSPP is secreted by both ameloblasts and odontoblasts. Highly charged, noncollagenous proteins are thought to bind collagen and form crystal nucleation centers, while hydrophilic (water-loving) glycosaminoglycans (GAGs) such as decorin and biglycan draw away water molecules, potentially concentrating mineral ions at the nucleation centers. Several good reviews of dentin formation are available.

Figure 1. Histological changes during early tooth formation.

These sections are of developing mouse molars: A) tooth initiation, B) bud stage, C) cap stage, D-F) bell stage, G) root formation. Note that the crown of the molar takes shape (the multicusp architecture is evident) prior to the onset of mineralization.
Prior to the onset of biomineralization, preameloblasts secrete enamel proteins on top of the predentin matrix. Some of the enamel proteins penetrate the predentin and are absorbed by odontoblasts. Immediately following the initial secretion of enamel proteins, the ameloblast basement membrane disappears, and ameloblast cell processes extend into irregularities on the predentin surface. Enamel crystallites are initiated within these irregularities, in close proximity to both the ameloblast cell membrane and collagen fibers protruding from the predentin. The ameloblastic processes appear to retreat back to the cell body, extending the incipient enamel crystallites as they go. This fills in the irregular (villus) surface of dentin with enamel crystallites and converts it into the smooth, undulating surface of aprismatic enamel, which is perforated by odontoblastic processes.

In the erupted tooth, odontoblastic processes that extend into the enamel layer are known as enamel spindles. These processes presumably act as receptors that detect changes in the enamel layer and potentially convey sensitivity. Dentin and enamel are intimately linked at the dentino-enamel junction. The collagen-based organic matrix gives dentin its tensile strength and flexibility, and allows it to cushion the more brittle enamel covering.

**Enamel Formation During the Secretory Stage: Crystal Elongation**

Secretory ameloblasts are tall, columnar cells with a proximally polarized nucleus and proximal and distal cell-cell junctions. After depositing the aprismatic enamel layer, secretory ameloblasts develop a specialized, cone-shaped Tomes’ process at their secretory (distal) ends. Mineral deposition occurs primarily at a mineralization front very near to the ameloblast cell membrane. Enamel crystallites extend in length at extracellular growth sites a short distance away from the secretory faces of the ameloblast cell membrane, in what appears to be a matrix comprised of assemblies of enamel matrix proteins. Because the mineralization front occurs close to the protruding Tomes’ process, the surface of developing enamel is indented.
The Tomes’ process organizes enamel crystals into rod and interrod enamel. Enamel crystallites that elongate near the tip of the Tomes’ process form the rod enamel. Crystallites that lengthen near the intercellular junctions form the interrod enamel. The border between rod and interrod enamel is distinct because part of the ameloblast membrane is “nonsecretory,” which creates gaps in the mineralization front (Figure 3). The rod enamel and interrod enamel differ solely in the orientation of their crystallites, and the border between these two regions of an enamel prism is indistinguishable where the mineralization front is uninterrupted.

During the secretory stage, enamel crystals do not grow continuously, but rather extend in increments. Each increment represents the amount of crystal elongation that occurs in a single day, and is manifested structurally as prism cross-striations. More prominent cross-striations occur in a regular period of about every nine days and are known as striae of Retzius or incremental lines (Figure 4). The striae of Retzius terminate at the enamel surface at the edge of tiny steps known as perikymata. The amount of enamel deposited in a given day may vary according to systemic factors and can lead to distinctive patterns of incremental lines that are faithfully reproduced in the enamel of all the teeth forming at a given time. At birth, the infant is suddenly separated from maternal nutrition and must establish its own system of diurnal rhythms. Birth is associated with a prominent incremental line known as the neonatal line, which may be associated with increased caries susceptibility.

Enamel Formation During the Maturation Stage: Crystal Thickening

During the maturation stage, enamel crystallites grow in width and thickness to replace the lost organic matrix, causing the enamel layer to harden. Most of the mineral in the enamel layer is deposited during the maturation stage. The ameloblasts move calcium, phosphate, and bicarbonate ions into the matrix and remove water. The bicarbonate is generated by carbonic anhydrase II, which is strongly expressed by ameloblasts, starting in the transition stage. A significant feature of the maturation stage is that the pH of the fluid surrounding enamel crystals oscillates from less than 6 to 7.2 (Figure 5). These pH fluctuations are similar to those the tooth will

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**Figure 3A. Consecutive sections of a developing porcine incisor showing the stages of enamel formation.**
The top section is stained with toluidine blue and the bottom section is immunostained with the 32-kDa enamelin antibody. Key: enamel E; dentin D; matrix formation stage F; transition stage T; maturation stage M; arrowheads mark the DEJ. Adapted from reference 57.

