Development of Tooth and Supporting Tissues
Tooth Development

A. Bud Stage
B. Cap Stage
C. Bell Stage
D and E. Dentinogenesis and amelogenesis
F. Crown formation
G. Root Formation and eruption
H. Function

Initiation of Tooth Development

The initiation of tooth development begins at 37 days of development with formation of a continuous horseshoe-band of thickened epithelium in the location of upper and lower jaws – **Primary Epithelial Band**

Each band of epithelium will give rise to 2 sub divisions:
1. Dental lamina and
2. Vestibular lamina

Figure from Ten Cate’s Oral Histology, Ed., Antonio Nanci, 6th edition
Stomatodeum

Maxillary Process

Stomatodeum

Dental lamina

Developing Tongue

Mandibular process

http://www.usc.edu/hsc/dental/ohisto/
Dental Lamina

- Dental lamina appears as a thickening of the oral epithelium adjacent to condensation of ectomesenchyme
- 20 areas of enlargement or knobs appear, which will form tooth buds for the 20 primary teeth
- Not all will appear at the same time. The first to develop are those of the anterior mandible region
- At this early stage the tooth buds have already determined their crown morphology
- Successional lamina: lamina from which permanent teeth develop
- The dental lamina begins to function at 6th prenatal week and continues to 15th year of birth (3rd molar)

Figures from: http://www.usc.edu/hsc/dental/ohisto/
Position of dental and vestibular lamina

- Nasal septum
- Palatal shelf
- Tongue
- Meckel’s cartilage
Vestibular Lamina

Figure from Ten Cate’s Oral Histology, Ed., Antonio Nanci, 6th edition
Tooth development is a continuous process, however can be divided into 3 stages:

1. Bud Stage
2. Cap Stage
3. Bell Stage
1. Bud Stage

- Bud stage is characterized by rounded, localized growth of epithelium surrounded by proliferating mesenchymal cells, which are packed closely beneath and around the epithelial buds.

http://www.usc.edu/hsc/dental/ohisto/
1. Bud Stage

In the bud stage, the enamel organ consists of peripherally located low columnar cells and centrally located polygonal cells.
2. Cap Stage

http://www.usc.edu/hsc/dental/ohisto/
2. Cap Stage

Condensation of the ectomesenchyme immediately subjacent to the tooth bud caused by lack of extracellular matrix secretion by the cells thus preventing separation. Histodifferentiation begins at the end of cap stage.

Epithelial outgrowth called **Enamel Organ** because it will eventually form the enamel

**Dental Papilla:** Ball of condensed ectomesenchymal cells (it will form dentin and pulp). The peripheral cells adjacent to the inner dental epithelium will enlarge and later differentiate into odontoblasts.
2. Cap Stage

Dental follicle or dental sac is the condensed ectomesenchymal tissue surrounding the enamel organ and dental papilla. This gives rise to cementum and the periodontal ligament (support structures for tooth)
Lateral Lamina: extension from the dental lamina that is connected to the enamel organ

Enamel niche: It is an artifact produced during sectioning of the tissue. It occurs because the enamel organ is a sheet of proliferating cells rather than a single strand and contains a concavity filled with ectomesenchyme

We can also see that the inner and the outer dental epithelium are being organized
Enamel Knot: Densely packed accumulation of cells projecting from the inner enamel epithelium into dental papilla. Exact role not known, but currently believed to be the organizational center for cusp development.

Dental organ or tooth germ is a term used to constitute the structure that has enamel organ, dental papilla and dental follicle.
Enamel knots are clusters of nondividing epithelial cells visible in sections of molar cap stage

Enamel knot precursor cells are first noted by expression of p21 gene expression

Enamel knot and enamel cord are temporary structures that disappear before enamel formation begins. It has been speculated that the function of the enamel knot and cord may be to act as a reservoir of dividing cells for the growing enamel organ
3. Bell Stage

- Continued growth leads to bell stage, where the enamel organ resembles a bell with deepening of the epithelium over the dental papilla

- Continuation of histodifferentiation (ameloblasts and odontoblasts are defined) and beginning of morphodifferentiation (tooth crown assumes its final shape)
3. Bell Stage (Early)

**Inner dental epithelium:** Short columnar cells bordering the dental papilla. These will eventually become ameloblasts that will form the enamel of the tooth crown by differentiating into tall columnar cells. The cells of inner dental epithelium exert an organizing influence on the underlying mesenchymal cells in the dental papilla, which later differentiate into odontoblasts.

