Tooth development ...from initiation until eruption

24.4.2024

Neural crest migration (towards the oral epithelium) Dental lamina formation (epithelial thickening)

> Bud stage Cap stage Bell stage

Aposition (hard tissues formation)

Root formation Eruption

Dental development initiation





Dental vs. Vestibular lamina





Ectodermal – to – (ecto)mesenchymal interactions



Ectodermal – to – (ecto)mesenchymal interactions



Complicated interaction system (reciprocal)

- Inner enamel epithelium induces differentiation of odontoblasts from ectomezenchyme
- Ectomezenchyme transplantation under oral ectoderm induces its conversion into enamel protein producing cells
- Transplantation of ectomezenchyme of incisor under molar ectoderm incisor
- Transplantation of molar ectomezenchyme under cutter ectoderm molar

Odontogenesis (dental development)

Primary dentition as well as permanent dentition develop from both ectoderm and ectomezenchyme

- Ectodermal origin enamel (ameloblasts), ERM
- Ectomesenchymal origin dental pulp, dentin, cementum, periodontium, alveolus
- Similar morphogenesis
- Proliferation, migration, embryonal induction, apoptosis

Primary information for tooth development (number, size) originates from ectomezenchyme (damage to cranial neural crest or its removal causes anodontia)

Ectomezenchyme induces proliferation and differentiation of oral ectoderm into odontogenic epithelium and primary dental lamina

The interaction of ectomezenchyme and oral ectoderm ultimately leads to the formation of a complete organ

Evolutionary conserved pathways

Do birds have teeth?







Do birds have teeth?

- Recent birds lost the ability to form teeth around 70–80 million years ago
- Still, birds retain the evolutionary conserved tooth-forming pathways in both oral epithelium and ectomesenchyme
- *In vitro* co-culture and *in vivo* transplantation experiments



Developmental stages of tooth - overview



Nature Reviews | Genetics

Primary dentition development

Kondenzace ektomezenchymu pod ektodermem dásňového valu na budoucí horní a dolní čelisti (po založení předsíně - vestibulum oris) - ve druhé polovině 6. týdne nitroděložního života

- **Ectomezenchymal condensation** under the oral ectoderm in the upper and lower jaw in the second half of the **6th week** of intrauterine life
- Ektomezenchyme stimulates the basal layer cells of the oral ectoderm to cell division which leads to primary **dental lamina** formation



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Bud stage

After setting up the maxillary and mandibular dental lamina, **10 dental buds** (primordia) are formed on both: maxilla and mandible

The buds proliferate on the side neighboring with ectomesenchyme and increase in size

Primordia formation:

end of the 7th to the beginning of 8th week

(mandibular - firstly)







Cap stage

9. - 10. week of development

Enamel knot formation in the middle of the bud leads to the epithelial overgrowth on sides and formation of the "Cap"

Ectomesenchyme is entrapped and forms dental papilla



Histological differentiation of cells: cells on the surface of the dental follicle become cubic to low cylindrical, while the inner cells become polymorphic (the origin of future stellate reticulum) Similar process in dental papilla Basal membrane - *lamina basalis ameloblastica*



Outer enamel epithelium (OEE)

Balogh and Fehrenbach 2011

FIGURE 6-7 Bell stage, which exhibits differentiation of the tooth germ to its furthest extent. Both the enamel organ and dental papilla have differentiated into various layers in preparation for the apposition of enamel and dentin.

