Ultrastructural Steps in Amelogenesis

- Maturation (~4 years)
  - Postsecretory (?) ameloblasts (they still form and secrete proteins in humans and other species)
    - Amelogenin, ameloblastin
  - Crystals mature, enlarge and enamel hardens
    - Slow process; can last as long as 5 years
  - Matrix proteins and fluid are replaced
  - Apoptosis of ameloblasts starts
  - **Modulation**: ruffle- and smooth-ended ameloblasts (maintenance of enamel integrity)
  - Secretion of basal lamina material that adheres and further protects enamel (hemidesmosomes)

Modulation during maturation proper stage of amelogenesis

Cyclic creation, loss and recreation of a highly invaginated ruffle-ended apical surface

Cells alternate between possessing a ruffled border or a smooth border

Modulation can be visualized by special stains and occurs in waves traveling across the crown of a developing tooth from least mature regions to most mature regions of the enamel (from cervical to incisal/occlusal direction)

Significance of modulation: Maintaining an environment that allows for accretion of mineral content and loss of organic matrix, in part through alteration in permeability of the enamel organ.
Ruffle-ended ameloblast

- Production of bicarbonate ions
  - Protect the enamel from decalcification
  - Maintenance of pH for matrix degrading enzymes
- Generally, ameloblasts do not resorb the matrix but release matrix resorbing enzymes
- Pumping of calcium to enamel

Smooth-ended ameloblast

- Leak small proteins and water

Basal lamina (nueva)

- Secreted by ameloblasts as they enter the modulation stage
- Formation of hemidesmosomes (HD)
- Laminin-5, amelotin, absence of type IV collagen
Summary of the many functions of the inner dental epithelium during its life cycle

1. Establishment of crown pattern of the tooth (morphogenesis)
2. Differentiate into ameloblasts (histodifferentiation)
3. Active secretion of enamel matrix, wherein they develop Tomes’ process
4. Short transitional phase leading to maturation phase
5. Ameloblasts exhibit modulation wherein ruffle-ended cells allow incorporation of inorganic material and smooth ended cells permits exit of protein fragments and water
6. Protective phase where the newly formed enamel is protected until time for tooth eruption

Proteins (table 7-2)

- Amelogenin
  - Main protein (90%)
  - Several (as many as 9) isoforms (mRNA spliced in numerous ways)
  - Genes present in both X and Y chromosomes. Therefore not homologous and sexual heterogeneity exists. Significance: not known.
  - Undergo minor and major extracellular processing resulting in tyrosine-rich and leucine polypeptides
  - Epithelial-mesenchymal events
  - Controls growth in thickness and width of crystals by forming nanospheres
  - Loss of function: enamel defects that affect overall thickness that lacks enamel rod structure.
  - Hypoplastic amelogenesis imperfecta (no rods)

Hypoplastic type
Generalized Pitted

Hypoplastic type
Generalized Pitted
Ameloblastin
Newly formed enamel; helps stability of DE junction

FUNCTION: Believed to assist ameloblasts in adhering to the forming enamel surface during secretory stage

Loss of function: terminal differentiating ameloblasts detach from the dentin, and enamel formation is aborted

Enamelin (the real one; old=albumin); Largest protein
FUNCTION: Promotes crystal elongation

Loss of function and mutant protein: Nb defined enamel layer

Proteins involved in post-secretory processing and degradation of amelogenins and non-amelogenins

Enamelysin: matrix metalloproteinase (MMP20)
- Found predominantly in newly formed enamel (secretory stage)
- Short term processing of enamel proteins
- Loss of function: Results in formation of a thin hypomatured enamel layer
- Amelogenesis imperfecta, hypomaturation type

Enamel matrix serine protease (kallikrein4)
- Secreted into full thickness enamel when ameloblasts lose their Tomes' processes and start their modulation cycles along the enamel surface
- Slowly degrades residual amelogenins and fragments from non-amelogenins
- Loss of Function: hypomature enamel

Hypomaturation Type
Diffuse X-linked

Hypomaturation Type
Diffuse X-linked (female)
Hypomaturation Type
Diffuse X-linked (female)

Light microscopy of mineralized thin sections through mandibular molars from 16-week-old mice.


Scanning electron microscopy of fractured incisors from AMELX and enamelin null mice.