**Outer dental epithelium:** Cuboidal cells that cover the enamel organ. Their function is to organize a network of capillaries that will bring nutrition to the ameloblasts. In preparation to formation of enamel, at the end of bell stage, the formerly smooth surface of the outer dental epithelium is laid in folds. Between the folds, adjacent mesenchyme of the dental sac forms papillae that contain capillary loops and thus provide nutritional supply for the intense metabolic activity of the avascular enamel organ.
3. Bell Stage (Early)

Stellate reticulum: Star-shaped cells with processes, present between the outer and the inner dental epithelium. These cells secrete glycosaminoglycans, which attract water, thereby swelling the cells and pushing them apart. However, they still maintain contact with each other, thus becoming star-shaped. They have a cushion-like consistency that may support and protect the delicate enamel organ. It is absent in the portion that outlines the root portions.

Stratum intermedium: Cell layer between the inner dental epithelium and stellate reticulum which have high alkaline phosphatase activity. They assist inner dental epithelium (ameloblasts) to form enamel.
**Dental Papilla:** Before the inner dental epithelium begins to produce enamel, the peripheral cells of the mesenchymal dental papilla differentiate into odontoblasts under the organizing influence of the epithelium. First, they assume a cuboidal shape and then a columnar form and acquire the specific potential to produce dentin. The basement membrane that separates the enamel organ and the dental papilla just prior to dentin formation is called the “membrana preformativa”
3. Bell Stage

Higher power view

http://www.usc.edu/hsc/dental/ohisto/
3. Bell Stage

**Cervical loop**: Area where the inner and the outer dental epithelium meet at the rim of the enamel organ. This point is where the cells will continue to divide until the tooth crown attains its full size and which after crown formation will give rise to the epithelium for root formation. Is also called “Zone of Reflexion”.

http://www.usc.edu/hsc/dental/ohisto/
Enamel cord: Pattern of enamel knot that extends between the inner and outer dental epithelium
3. Bell Stage

Dental lamina (and the lateral lamina) will disintegrate and loose contact with oral epithelium. Sometimes, these epithelial cells will persist when they are called “epithelial pearls” or “cell rests of Serre”

Clinical significance: Cysts will develop in these (eruption cysts) and prevent eruption, or they may form odontomas (tumors) or may form supernumery teeth

http://www.usc.edu/hsc/dental/ohisto/
Eruption Cyst
Future crown patterning also occurs in the bell stage, by folding of the inner dental epithelium. Cessation of mitotic activity within the inner dental epithelium determines the shape of a tooth.
Vascular and Nerve Supply during Tooth Development

**Vascular Supply:** Clusters of blood vessels in dental follicle and papilla

- Clustering of vessels in papilla coincide with position of root formation
- Enamel organ is avascular, however vessels seen in close association in the follicle

**Nerve Supply:** Initially noted in the dental follicle during bud to cap stage

- However after start of dentinogenesis, seen in dental papilla
- Nerve fibers do not enter enamel organ
Clinical Correlation. Several odontogenic cysts and tumors can arise from developing tooth structures. Two such conditions are:

1. Ameloblastoma – which are tumors of odontogenic epithelium that may arise from cell rests of enamel organ or from the developing enamel organ among other things
Histology resembles enamel organ epithelium with peripheral columnar ameloblast-like cells surrounding loosely arranged stellate-reticulum-like cells.
2. Odontogenic Myxoma: Tumor of the jaw that arise from odontogenic ectomesenchyme. Histologically, looks similar to mesenchymal portion of a developing tooth (dental papilla).
Formation of Permanent Dentition

The tooth germs that give rise to permanent incisors, canines and premolars form as a result of further proliferative activity within the dental lamina, lingual to the deciduous tooth germ.