TOOTH GERM/DENTAL ORGAN = ENAMEL ORGAN + DENTAL PAPILLA + DENTAL FOLLICLE



Youtube channel



Hack Dentistry Published on 9 Jan 2019



Youtube channel



Hack Dentistry Published on 9 Jan 2019

Bell stage

10. - 12. week Enamel organ in contact with dental papilla

Enamel organ have 4 layers

Inner enamel epithelium – On the border with ectomezenchyme on dental papilla surface. Tall columnal cells (up to 50 μ m) about 4 μ m thick. Cells annealed to *I. basalis ameloblastica.*

Stratum intermedium – contains 3-5 layers of flattened cells divided by intercellular space and connected with desmosomes

Stellate reticulum – epithelial cell reticulum, star-shaped cells

Outer enamel epithelium – have its own basal lamina, in the beginning cubical and later flattened cells





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Enamel knot

Epithelial signals

Dental Enamel lamina knot BMP BMP PITX2 FGF FGF Shh Shh Wnt Wnt BMP BMP FGF FGF Activin Wnt Odontogenic Dental papilla mesenchyme MSX1 PAX9 RUNX2 Mesenchymal signals

Developmental stages (timing)



Developmental stages (timing)



Aposition

- Period of hard tooth tissue formation
- Begings during the second half of 4th month of foetal development



Mineralization starting poing

- <u>Crown</u> formed firstly (dentin, enamel)
- Enamel and dentin aposition begins in the place of signaling center and spread apically
- Enamel formation = amelogenesis
- Dentin formation = dentinogenesis



<u>**Root**</u> – emegres later (dentin and cementum) Cementum formation = **cementogenesis**



https://www.youtube.com/watch?v=QLNBjHgUHSU



Chronology of primary and secondary dentition formation

Chronology of the deciduous dentition					Chronology of the permanent dentition				
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12-16

17-21

18-25

8-10 years

8

Crown development

- **Polarity change** of cells in inner enamel epithelium (IEE)
- **Preameloblasts** differentiate as a first ones (epithelium differentiate faster then ectomesenchyme)
- Preodontoblasts differentiate based on mutual interactions with preameloblasts
- Lamina basalis ameloblastica degradation
- Maturation of preodontoblasts into odontoblasts = dentin synthesis initiation
- Followed by maturation of **preameloblasts** into ameloblasts = enamel synthesis initiation







FIGURE 6-9 Close-up of inner enamel epithelium of the enamel organ differentiating into the preameloblasts, the future cells that will secrete enamel matrix. A: Inner enamel epithelial cells, with their central nuclei, line up along the basement membrane. B: Inner enamel epithelial cells that have elongated and repolarized their nuclei to become preameloblasts. Note the outer cells of the dental papilla

Balogh and Fehrenbach 2011



FIGURE 6-10 Close-up of the outer cells of the dental papilla, which are induced to differentiate into the odontoblasts and form predentin after the formation of preameloblasts from the inner enamel epithelium. A: Outer cells of the dental papilla line up along the basement membrane with repolarization of their nuclei to become odontoblasts. B: Odontoblasts start dentinogenesis, the apposition of predentin on their side of the basement membrane (*arrows*).

Repolarization of inner enamel epithelium \rightarrow **preameloblasty**

Repolarization of dental papilla cells \rightarrow preodontoblasty

Lamina basalis ameloblastica degradation – by maturation of preameloblasts into ameloblasts



FIGURE 6-12 Preameloblasts being induced to differentiate into ameloblasts and beginning amelogenesis from Tomes' process (*large arrow*), with the apposition of enamel matrix on their side of the basement membrane. Later this membrane will disintegrate and mineralize to form the dentinoenamel junction. Note that the predentin is thicker than the enamel matrix because the odontoblasts differentiate and start matrix production earlier than the ameloblasts. The predentin forms around the dentinal tubules that contain the odontoblastic process attached to the odontoblasts.

Amelogenesis

Amelogenesis

Apexes of differentiated ameloblasts after repolarization are directing towards the deposited dentin matrix and odontoblasts. Base (with nuclei) is located next to the stratum intermedium

Thin and long cells (50 μ m), apical part of cells contain Golgi aparatus and rER (secretory grain formation)

Ecrine secretion of granules above the junction complexes

Granules contains proteins essential for enamel backbone formation:

- a) Amelogenins (90 %)
 - Main product of ameloblasts secretory stage
 - Spherical polymers, regulation of enamel prisms growth

b) Non-amelogenins

- Enamelin Nucleation and direction of growth regulation of crystals
- Ameloblastin Adhesive molecule
- Kalikrein 4 Protease secreted by ameloblasts in the final sectretory stage
- Tuftelin Stabilizes connection to dentin

c) Proteins with enzymatic activity

- Metaloproteases (MMP20) amelogenin degradation
- Alkalic phosphatase, phosphomonoesterase and serinprotease 1



Amelogenesis

All ameloblasts are gradually engaged in enamel secretion and each creates single prism.

