Tooth Development - I

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Developmental histology of tooth

Ex) human > heart > muscle > myocyte > nucleate

Gross anatomy
Microscopic anatomy (histology)

Methods
Naked eye
Light microscopy
Electron microscopy

This lecture is about the microscopic anatomy of the developmental process of teeth.

Two types of cells give rise to teeth

Two types of embryonic cells undergo proliferation, migration, and differentiation to form teeth.

Developing tooth

Dental follicle

Ameloblasts forming enamel
Odontoblasts forming dentin
Cementoblasts forming cementum

Proliferation
Increase in number

Migration
Change in location

Differentiation
Specialization

Early embryonic development

Three layers of embryonic cells (ectoderm, mesoderm and endoderm) will be developed to specified tissues in the future.
Tissues originated from ectoderm besides epidermis

- Ectoderm
- Neural tube
- Neural crest
- Ectomesenchymal origin
- Embryonic connective tissue

Ectoderm undergoes invagination to mesodermal side and form two more differentiated structures; neural tube and neural crest. Neural crest gives rise to sensory ganglia and connective tissues of head and neck, including a part of tooth. Therefore, teeth are originated from two types of cells:
1. Ectodermal origin → generate enamel
2. Ectomesenchymal origin → generate dentin, cementum, and pulp

Stages of tooth development
1. Bud stage
2. Cap stage
3. Bell stage
4. Appositional stage (mineralization)
5. Root formation
6. Eruption

Tooth development by complicated continuous processes of proliferation and histomorphological differentiation. For convenience of explanation, the stages of tooth development are separated as presented. However, there is no clear cut between stages.

* The figure only displays changes in the epithelium.
* Note the disintegration of dental lamina in panel d.

Location of tooth formation

Tooth formation occurs at the oral epithelium of maxillary and mandibular processes of embryo.

Stages of tooth development
- Outgrowth of horseshoe-shaped epithelial band from oral ep. (oralarch)
- 10 tooth buds/arch

Bud stage
- Outgrowth of dental lamina from oral epithelium.
- Outgrowth of vestibular lamina from oral epithelium.
- Outgrowth of tooth buds from dental lamina.
Purple dots represent nucleus of cells. Higher density of purple dots represent more dense distribution of cells. Note the higher density of ectomesenchymal cells around the tooth bud.

This is a tooth germ of maxillary tooth. At this stage, epithelial cells proliferate to form enamel organ. Ectomesenchymal cells form two different structures, dental papilla and dental sac.
Future tooth products

Each structure will form tooth and periodontal structures in the future.

Tooth germ means

Tooth germ = enamel organ + dental papilla + dental follicle.

Differentiation of enamel organ

Transformation of a cell mass into morphologically and functionally distinct components

- Enamel organ
- Dental papilla
- Dental sac

Differentiation represent the changes in the properties of the cells. In contrast, proliferation represent the increase in number. At this stage, proliferation and differentiation occur at the same time. Differentiation is obvious in a mass of epithelial cells (enamel organ). Differentiation may start by the secretion of proteoglycan from the epithelial cells to the extracellular space. The absorption of water by the proteoglycan increases the extracellular space. The cells in the epithelial layers are not separated but still maintain contacts between cells via the tight intercellular junction by desmosome. Such changes eventually make the cells look star-shaped. Inner (a side facing dental papilla) enamel epithelium undergoes independent differentiation processes to make them taller and polarized.

- The height of the cells: Squamous < cuboidal < columnar
- Polarized: the nucleus is located at one side of the cell.

Enamel organ of cap stage

1. Inner enamel ep: a layer facing dental papilla. The cells are tall and polarized.
2. Outer enamel ep: a layer facing mesenchyme. The cells are cuboidal or squamous.
4. Stellate reticulum: a mesh like structure composed of star-shaped cells.
5. Enamel knot: a condensation of stellate reticulum at the deepest part of enamel organ.
7. Enamel navel: a depression on the outer enamel ep to which enamel cord is directed.

5-7 are transiently observed during cap stage.
Inner enamel ep is the most important structure since it will generate enamel in the future. Cervical loop will proliferate further to form the shape of crown and eventually guide the formation of root. Stellate reticulum and outer enamel ep might play a role to protect inner enamel ep layer. Enamel knot may be a site where the calcification is initiated and therefore the location of future cusp tip.

Basement membrane: a structure composed of collagen fibers and attachment proteins that connects connective tissue and epithelium. It is a universal structure between connective tissue and epithelium, not a unique structure of the tooth germ.

Proliferation and differentiation of epithelial and ectomesenchymal cells continue and form a larger tooth germ with more complicated structures.