The developing permanent molars have no deciduous predecessor and their tooth germs originate from the dental lamina that extends posteriorly beneath the oral epithelium after the jaws have grown.

http://www.usc.edu/hsc/dental/ohisto/
Essentials of Oral Histology and Embryology,
A timetable to remember
Entire primary dentition initiated between 6 and 8 weeks of embryonic development.

Successional permanent teeth initiated between 20th week in utero and 10th month after birth

Permanent molars between 20th week in utero (first molar) and 5th year of life (third molar)
The development of hard tissues will also be discussed by Dr. Sandra Myers, however

You must remember the following:

- Hard tissue formation starts at the late stages of the bell stage
- Differentiation of cells into odontoblasts and ameloblasts
- Dentin is formed before enamel
- Dentin initiates the formation of enamel
Bell Stage

Deposition of dental hard tissues is called “apposition”

After the crown attains its final shape during cap to early bell stage, the inner dental epithelial cells stop to proliferate, except the cells at the cervical loop

First layer of dentin appears at the cusp tips and progresses cervically, and the columnar cells of the inner dental epithelium become elongated and show reverse polarization, with the nuclei adjacent to stratum intermediate (ameloblasts)

The boundary between the odontoblasts and inner dental epithelium defines the future dentino-enamel junction

http://www.usc.edu/hsc/dental/ohisto/
For dentinogenesis and amelogenesis to take place normally, the differentiating odontoblasts and ameloblasts will receive signals from each other – “reciprocal induction”

Stages of Apposition

1. Elongation of inner dental epithelium
2. Differentiation of odontoblasts
3. Formation of dentin
4. Formation of enamel
At the same time or soon after the first layer of dentin (mantle dentin) is formed, the inner dental epithelial cells differentiate into ameloblasts and secrete enamel proteins. These proteins further will help in the terminal differentiation of odontoblasts. The ameloblasts will then start laying down organic matrix of enamel against the newly formed dentinal surface. The enamel matrix will mineralize immediately and form the first layer of enamel. The formation of enamel is called amelogenesis.

http://www.usc.edu/hsc/dental/ohisto/
Apposition

At the same time when the inner dental epithelium is differentiating, the undifferentiated ectomesenchymal cells increase rapidly in size and ultimately differentiate into odontoblasts.

The increase in size of the papillary cells leads to elimination of the acellular zone between dental papilla and inner dental epithelium.

Differentiation of odontoblasts from ectomesenchymal cells are induced by influence from the inner dental epithelium.

Experiments have shown that if there is no inner dental epithelium, there is no dentin formed.

http://www.usc.edu/hsc/dental/ohisto/
Dentinogenesis

Dentin is formed by odontoblasts that differentiate from ectomesenchymal cells of dental papilla with influence from the inner dental epithelium.

Differentiation of odontoblasts is mediated by expression of signaling molecules and growth factors in the inner dental epithelial cells.

http://www.usc.edu/hsc/dental/ohisto/
Immediately after the inner dental epithelial cells undergo reverse polarization, ectomesenchymal cells immediately subjacent to the acellular layer, will rapidly enlarge and elongate to become odontoblasts and appear like protein-producing cell. The acellular layer is eliminated and the odontoblasts will occupy this zone

Odontoblasts are highly polarized with the nuclei away from inner dental epithelium.

Following differentiation of odontoblasts, first layer of dentin is produced, characterized by appearance of large-diameter type III collagen fibrils (0.1 to 0.2 µm in dia) called von Korff’s fibers, followed by type I collagen fibers – MANTLE DENTINE

At the same time as initial dentin deposition, the odontoblasts will develop stubby processes at the side close to the inner dental epithelium which extend into forming extracellular matrix

As the odontoblasts move pulpward, the odontoblast process (Tomes´ fiber) will elongate and become active in dentine matrix formation

It is initially called predentin and following mineralization is called dentin
Dentinogenesis/Amelogenesis

Essentials of Oral Histology and Embryology,
The odontoblasts as they differentiate will start elaborating organic matrix of dentin, which will mineralize. As the organic matrix of dentin is deposited, the odontoblasts move towards the center of the dental papilla, leaving behind cytoplasmic extensions which will soon be surrounded by dentin. Therefore, a tubular structure of dentin is formed.

