Enamel: Composition, Formation & Structure

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ENAMEL

- It is the hardest calcified matrix in the body.
- Ameloblasts are the cells responsible for enamel formation.
- These cells are lost as the tooth erupts into the oral cavity and hence, enamel cannot renew itself.
- Enamel has a complex structure and high degree of mineralization when compared to other mineralized tissues like cementum, dentin and bone.
- Can withstand mechanical forces during tooth function.
Physical characteristics

- Fully formed enamel has approximately
  - 95% of inorganic mineral
    - Crystalline calcium phosphate (hydroxyapatite)
    - Can be substituted with ions like carbonate, strontium, magnesium, lead and fluoride, if present during enamel formation. These ions affect the susceptibility to dissolution.
  - 4% of organic material/proteins
  - 1% of water
Physical characteristics

- Enamel is translucent
- Varies in thickness – **2.5mm over cusp tips** to feather edge in cervical line
- Color varies from light yellow to gray white. Underlying yellow dentin could be seen in thinner regions.
- Extremely hard
- Brittle in nature. Underlying resilient dentin is necessary to maintain enamel integrity.
  - Loss of dentin by caries/cavity preparation leads to unsupported enamel which fractures easily.
Structure of enamel

- Structure is difficult to study
- Demineralized sections show empty space occupied by mature enamel.
- Fundamental organizational units are the rods (prisms) and inter-rod enamel (inter-prismatic substance).
Structure of enamel

- Enamel rod was first described as hexagonal and prism-like in cross section. But these rods do not have regular geometry and are not prismatic.

- Enamel has closely packed, long, ribbon-like carbonatoapatite crystals (60-70nm in width, 25-30nm in thickness).

<table>
<thead>
<tr>
<th>Salt</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apatite</td>
<td>(Ca$_{10w}$)$_8$(PO$_4$)$_6$(OH)$_2$</td>
</tr>
<tr>
<td>Carbonated apatite</td>
<td>(Ca$_{18w}$Na$_2$)PO$<em>4$$</em>{(y)}$(CO$<em>3$)$</em>{(x)}$(OH)$_2$</td>
</tr>
<tr>
<td>Fluor-hydroxyapatite</td>
<td>(Ca$<em>{10w}$)PO$<em>4$$</em>{(y)}$(F$</em>{(z)}$(OH)$_2$</td>
</tr>
<tr>
<td>w = Na, Mg, K, Sr</td>
<td>x = CO$_3$ or HPO$_4$</td>
</tr>
<tr>
<td>y = Cl or F</td>
<td>z = P$_2$O$_5$ or CO$_3$</td>
</tr>
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</table>
Structure of enamel

- Calcium phosphate unit has a hexagonal symmetry.
- Fully mature crystals are irregular in outline as they are pressed against each other during final growth.
- Rod directions can be studied under electron microscopy.
Structure of enamel

- **Enamel rod** is shaped like a cylinder with crystals running along the long axis.
- **Inter-rod region** surrounds each rod and crystals are oriented in a different direction.
- The narrow space containing organic material between rod and inter-rod enamel is called **rod sheath**.
Structure of enamel

- In some areas, there is no space or rod sheath between rod & inter-rod enamel.
- The rod crystals are confluent with inter-rod enamel. This is compared with the *shape of a key-hole*.
- Hence, basic pattern in mammalian enamel are **cylindrical rods embedded in the inter-rod enamel**.
Amelogenesis

- Amelogenesis or enamel formation is a **two-step process**.
- When enamel forms, it is only partially mineralized (30%)...
Light Microscopy of Amelogenesis

- Short columnar cells of the IEE show **reversal of polarity** towards the stratum intermedium.
- Induces differentiation of ectomesenchymal cells to odontoblasts.
- Dentin formation initiated.
- Induces the ameloblasts to lay down the initial layer of enamel protein without rods.
- Ameloblasts move away from dentin surface.
At the late bell stage, the IEE differentiates into ameloblasts and as it differentiates, it induces the undifferentiated ectomesenchyme to form odontoblasts.

Odontoblasts forms the initial dentin which will in turn induce ameloblasts to secrete enamel.

IEE have been shown to express and secrete several growth factors – TGF β1, BMP2, IGF, Enamel proteins.