**Figure 3B. Secretory and nonsecretory faces of the Tomes’ process.**
This histologic section is immunostained for the enamelin C-terminus. Note the enamelin is concentrated at the mineralization front beneath the secretory face of the Tomes’ process. The nonsecretory face of the Tomes’ process represents a discontinuity at the mineralization front and causes a partial, yet distinct, separation between rod and interrod enamel. Key: Tomes’ process TP; secretory face SF; nonsecretory face NF; endoplasmic reticulum ER; enamel E. Adapted from reference 37.
experience naturally, following its eruption into the oral cavity. The developing enamel crystals are not structurally homogeneous. Crystals that are more susceptible to acid dissolution (i.e., those with high carbonate content) are selectively removed during the low pH part of the cycle. Therefore, during the maturation stage, an evolutionary process occurs in which relatively acid labile mineral is replaced by more acid-resistant apatite. This process also occurs in the oral cavity following eruption, so that enamel generally becomes more caries-resistant over time.

In some dental procedures, the crown of a tooth may become exposed to the oral cavity prematurely. This happens, for instance, when an unerupted third molar with open root apices is transplanted into the socket of an extracted first molar. Such a tooth would be expected to decay rapidly due to the incomplete state of enamel maturation, and should be treated with fluoride and sealant as soon as possible after the transplantation procedure. At the end of the maturation stage, about 90 percent of the enamel volume is mineral, which contains less than 1 percent residual protein. Vital bleaching is a popular esthetic treatment that uses high concentrations of peroxide to remove extrinsic stains and which may also remove residual organic material from superficial enamel. Despite this, vital bleaching has not been associated with the development of structural weaknesses in the enamel. This suggests that enamel degradation products retained in the finished enamel do not contribute significantly to enamel’s structural properties.

Enamel Proteins

Ameloblasts actively secrete enamel proteins, starting just prior to the onset of dentin biomineralization and continuing strongly until the end of secretory stage. The three major structural proteins are amelogenin (80-90 percent of total enamel protein), ameloblastin (5-10 percent), and enamelin (1-5 percent). These proteins are secreted at the mineralization front where they appear to form assemblies responsible for incremental increases in the lengths of existing crystallites.

Amelogenin (18- to 25-kDa)

Amelogenin is the most abundant enamel protein. Amelogenin is expressed from genes on the X and Y chromosomes, with about 90 percent of all
RNA transcripts coming from the X-chromosome. The X and Y copies of the amelogenin gene do not undergo homologous recombination (the trading of DNA that occurs between equivalent segments on paired chromosomes). Because of this, the amelogenin gene is the preferred genetic marker for sex determination in forensics.\(^{41,42}\) Mutations that knock out the human X-chromosomal copy of the amelogenin gene result in inherited enamel malformations known as X-linked amelogenesis imperfecta or AI. In females with this condition, there is a Lyonization effect; that is, cohorts of ameloblasts that inactivate the X-chromosome carrying the wild-type amelogenin gene produce defective enamel, while cohorts of ameloblasts that inactivate the X-chromosome carrying the mutated amelogenin gene produce normal enamel. Clinically, the teeth of affected females show alternating vertical bands of defective and normal enamel.
Because amelogenin is the predominant protein in the developing enamel matrix, it is believed to be the most critical component and is the best characterized. Amelogenin is represented in the matrix by a number of different isoforms, generated by alternative splicing of the primary amelogenin RNA transcript. Most of the isoforms can self-assemble into spherical structures, each containing about 100 molecules of amelogenin. These spheres average about twenty nanometers in diameter and have been referred to as nanospheres. There is a good correlation between the size of the nanospheres and the spacing of enamel crystallites. Since enamel crystals eventually grow until they contact adjacent crystals, the nanosphere dimension may ultimately dictate the width and thickness of enamel crystals (Figure 5).