http://www.usc.edu/hsc/dental/ohisto/
Odontoblasts with cytoplasmic processes forming dentinal tubules

2 steps of dentinogenesis:
1. Formation of collagen matrix
2. Deposition of calcium and phosphate (hydroxyapatite) crystals in the matrix

http://www.usc.edu/hsc/dental/ohisto/
Amelogenesis is also a two-step process:

1. First step produces a partially mineralized matrix (~30%)

2. Second step involves influx of additional mineral coincident with removal of organic material and water to attain greater than 96% mineral content
Amelogenesis

Stage 1: Matrix production  II: Transitional  
III: Preabsorptive  
IV: Early maturation  
V: Late maturation

Enamel
Dentin
Interameloblast and proximal compartments communicate

Interameloblast and distal compartments communicate
Distal compartment reestablished
Amelogenesis


FIG. 5-10  Diagram of rodent dentin and enamel formation.  
Amelogenesis

Amelogenesis begins after a few µm of dentin deposition at the dentinoenamel junction

Ameloblasts goes through following functional stages:
1. **Morphogenetic.** During this stage the shape of the crown is determined.

2. **Histodifferentiation.** The cells of the inner dental epithelium is differentiating into ameloblasts. The above two stages are the presecretory stages, where the cells differentiate, acquire phenotype, change polarity, develop an extensive protein synthesis machinery, and prepare to secrete an organic matrix of enamel.

3. **Secretory stage:** Ameloblasts elaborate and organize the entire enamel thickness. Short conical processes called Tomes´ processes develop at the apical end of the ameloblasts. The main protein that accumulates is amelogenin.
Terminal bar apparatus

Tomes’ process

Essentials of Oral Histology and Embryology,
Amelogenesis

4. **Maturation stage:** Ameloblasts modulate and transport specific ions required for the concurrent accretion of mineral. At this stage, ameloblast becomes more active in absorption of the organic matrix and water, which allows mineralization to proceed. After the ameloblasts have completed their contributions to the mineralization phase, they secrete an organic cuticle on the surface of the enamel, which is called developmental or primary cuticle.

5. **Protection:** The ameloblast are shorter and contact the stratum intermedium and outer dental epithelium and fuse to form the reduced dental (enamel) epithelium. The reduced enamel epithelium remains until the tooth erupts. As the tooth erupts and passes through the oral epithelium, the incisal part of the reduced dental epithelium is destroyed but the epithelium present cervically interacts with oral epithelium to become the junctional epithelium.
Advanced Stages of Apposition

http://www.usc.edu/hsc/dental/ohisto/
Amelogenesis Imperfecta

Hypomaturation

Hypoplastic

Hypocalcified
Summary of Tooth Development
(So Far)

1. The epithelium is separated from the dental papilla by an acellular zone

2. Inner dental epithelial cells are elongated, and the acellular zone is lost by differentiation of odontoblasts

3. Odontoblasts retreat toward the center of the pulp, leaving behind dentin

4. Ameloblasts begin to migrate outward and leave behind formed enamel
## Time Line of Human Tooth Development

*Table 5-2 in Text book*

<table>
<thead>
<tr>
<th>Age</th>
<th>Developmental Characteristics</th>
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</thead>
<tbody>
<tr>
<td>42 to 48 days</td>
<td>Dental lamina formation</td>
</tr>
<tr>
<td>55 to 56 days</td>
<td>Bud stage; deciduous incisors; canines and molars</td>
</tr>
<tr>
<td>14 weeks</td>
<td>Bell stage for deciduous teeth; bud stage for permanent teeth</td>
</tr>
<tr>
<td>18 weeks</td>
<td>Dentin and functional ameloblasts in deciduous teeth</td>
</tr>
<tr>
<td>32 weeks</td>
<td>Dentin and functional ameloblasts in permanent first molars</td>
</tr>
</tbody>
</table>
Incremental pattern of dentin and enamel formation from initiation to completion

Growth areas of developing crown. Growth at cusp tip, intercuspal region, and cervical region

Development of root begins after the enamel and dentin formation has reached the future cementoenamel junction.