Proteins

- Amelotin
  - Modulating ameloblasts
  - Resides on basal lamina and junctional epithelium

- Odontogenic ameloblast-associated (ODAM)
  - Modulating ameloblasts
  - Calcifying epithelial odontogenic tumor

Differences from other hard tissues

- There is no pre-enamel
- Crystals grow against the secretory surface of ameloblasts
- Enamel proteins do not play any major structuring function
- No matrix vesicles
- Enamel lacks mineral modulating molecules, i.e. promoters and inhibitors

Mineralization of Enamel
Calcification (in general-not enamel) mechanism

- Not-well understood process
- Deposition of calcium salts on collagen fibrils
- Induced by high-affinity calcium-binding proteins and proteoglycans
- Within cytoplasmic vesicles and released when necessary
- Aided by alkaline phosphatase

Mineralization

- Calcium moves from blood through intercellular and transcellular routes
- Formation of crystallites against mantle dentin.
- Dentin may also provide nucleation of enamel crystallites.

Recent microangiography and computer enhancement studies have suggested that enamel mineralization might actually involve several stages rather than 2 steps. These stages (4 stages) result in a creation of an enamel layer that is:

1. Surface enamel → most highly mineralized
2. Degree of mineralization decreases towards the DE junction
3. Mineralization again increased at the innermost layers
4. "Secondary enamel" forms
5. "Tertiary enamel" forms

Summary of enamel mineralization

1. Cells secrete enamel proteins which immediately participate in mineralization to generate enamel that is ~30% mineralized.
2. Once entire thickness of enamel is formed and structured, it then acquires a significant amount of additional mineral coincident with bulk removal of enamel proteins and water to yield a unique layer consisting of 95% mineral.
3. Above step is under complex cellular control, and associated morphological changes including modulation.
Relationship of Rods

- Circumferential arrangement around the long axis
- Not a straight course except in the cervical
- In the inner 2/3s adjacent groups intertwine
- Generally, perpendicular direction to dentin surface
- Slight inclination toward the cusp
- Near the cusp tip vertical and twisted forming gnarled enamel
- At the cervix, parallel
- At the CE junction they deviate

Striae of Retzius

- Ground sections of calcified teeth
- Incremental lines or weekly rhythm of enamel production
- Neonatal line (is an enlarged striae of Retzius)
- Accentuated lines produced by systemic disturbances.

As crown becomes bigger, new cohorts of ameloblasts are added cervically to compensate for the increase in size. These cell undergo decussation (crossing) as the enamel grows in thickness at a more coronal position. The demarcation between the enamel produced by different cohorts may appear as a line of Retzius.
**Perikymata**
- Surface of enamel
- End points of striae of Retzius
- Circumferentially horizontal lines
- Shallow furrows
- Pellicle

**Cross striations**
- Human enamel (4µm/day)
- Better defined by SEM
- Define changes in the organization of the crystals
- Structural interrelations of interrod and rod enamel

**Bands of Hunter-Schreger**
- Optical phenomenon
- Changes in rod directions
- Ground sections
- Inner 2/3
- Light and dark zones
Gnarled Enamel

Present over cusps of teeth

Rods appear twisted around each other

Complex arrangement

Tufts

• Faults (no clinical significance)
• From DE junction to short distance into the enamel
• Developmental process due to abrupt changes in the direction of groups of rods that arise from different regions of DE junction
• Branched
• Greater concentrations of enamel protein

Lamellae

• From the surface to varying distance within the enamel
• Defects filled with organic material (trapped enamel organ)
• Sometimes throughout enamel
• Different from cracks (cracks don’t contain organic material)
### DE junction – Enamel spindles

- **DE**: Scalloped pattern in cross section
- Spindles: entrapped cytoplasmic processes of odontoblasts
- DE junction: ridges more prominent in the coronal enamel where occlusal stresses are greatest

### Age changes

- Lack of regeneration
- Environmental structural changes
  - Progressively worn – attrition
  - Bruxism
  - Abrasion
- Discoloration – Extrinsic & Intrinsic
- Decreased permeability
- Progressive increase in FL content

### Attrition

![Attrition Image]

### Abrasion

![Abrasion Image]
Enamel Defects

- Hereditary – Amelogenesis imperfecta
- Developmental
  - Enamel Hypoplasia
    - Accidents, febrile disease, fluorosis
    - Fluoride makes the crystals more resistant to acid dissolution by precipitating calcium phosphate
    - Fluoride application in children
  - Tetracycline staining

Enamel hypoplasia due to exanthematous fever (measles, rubella)
Turner's hypoplasia

Tetracycline-related discoloration of enamel

Fluorosis: Chronic ingestion excess of 5ppm
- Acid etching
  - Fissure sealants, bonding, cementing
  - Removal of plaque and increase of enamel porosity
  - Three patterns (depend on crystal orientation)
    - Type I: Loss of rods
    - Type II: Loss of interrod enamel
    - Type III: Haphazard
Crystals lying perpendicular to the enamel surface are most affected to etching.