Amelogenin nanospheres may interact with other structures. Amelogenins bind N-acetyl-glucosamine, a sugar group commonly found in glycoproteins. Amelogenin has two other properties worth mentioning. First, intact amelogenin binds tightly to enamel crystals. This binding is mediated by the C-terminal segment, which is removed by proteinases a short time following amelogenin secretion. As a consequence, amelogenin cleavage products have a low affinity for enamel crystals. Second, amelogenin is rich in the amino acid histidine, which is capable of absorbing hydrogen ions and thereby buffering the enamel fluid.

Ameloblastin (65-kDa)

Ameloblastin is an O-linked glycosylated protein that is proteolytically cleaved in its N-terminal half a short time after its secretion. Proteolytic cleavage products containing its C-terminus disappear rapidly and can only be detected within thirty microns of the developing enamel surface and only in the rod and interrod enamel. Ameloblastin cleavage products not containing the C-terminus accumulate in the sheath space throughout the entire thickness of developing enamel, and are relatively absent from the rod and interrod enamel. In contrast to amelogenin, ameloblastin does not appear to engage in protein-protein interactions either with itself or with other enamel proteins. At least two ameloblastin isoforms are generated by alternative RNA splicing.

Enamelin (180- to 190-kDa)

Enamelin is the largest enamel protein. About a third of its apparent molecular weight comes from glycosylations. Intact enamelin is restricted in its distribution to the mineralization front, which suggests that it may be directly involved in the catalysis of crystal elongation. Enamelin is first cleaved proteolytically near its C-terminus. Most enamelin cleavage products are unstable and do not accumulate in the enamel matrix. Those that do accumulate localize to the rod and interrod enamel from the DEJ to the enamel surface and are relatively absent from the sheath space. The human enamelin and ameloblastin genes are located on chromosome 4q in a region previously linked to amelogenesis imperfecta. Both are candidate genes in the etiology of autosomal forms of AI.

Enamel Proteinases

Enamelysin (MMP-20, 45- and 41-kDa) functions during the secretory stage to process enamel proteins into a series of cleavage products that accumulate as the enamel crystallites grow in length. During the early maturation stage, kallikrein-4 (KLK4, 34- and 31-kDa), which was originally isolated from developing teeth and designated enamel matrix serine proteinase 1 (EMSP1), degrades the enamel protein matrix, which facilitates its reabsorption into ameloblasts.

Diagnosis of Developmental Defects of Enamel

The diversity of enamel malformations observed in patients with amelogenesis imperfecta is believed to reflect differences in the timing, during amelogenesis, when the disruptions occur. Flaws incorporated during formation of the dentino-enamel junction can result in an enamel layer that shears easily from the underlying dentin. Secretory stage defects result in insufficient crystal elongation and leave the enamel layer pathologically thin, or hypoplastic. Maturation stage defects, such as those that might occur if the enamel matrix is not properly degraded and reabsorbed, produce an enamel layer that is of
normal thickness, but is pathologically soft. Enamel defects that are not inherited usually reflect a systemic disturbance. Only teeth actively forming enamel at the time of the illness are affected. The timing of tooth calcification and eruption is known,\(^{37,51}\) so that the timing of systemic disturbances that affect enamel mineralization can be estimated.\(^{52,53}\) The chronology of calcification and eruption of the dentition is also used to estimate age at the time of death in forensics\(^{54}\) and anthropology.\(^{55}\)

The Future

At the present time, the genes responsible for amelogenesis and dentinogenesis, imperfecta, odontodentia, and many other disorders affecting the hardness, color, size, shape, and number of teeth are being identified. In the future, genetic testing will be available to identify the specific mutation that causes the inherited disease in a given family. Dental professionals will order these tests, first, to permit the positive diagnosis of each member of a kindred and, second, to improve treatment decisions. For instance, not all forms of amelogenesis imperfecta respond favorably to enamel bonding.\(^{56}\) It may be that successful bonding will correlate to which genes are affected. That is, it may be found that mutations in the KLK4 gene, for instance, result in enamel malformations that do not respond to etching and bonding, while those caused by defects in the enamelin or ameloblastin genes do.

As the molecular mechanisms of dental enamel formation become understood, it is likely that synthetic enamel will become available as a restorative material. Recombinant enamel proteins are currently being tested for their effects on crystal growth, but significant breakthroughs are needed before anything like dental enamel can be synthesized in the lab. In the longer term, stem cell research combined with advances in our understanding of tooth development may lead to the growth of teeth in culture for use as dental implants. This would require a significantly improved understanding of the dauntingly complex processes of tooth development, but is theoretically possible.

REFERENCES