Enamel organ is further differentiated into four distinct layers.
Stratum intermedium represent layers of cells on the inner enamel epithelium that are derived from the retraction of stellate reticulum.

The cells in inner enamel epithelium at this stage are composed of a spectrum of cells with different degree of proliferation and differentiation.

1. The cells at the cervical loop – mainly proliferating
   The ‘proliferation’ of inner enamel epithelium mainly occur at the cervical loop. These cells are younger (recently proliferated and undifferentiated) and the shape is round and short. As the cells proliferate, the cells migrate to the direction of the future root.

2. The cells at the deep part of enamel organ – mainly differentiating
   The cells at the deepest part of the enamel organ stop proliferating and migrating. Instead, the cells mainly undergo ‘differentiation’ to prepare forming enamel. The cells become taller and polarized.

* Note the relative distance from inner enamel epithelium to outer enamel epithelium become shorter as the tooth germ develops.
* The absolute size of the tooth germ become bigger as the development continues. The presented examples are scaled to the similar size for better contrast of the morphological changes.
At the appositional stage, the mineralization starts at the deepest part of the enamel organ. Note that blood vessel does not exist within the enamel organ.

Epithelial and mesenchymal cells interact each other throughout the tooth formation.
1. Mesenchymal cells underlying oral epithelium induce the proliferation of oral epithelium to form dental lamina.
2. Pre-ameloblasts (differentiated inner enamel epithelial cells to form enamel in the future) induce the differentiation of ectomesenchymal cells to preodontoblasts that will synthesize the matrix of dentin.
3. Dentin matrix (uncalcified predentin) induce the differentiation of preameloblast to ameloblast which synthesize the matrix of enamel.
Please remember the order of differentiation.

Preameloblasts → preodontoblasts → odontoblasts → dentin → ameloblasts → enamel

Developmental lobes

Each tooth has multiple starting points of mineralization. The unit mineralizing segment is called a developmental lobe. Coalescence of lobes results in grooves and pits.

Biomineralization

The formation of tooth structure is initiated by the synthesis of matrix protein (collagen in dentin and amelogenin in enamel). The inorganic crystals are accumulated in the matrix protein to form hard tissues. Note that the matrix of enamel (amelogenin) disappears during the mineralization process to allow dense packing of inorganic crystals. In contrast, collagenous matrix of dentin is not resorbed.
Essential components of calcification

\[
\text{Ca}^{2+} + \text{PO}_4^{3-} \rightarrow \text{Ca}_2\text{(PO_4)_6(OH)_2}
\]

Matrix vesicles
- Calcium and phosphate form hydroxyapatite crystals.
- Alkaline phosphatase increases the local concentration of phosphate by the cleavage of organic phosphate at the site of mineralization. Although calcium, phosphate and alkaline phosphatase present abundantly in many tissues, calcification does not occur in every tissue since 'pyrophosphate' inhibits calcification even in the presence of calcium and phosphate. Therefore, inhibition of the role of pyrophosphate by pyrophosphatase is also necessary for the mineralization process.
- The odontoblasts and osteoblasts release matrix vesicles for mineralization. Matrix vesicles contain not only the essential components of calcification but also seeds of inorganic crystals where the growth of crystals can occur easily within organic matrix.

Calcification process

- Appositional stage
  - Ameloblasts and odontoblasts keep moving away from the dentino-enamel junction as they synthesize matrix.
  - Ameloblasts die away once they synthesize a full thickness of enamel. But odontoblasts remains and continuously synthesize dentin in the pulp.
  - Stratum intermedium contains high concentration of alkaline phosphatase.
  - Aprismatic enamel: a layer of first synthesized enamel
  - Mantle dentin: a layer of first synthesized dentin
  - Korff's fiber: collagen fibers of mantle dentin

Acid can reverse calcification

\[
\text{Ca}^{2+} + \text{PO}_4^{3-} \rightarrow \text{calcification}
\]

\[
\text{acid} \quad \text{acidogenic bacteria} \quad \text{Ca}_2\text{(PO_4)_6(OH)_2}
\]

- Dental caries: decalcification of inorganic tooth substances by acid produced by bacteria
- Only very limited remineralization can occur in tooth.
- Bone maintains a balance between mineralization and demineralization.

This is a sketch of electronmicroscopic structures.

Ameloblasts and odontoblasts are actively synthesizing the matrix to form enamel and dentin. Large part of cytoplasm is filled with cellular organelles, such as endoplasmic reticulum and golgi complex, for synthesizing protein matrix.

Ameloblasts and odontoblasts keep moving away from the dentino-enamel junction as they synthesize matrix. Ameloblasts die away once they synthesize a full thickness of enamel. But odontoblasts remains and continuously synthesize dentin in the pulp.