Reciprocal Induction
Light microscopy of amelogenesis

- Can be seen in **late bell stage** of tooth formation.
- The low columnar cells of inner enamel epithelium gradually become taller and columnar as it is traced coronally.
- Nuclei become aligned to proximal ends adjacent to stratum intermedium.
- Ameloblasts lay down partially-mineralized, initial enamel without any rods.
- After this first increment, ameloblasts move away from dentin surface.
- Enamel can be seen as a deep-staining layer.
Light microscopy of amelogenesis

- **Tome's process** is the cytoplasmic extension of ameloblasts giving a **picket-fence or saw-toothed appearance**.
- Blood vessels invaginated deep into the enamel organ to form a convoluted structure called **papillary layer**.
- Ameloblasts and papillary layer regress after enamel maturation.
- **Reduced enamel epithelium** is formed that remains till the tooth erupts and interacts with oral epithelium to form junctional epithelium in cervical region.
Life cycle of ameloblast

1. Morphogenetic stage
2. Histo-differentiation stage
3. Formative stage
   - Initial secretory stage (no tome’s process)
   - Secretory stage (tomes process)
4. Maturative stage
   - Ruffle ended ameloblast
   - Smooth ended ameloblast
5. Protective stage
6. Desmolytic stage
Morphogenetic Stage

- Cells are **short columnar** with **large oval nuclei in the center**.
- Golgi apparatus and centrioles are located in the proximal end of the cell.
- **Mitochondria are evenly dispersed** throughout the cell.
- During differentiation, mitochondria migrate to the proximal part of the cell.
- IEE is separated from the dental papilla by a **delicate basement membrane**.
Organizing stage

- IEE differentiate into ameloblasts.
- Ameloblast induces differentiation of dental papilla into odontoblast.
- Ameloblast elongate, *nucleus shift proximally* toward the stratum intermedium (Reversal of polarity).
- Basement membrane fragmented by cytoplasmic projection and mantle dentin formation.
Organizing stage

- Golgi complex migrates distally from its proximal position.
- RER increases significantly.
- Mitochondria cluster in the proximal region.
- Distal junctional complex develops compartmentalizing the ameloblast, into a body and distal extension called Tomes process.
- During the terminal phase of the organizing stage, mantle predentin is formed by odontoblast.
Organizing stage

- Reversal of the nutrition stream occurs due to the deposition of mineralized dentin below the ameloblast.
- Proliferation of capillaries of the dental sac along with the deduction and gradual disappearance of the stellate reticulum occurs.
- Thus, the distance between the capillaries and the stratum intermedium and the ameloblast layer is shortened.
Importance of Junctional Complex

- Alignment of adjacent ameloblasts are maintained by JC.
- These complexes encircle the cells at their distal and proximal extremities.
- Fine actin containing filaments radiate from the JC into the cytoplasm of the ameloblast.
- These JC play a **major role in amelogenesis** by determining at different times, what may or may not pass between ameloblasts (to enter or leave the enamel).
Formative stage

- After the first layer of predentin has been formed, ameloblast enter into this stage.
- Golgi complex is extensive,
- Numerous cisternae of RER occupy a large part of the distal region of the cell.
- The mRNA for enamel protein is translated by ribosomes on the membrane of RER and the synthesized protein is translocated into the ER.
Formative stage

- Enamel proteins progress through the golgi complex for continued post translational modification and are packaged in the membrane bound secretory granules.
- These granules migrate into distal extremity of the cell - into Tomes Process.
- Secretory granules are released against the mantle dentin along the surface of the process to form an initial layer of enamel.
Maturation Stage:

- Enamel maturation occurs after most of the thickness of enamel matrix has been formed in the occlusal or incisal area.
- In the cervical part, enamel matrix formation is still progressing.
- Reduction in height of the ameloblasts
- Decrease in the volume and organelle content.
**Maturation Stage:**

- Ameloblasts undergo programmed cell death *(apoptosis).*
  - 25% of the cells die during transitional phase and another 25% die as enamel maturation proceeds.