Epithelial cells of the inner and outer dental epithelium proliferate from the cervical loop of the enamel organ to form the *Hertwig’s epithelial root sheath*. The root sheath determines if a tooth has single or multiple roots, is short or long, or is curved or straight. 

http://www.usc.edu/hsc/dental/ohisto/
Eventually the root sheath will fragment to form several discrete clusters of epithelial cells known as *epithelial cell rests of malassez*. *These will persist in adults within the periodontal ligament*.
Epithelial Cell Rests of Malassez

The epithelial rests appear as small clusters of epithelial cells which are located in the periodontal ligament adjacent to the surface of cementum. They are cellular residues of the embryonic structure known as Hertwig's epithelial root sheath.

http://www.usc.edu/hsc/dental/ohisto/
Primary apical formen

Epithelial diaphragm: the proliferating end of the root sheath bends at a near 45-degree angle. The epithelial diaphragm will encircle the apical opening of the dental pulp during root development.

http://www.usc.edu/hsc/dental/ohisto/
Secondary apical foramen form as a result of two or three tongues of epithelium growing inward toward each other resulting in multirooted teeth
Direction of root growth versus eruptive movement of tooth

Tooth eruption and Development of supporting structures

Soon after root formation begins, tooth begins to erupt until it reaches its final position.

While roots are forming, the supporting structures of tooth also develop – periodontal ligament and cementum.

As the root sheath fragments, the dental follicle cells will penetrate between the epithelial cells and lie close to the newly formed root dentin.

These cells will differentiate into cementoblasts, which will make cementum.

Fibers of the periodontal ligament, which will also form from the cells of the dental follicle will get anchored in the organic matrix of the cementum which will later get mineralized.
Oral Ectoderm and Tooth Patterning

Is the epithelium or the mesenchyme responsible for tooth morphology?

Recombination experiments show that at early (E10.5) time point the epithelium directs patterning, however at later time (E11.0) mesenchyme directs patterning – Reciprocal Signaling
What are the molecular mediators of this patterning information?

Future oral ectoderm has acquire a concept of “pre-pattern” through the nested expression of fibroblast growth factors (Fgf), sonic hedgehog (Shh) and Bmps. These signals are then interpreted and refined by the underlying mesenchyme into spatially restricted domains of homeobox gene expression, which in turn regulate other signaling molecules (Bmp, Wnt and Fgf) that induce the epithelial folding and invagination that signal the initiation of tooth development.

Proximal-distal patterning (what decides a tooth is incisor or molar)?
Rostral – caudal patterning
Tooth Number
Shape of the resulting tooth
Dlx and Barx – Molar region
Msx – Incisor region
FGF8 activates LIM homeobox genes (*Lhx* 6 and 7), which is unaffected by BMP4. So *Lhx*6 and 7 are expressed throughout the oral half of the mesenchyme.

Therefore the jaw is divided into a tooth-forming LHX-positive domain and a non-tooth-forming GSC-positive domain. In mice, in which Gsc has been knocked out, the teeth form normally but the supporting skeletal structures in the aboral region are absent — thus defining the rostral-caudal patterning.

Overall called **Odontogenic Homeobox Code**
What are the signals responsible for initiation of tooth bud?

In the epithelium: SHH expression marks the sites of tooth development.

In the mesenchyme, PAX9 and Activin expression marks the sites of future epithelial invagination.

*Pax9* KO - tooth development arrested at the bud stage.

*Runx2* KO – arrest at bud stage however upper molars make it to an aberrant cap stage. Difference explained by the expression of *Runx3* in the maxilla and not mandible.
What is responsible for shape of tooth?

Enamel knots express a wealth of signaling molecules such as SHH, members of FGFs, BMPs and WNT families along with p21(cell cycle) and MSX2 (transcription factor).

High levels of apoptosis also occurs at the enamel knot thereby leading to loss of structure and signaling center at late-cap to early bell stage.

Secondary and tertiary enamel knots develop in molar tooth germs but not in incisor tooth germs – thereby more cusps.

These extra enamel knots express $Fgf4$. 
Genes expressed during tooth development

Website: http://bite-it.helsinki.fi/