Prisms grow from the apical ends of the ameloblasts.

Prisms doesn't grow continuously, but periodically (with regular alternation of the maximum secretory activity phase and the resting phase)

During one cycle the prism is prolonged by about 15-30 micrometers with the manifestation on the dental cuts – the Retzius lines – (ending by perikymata)

Period lenght: about 4 days

Prisms formation - 3 phases:

- **Organic backbone formation** proteins, glykopolysacharides, lipids
- **Mineralization** crystallization centers are formed in the matrix. Hydroxylapatite is deposited here in the form of submicroscopic crystals
- Maturation crystal growth associated with organic matrix loss

Retzius lines



Perikymata





Nutrition of ameloblasts

Develops before the formation of prisms - focal death of cells from the outer enamel epithelium (apoptosis)

Through these openings in the outer enamel epithelium, blood vessels penetrate into the stellate reticulum - providing ameloblast nutrition

Reduction of epithelial reticulum and intracellular mucoid substance

Finally stays only: Stratum intermedium + Ameloblasts





https://pocketdentistry.com/7-enamel-composition-formation-and-structure/



Schematic representation of the various functional stages in the life cycle of ameloblasts as would occur in a human tooth

- 1) Morphogenetic stage
- 2) Histodifferentiation stage
- 3) Initial secretory stage (no Tomes' process)
- 4) Secretory stage (Tomes' process)
- 5) Ruffle-ended ameloblast of the maturative stage
- 6) Smooth-ended ameloblast of the maturative stage
- 7) Protective stage

https://pocketdentistry.com/7-enamel-composition-formation-and-structure/

Before the end of secretory activity, the ameloblasts form a thin layer of organic substance on the enamel surface - **cuticula dentis**



After the end of the secretion, the ameloblasts shorten and blend into the stratum intermedium cells, resulting in the so-called **reduced enamel epithelium** - protects the crown during its pruning.

Crown shape

Zodpovědné procesy:

- 1. Primary patterning of tooth shape enamel knots
- 2. The number of **growth centers** in the dental cup the sites where cells start secretory aktivity. They are defined during the differentiation of ameloblasts by the mechanisms of embryonic induction by odontoblast signalling molecules
- 3. Ameloblasts nutrition during enamel production (Häkkinen et al., 2019 BioRxiv)



Dentinogenesis

Dentinogenesis

The dentine matrix is secreted by the odontoblasts that originate from the surface layer of the ectomezenchyme of the dental papilla

Odontoblasts located on the **growth center** (top of the dental papilla) initiates secretory activity

Dentine matrix precursors are collected in odontoblasts' apexes located opposite to ameloblasts' apexes

Firstly formed matrix is non-calcified composed of **Collagens (I + III)** and **proteoglycans** (versican, keratansulphate, decorin, chondroitisulphate)

Odontoblasts and ameloblasts are moving away from each other during matrix formation



In addition to the odontoblasts on the growth center, also odontoblasts of other dental papilla compartments are gradually involved into matrix production

All odontoblasts forms together a dentine base of the crown (and later root)





Vývoj subu. Dočasný zub ve stadiu sposice (A), časné stadium vývoje zubu trvalého (B). Embní papils (1), destin (2), sklere (3), orgán skloviny (4).