- Ameloblasts show modulation, the cyclic creation, loss and recreation of a
  - **highly invaginated, ruffle ended, apical or**
  - **a smooth surface.**

- The cells of the **stratum intermedium** lose their **cuboidal shape** and regular arrangement to assume a spindle shape.
Protective Stage:

- Ameloblast cease to be arranged in a well defined layer and can no longer be differentiated from the cells of the stratum intermedium and outer enamel epithelium.

- These cell layers then form a stratified epithelial covering of the enamel, so called Reduced Enamel Epithelium (A+SI+OEE).

- REE protects the enamel by separating it from the connective tissue until the tooth erupts.
Desmolytic Stage:

- REE proliferates and **induce atrophy of the connective tissue** separating it from the oral epithelium,
- There is **fusion of REE with oral epithelium**.
- Enzymes that are elaborated by the epithelial cells may destroy connective tissue fibers.
- Premature degeneration of REE may prevent the eruption of tooth.
Lifecycle of Ameloblast

Morphological Stage of Ameloblast:

0. Morphogenetic
   (Inner dental epith.)
1. Differentiating (preameloblasts)
2. Secretory ameloblasts
3. Transitional ameloblasts
4. Maturation ameloblasts
5. Protective
   (reduced dental epithelium)

* Main Functional Activities in Enamel:

   Active synthesis and secretion of enamel proteins and initial mineralization
   Enamel reach its full thickness
   Resorption of enamel proteins
   Massive influx of minerals, calcium and phosphates, and selective loss of enamel proteins and water.
   Protect mature enamel from contact with dental sac (follicle) cells.

* Note that many activities take place during each stage of enamel formation but only the main activities for each stage are listed above.
Electron microscopy of amelogenesis

- Ameloblasts have multiple activities throughout their life cycle.
- It is described as 6 phases, with 3 main functional stages,
  - **Pre-secretory** – differentiating ameloblasts show polarity change, develop protein synthetic apparatus and prepare to secrete organic matrix.
  - **Secretory or formative** – ameloblasts secrete and organize the entire enamel thickness
  - **Maturation** – ameloblasts modulate and transport specific ions for mineral deposition.
Electron Microscopy of Amelogenesis

- Pre-secretory stage
  - Morphogenetic phase
  - Differentiation phase
- Secretory stage
- Maturation stage
  - Transitional phase
  - Maturation proper
- Protective stage
- Mineral pathway
SECRETORY STAGE

- **Secretory granules** are stored at the cytoplasm.
- **Tomes process** *initially has only a proximal portion*.
- Content of secretory granules is **released against the mantle dentin** along the surface of the process to lay down initial layer of the enamel which lacks enamel rods.
- **Ameloblasts migrate away from the dentin and develop the distal portion of Tomes process**
Once the distal portion of Tomes’ process is developed, the secretion of enamel proteins is confined to **two sites**.

Secretion from the **proximal part** of the process that is close to JC, around the periphery of the cell along with that from adjoining ameloblasts, results in the formation of **Inter-rod enamel**.

Secretion from one face of the **distal portion** of the Tomes’ process results in the formation of **Rod enamel**.
- Distal portion of Tomes process increases in length but becomes thinner as the rod growing in diameter press it against the wall of inter-rod cavity.
- Distal process **finally disappears** creating a narrow space between rod and inter-rod enamel that fills with organic material forming the **rod sheath**.
- The final layer of enamel protein is formed without the distal extension of Tomes process and lacks the rod and inter-rod configuration.
Maturation Stage

Transitional Phase:

Maturation Proper:

- Modulation of Ameloblasts.
- Cells alternate between ruffle border or a smooth border.
- **Ruffle border** with
  - Proximal Junctions towards stratum intermedium - **leaky**
  - Distal Junctions towards enamel - **tight**.
Transitional phase

- Once full thickness of immature enamel is formed, the ameloblast changes in morphology to prepare for next phase – enamel maturation.
- It involves reduction of height, volume and organelle content of ameloblasts.
- Around 25% of ameloblasts die during transitional phase and 25% die during enamel maturation – cell death by apoptosis & necrosis.
- Apoptosis also occurs in enamel knot and helps in morphogenesis.
- Bcl-2 family of apoptosis regulatory proteins play a key role.
Maturation stage

- Cells alternate with a
  - Ruffled border – introduction of inorganic material
  - Smooth border - removal of organic material
- Water and organic material are selectively removed from the enamel
- Additional inorganic material is introduced by alternate bursts of activity.
Maturation stage

- **Ruffle border** ameloblasts contain
  - lysosomes
  - calcium binding proteins
  - membrane associated calcium ATPases - promote the pumping calcium ions into maturing enamel.

- **Smooth border** ameloblasts
  - secrete enzymes that degrade various matrix proteins extracellularly into small polypeptide fragments.
  - These small fragments leave the enamel layer through leaky distal junctions.
As the ameloblasts begin their modulation cycles, they **deposit a basal lamina** at their flattened apex.

- Basal lamina are rich in glyco-conjugates.
- These glycosylates molecules **help regulate the movement of material into and out of the enamel layer**.
- Ameloblasts attach to it by **hemi-desmosomes**.
Mineral pathway

- Mineralization **spans both secretory and maturation phases** of enamel formation.
- Massive influx of mineral in a short period of time during maturation phase.
- Ameloblasts control the influx of Calcium into mineralized enamel.
- **Calcium moves** from blood vessels thru the enamel organ to enamel.
  - **Intercellularly** in secretory phase
  - **Transcellularly** thru the ruffle ended ameloblast during maturation.
Mineralization of enamel

- **No vesicles** in enamel formation.
- **Immediate formation of crystallites** in newly secreted enamel proteins.
- **No equivalent of pre-dentin / pre-osteoid**.
Mineralization of enamel

- In **four** stages,
  - Primary mineralization
  - Secondary mineralization
  - Tertiary mineralization
  - Quaternary mineralization
Mineralization of enamel

- **Primary** – partially mineralized enamel matrix – 30% mineralization (inner most 8µm next to DEJ, is heavily mineralized – enamelin)

- **Secondary** – starts at the surface, sweeps rapidly into deeper layers until the 8µm layer.

- **Tertiary** – increase in mineral rebounding from the innermost layer out toward the enamel surface. Surface layer is 15µm wide and mineralized more slowly.

- **Quaternary** – outer layer mineralizes rapidly and becomes the most mineralized part of the enamel.
Mineralization of enamel

- Thus enamel is
  - Highly mineralized at its surface
  - Degree of mineralization decreasing towards the DEJ until the innermost layer is reached,
  - 8µm layer has increased mineralization.
<table>
<thead>
<tr>
<th>Name of protein</th>
<th>Features</th>
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| Amelogenin (AMELX, AMELY)                           | • Main protein present in forming enamel  
• Expression stops when enamel has reached full thickness  
• Is a low molecular weight protein that inhibits lateral growth of hydroxyapatite crystals |
| Ameloblastin (AMBN)                                 | • Smaller amounts than amelogenin but larger molecular weight.  
• Seen in newly formed enamel and on outer surface than near DEJ.  
• Assist ameloblasts in adherence to forming enamel surface.  
• Amelin/Sheathlin is the older term |
| Enamelin (ENAM)                                     | • Largest and least abundant  
• Binds strongly to mineral and modulates mineralization and promote crystal elongation. |
| Odontogenic ameloblast-associated (ODAM) and Amelotin (AMTN) | • Secreted by ameloblasts and seen throughout maturation in basal lamina |
| Enamelysin (MMP20)                                  | • Calcium dependant metalloproteinase found in newly formed enamel.  
• Cleaves amelogenin, ameloblastin and enamelin. |
| Enamel matrix serine protease (KLK4)                | • Serine protease belonging to kallikrein subfamily.  
• Secreted when enamel has reached full thickness |
Inner Enamel Epithelium

- Differentiates into Ameloblasts
  - Induces the dental papilla

Ameloblasts

- Differentiation Phase
  - Nucleus, mitochondria - Proximal
  - Golgi ER - Distal,
  - Distal JC is formed
  - Polarized Ameloblast

Odontoblasts

- PreDentin

...contd
Induced Ameloblasts → Initial Enamel Layer → Migration of Ameloblast away from the Dentin surface → Tomes’ Process → Proximal Process → Distal Process

- Extensive Golgi Complex and RER.
  - Protein is synthesized by translation of mRNA
  - Secretory proteins migrate to the proximal part of Tomes’ Process.