In the first layers of matrix are only reticular fibres

Radial bundles – Korff's bundles - can be visualized by silver salts (in mantle dentine)

Only after depositing the Korff's bundles, odontoblasts will begin to deposit collagen fibers that run longitudinally - perpendicular to the dentinal tubules

Calcification of the dentin matrix is a complex process of participation of the alkaline phosphatase enzyme. Its aktivity had beed demonstrated both in the bodies and the processes of odontoblast.

4 day increments calcify together (subcrystalline crystallization centers → calcispheres and interglobular regions → calcisphere merging)

Dentine matrix in the closest proximity of odontoblasts never calcify

= predentin





Periodical matrix formation

4-8 um/day

(Ebner's lines on decalcified slides)

+- 4 day increments calcify together (Owen's line on dental cuts)





As the dentine matrix growths, the apical parts of the odontoblasts are pulled out into thinner and thinner processes, and after calcification of the matrix, they are permanently embedded as Tomes' fibers in the dentin channels



Odontoblasts development



Matrix deposition progression

Root development

Root development

- The root dentine begins to develop only after the crown dentine is deposited
- Development is under the supervision of the enamel organ
- Cells from the **cervical loop** proliferate towards the apex of the future root
- Proliferating and extending part of the dental cup, consisting only of IEE and OEE = Hertwig's epithelial root sheath (HERS)



Cervical loop





- Maintaining of Stem cell niche
- Extensive signalization both inside and outside the epithelium
- Model system for the study of tissue regeneration and repair





Dentinogenesis (root)

The inductive effect of the Hertwig's epithelial root sheath (HERS) provide differentiation signals to ectomezenchyme to differentiate into odontoblasts that initiate the deposition of the dentin matrix of the root

Once the dentine of the root is formed, HERS disintegrate and cells of ectomesenchymalorigin from dental sac migrate near the root to form cementum and periodontium

Remnants of the Hertwig sheath in periodontium exist as Epithelial Rests of Malassez (ERM)

Shape of root depends on the shape of apical opening

Apical opening of HERS can be:

- circular one root
- divided by horizontal discs called diaphragms on several secondary apical holes

The number of diaphragms determines the number of roots (branches) of the tooth (in multi-root tooth diaphragm divides papilla into sections)



FIGURE 6-21 Apical view of multiroot development from horizontal extensions (*arrows*) of the cervical loop for (A) a two-rooted tooth, and (B) a three-rooted tooth. Cross section that shows the division that produces three roots (C) on a permanent maxillary molar.





Schematický nákres kořenové diafragmy při pohledu zespodu ukazuje její tvar a změny při vývoji zubu s jedním, dvěma a třemi kořeny.



25.2 Apices of developing roots. (a) Two-rooted tooth; (b) three-rooted tooth.

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Cementogenesis

Dental cementum

- Hard, bone-like tissue covering the root of the tooth
- Yellowish color
- Avascular tissue
- Does NOT rebuild (as opposed to bone tissue)
- Can be resorbed by cementoclasts - during the tooth replacement
- During life, is being replaced by the apposition of new layers of vital tissue

Composed of:

- Cellular part
- ECM



Cementogenesis

Begins after the disintegration of the Hertwig's epithelial sheath

Its place will be taken by ectomezenchymal cells that form a **cementogenic mantle** around the root of the dentine base

By differetniation of ectomesenchymal mantle – cementoblasts are formed

In the beginning, the cementum is deposited very slow, so cementoblasts have time to relocate into superficial layers

- acellular (primary) cementum

In the period just before eruption, cementoblasts forms the matrix much faster which prevents cells to migrate on the surface and they will remain permanently entrapped inside

- Cellular (secondary) cementum



Microscopic anatomy



Cementum matrix

Collagen fibres and calcified amorphous mass Collagen fibres run in bundles (orientation is determined by the forces on teeth)

Cementum is divided by origin into:

Primary (acellular)

Does not contain cementocytes In the range of the entire tooth root Directly connected to the dentine Thickness: **10 to 200 µm**

Secondary (cellular)

Contains cementocytes Especially on dental apexes Grows **up to 500 µm thick**







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