No rod and inter rod configuration... contd
Proximal Process

Inter rod substance

Distal Process

Enamel

Distal process goes out of existence

Narrow space filled with org material called Rod Sheath

Enamel rod

Ameloblast become shorter

Final enamel formation

Without rod and inter rod partitions... contd
Transitional Phase

Maturation Stage

Modulation

Ruffle border
- Lysozyme, calcium binding proteins, calcium ATPase pump calcium ions into the enamel.
- Some degraded proteins can be absorbed.

Smooth border
- Interstitial fluid leak to neutralize the enamel fluid.
- Matrix proteins digested by bulk degrading enzymes into polypeptide fragments to leave the enamel through leaky distal junction.

Calcification

Reduction in organelle content and height of Ameloblasts.

Inorganic material replaces water and organic material.
Calcification

Protective Stage

Reduced Enamel Epithelium

Ameloblasts decrease in height and deposit a Basal lamina at their Flattened apex

Ameloblasts fuse with the stratum intermedium and outer Epithelium.

...contd
Life cycle of Ameloblast
LM of Enamel

- Striae of Retzius
- Hunter-Schreger Bands
- Enamel lamellae
- Enamel tufts
- Enamel spindle
- Gnarled enamel
- DEJ
- CEJ
- Enamel cuticle
- Enamel surface features
Striae of retzius

- Incremental growth lines.
- Successive apposition of layer of enamel during formation of enamel.
- Brown bands in ground section.
Striae of Retzius

- In longitudinal section – surround the tip of dentin.
- In cervical part – run obliquely
- DEJ to enamel surface-deviate occlusally.
- In Transverse sections – appear as concentric circles which are compared to growth rings in a tree.
Striae of retzius

- Striae of retzius occurs as a result of temporary constriction of tomes processes, associated with a corresponding increase in secretory face forming inter-rod enamel.
- Lines of retzius represent change in enamel growth- accentuated by disease or change in nutrition.
Nutrition changes prenatally and after birth.

Enamel formed after birth shows accentuated lines of retzius-dividing the prenatal enamel from that produced after birth – NEONATAL LINE.
Hunter Schreger Bands

- Series of alternating dark & light bands seen in L.S section of enamel, when viewed by reflected light.

- Change in direction of rods is responsible for their appearance.

- Originate from DEJ & run perpendicular or oblique to striae of retzius
Hunter Schreger Bands

- Careful decalcification & staining - alternate zones having different permeability & content of organic material.
- **Diazones** - Bands in which rods are sectioned more transversely, appear dark,
- **Parazones** - in which rods are sectioned more longitudinally, appear light.
- It is not just an optical phenomenon.
Enamel Lamellae

- Thin leaf like structures – extending from enamel surface towards DEJ.
- May develop in plane of tension & consist of organic material.
- Lamellae was thought to act as foci of caries - creating a hypomineralized area containing cellular debris + other particles from oral cavity.
- Distinguished from artifactual cracks by decalcification - cracks disappear & lamellae persist.
Three types of lamellae:
- Type a: lamellae composed of poorly calcified rod segment
- Type b: lamellae consisting of degenerated cells.
- Type c: lamellae arising in erupted teeth where cracks are filled with organic matter (saliva).

Type a – within enamel
Type b & c: reach dentin.
Enamel Tufts

- Arises from DEJ –reaches into enamel to about 1/5th -1/3rd of its thickness.
- Appeared to be branched & contain greater concentration of enamel proteins.
- Formed as rows with same orientation as the lamellae.
- Hence seen in abundance in horizontal than the L .S .
Enamel Tufts

- Tufts consists of hypocalcified enamel rods & interprismatic substance.

- Occur developmentally because of abrupt changes in direction of rods that arises from different region of the scalloped DEJ.

- Tufts & lamellae – no clinical significance & do not appear to be sites of increased vulnerability to caries attack.
Enamel Spindles

- The slender projection that transverse the DEJ from underlying odontoblast.
- Project at right angles to DEJ & thereby form an oblique angle to the direction of enamel rods.
- Theory 1: Elongated odontoblastic processes that have insinuated between ameloblast during formative period of enamel production.
- Theory 2: Another theory it has been noted that the processes of ameloblast may project well into the dentin matrix Enamel matrix is deposited in the territory of dentin & will, therefore surround the processes of ameloblasts.
Gnarled Enamel

- When direction of enamel rod in cuspal region of crown become irregular & twisted – **GNARLED**.
- Seen as series of ridges that probably increases the adherence between enamel and dentin.
- This configuration provides strength & resistance to the crushing & shearing stress of mastication.
Dentino-Enamel Junction (DEJ)

- Surface of dentin at DEJ is pitted.
- Into the shallow depression of the dentin, fits the rounded projections of the enamel – Tight grip of enamel
- Appears not as a straight but **scalloped line** – convexities of the scallop directed towards dentin.
Cemento-enamel junction

- Relation between cementum & enamel at cervical region is variable.
- 30% of teeth cementum meet cervical end of enamel.
Cemento-enamel junction

- 10% of teeth cementum & enamel don`t meet.

- Occurs when enamel epithelium in cervical portion of root is delayed its separation from dentin.
Cemento-enamel junction

- 60% - cementum overlaps the cervical end of enamel.

- Occurs when enamel epithelium degenerates at its cervical termination, permitting connective tissue to come in contact with enamel surface.
Cross-striation

- Human enamel is formed at a rate of approximate - 4µ a day.
- Along the length of rod, cross striation – observed at an interval of 4µ - rod a segmented appearance.
- Represent transverse zone or area of organic content.
- Crystallite orientation or localized width difference - creates optical effect of striated marking.
Enamel surface features

- **Perikymata** - striae of retzius often extends from DEJ to outer surface of enamel ending in shallow furrows.
- Run in circumferentially horizontal lines, across the face of crown.
Pellicle

- It is a precipitate of salivary proteins.
- Reforms after an hour—mechanically cleaned.
- Becomes colonized by microorganism to form plaque.
Mammelons

- Developmental structures present in newly erupted incisors.
- Seen as three prominences or scallop along incisal edges.
- Worn off early in life.
Cracks

- appear as jagged lines in various regions of tooth surface.
Fissures & grooves

- Seen on tooth surface of crown.
Clinical implications

• Fluoridation
• Acid etching
• Enamel hypoplasia
• Age changes
Fluoridation

- Fluoride ion into hydroxyapatite crystal - More resilient to acid dissolution.
- Prevents demineralization and in caries prevention.
- If fluoride is present during enamel formation – resistant to acid dissolution.
Fluoridation

- **Semi permeable** nature of young enamel
- Enables topical fluorides, fluoridated toothpaste & fluoridated drinking water to provide an increased higher concentration in enamel surface of erupted teeth.
- Enhances chemical reaction that precipitate calcium phosphate.
ACID ETCHING

- Acid etch or enamel conditioning has become an important technique in clinical practice.
- Use of tissue sealants, in bonding of restorative material & cementing of orthodontic bands to tooth surface.
- Effect is achieved by:
  - removal of plaque & other debris
  - increasing the porosity of exposed surfaces through selective dissolutions of crystals for better bonding surface.
Etching pattern in enamel

- Etching pattern depends upon crystal orientation.
- Ultrastructural studies of crystal dissolution indicate crystal dissolve more at ends than on sides.
- Crystals lying perpendicular to enamel surface are more vulnerable.
Age changes

- Enamel is a non vital tissue incapable of regeneration.
- Masticatory attrition.
- Older patient wear facets & portion of enamel & dentin erodes.
- Discoloration: teeth darken with age.
  - addition of organic material from environment
  - deepening of dentin color,
  - visible through the translucent enamel.
- Permeability decreases with age.
Amelogenesis Imperfecta

- Hereditary enamel defect
- It can be classified according to the stages in which enamel is formed:
  - Hypoplastic
  - Hypocalcified
  - Hypomaturative
Defects in enamel can be caused by febrile diseases.
- Characterized by distinctive bands of malformed enamel.

Defects can be formed by tetracycline induced disturbances in teeth.
- Band of brown pigmentation or even total pigmentation.

Defects in enamel can be caused by interference of Fluoride ion with amelogenesis.
- White patches of hypo mineralized and altered enamel.