

Chapter 2

Tooth Development

Experimental research on tooth development or odontogenesis is based very largely on the teeth of murine rodents (Butler 1967). Pioneering work by Shirley Glasstone on rat tooth germ cultures gave detailed information about structural, histological, and self-differentiating interactions of explants (Glasstone 1936). However, the dentition of the mouse and that of many other mammals is very different. Mice have highly specialized incisors, distinctive molar patterns, a small number of teeth, and they do not have tooth replacement (Butler 1967).

In vertebrate embryos, a group of cells, called neural crest (NC) cells, separate from the neural tube and migrate away from their parental epithelium to reaggregate with other cells. In the developing embryo, almost all organs, glands, and tissues, such as craniofacial skeleton, cornea, teeth/dentin, thyroid gland, thymus, cardiac septa, adrenal gland, melanocyte, autonomic nerve, sensory nerve, and Schwann cells, have these basic cells (Crane and Trainor 2006; Alberts et al. 2008; Lee et al. 2010a). In addition to the unique invasiveness of NC cells, their contribution toward building the head of vertebrates has been considered to be a turning point in the evolution of the vertebrates (Gans and Northcutt 1983).

Although NC cells are of ectodermal origin, it has been suggested to call them “mesectoderm” or “ectomesenchyme” since they undergo “mesenchymalization.” This property is important to discussions regarding mesenchymal stem cells since their origin is mesenchyme, which is derived from the mesodermal germ layer (Le Douarin et al. 2004). On the other hand, along with the cranial skeleton and other tissues of the head and neck, odontoblasts and tooth papillae are derived from mesectoderm or ectomesenchym (Le Douarin et al. 2004). Oral ectomesenchymal and ectodermal inductive interactions are the earliest expressions recorded in vertebrate fossils. These epithelial (oral ectoderm) and mesenchymal (ectomesenchyme/mesectoderm) interactions phylogenetically precede the origin of odontogenesis (Moss 1969).

Teeth share similarities with the other ectodermal organs, such as hair, feathers, scales, beaks, and many exocrine organs, in the placode and bud stage. These similarities disappear by morphogenesis. Initiation of tooth development includes a series of sequential reciprocal inductive molecular interactions between the dental epithelium and the underlying ectomesenchymal

cells (Jernvall et al. 2000). While the first signal inducing differentiation comes from the mesenchyme in all ectodermal organs, in tooth development, morphogenetic events are started by the signals from the epithelium (Pispa and Thesleff 2003). Several recombination studies revealed that only the very early first arch epithelium cells (occurring during the 8–11.5th embryonic days (ED) of mouse development) and ectomesenchyme (ED 12) have odontogenic potential (Mina and Kollar 1987; Lemus 1995).

The molecular aspects of tooth development are similar to those in the development of other organs and include epithelial–mesenchymal interactions. A plethora of molecules (approximately 300) are involved in tooth development such as fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), sonic hedgehog (SHH), and wingless integrated (WNTs). As mentioned previously, BMP4 acts antagonistically with FGF8 and this interaction plays a role in the periodic patterning (Neubuser et al. 1997). Irma Thesleff's group from the University of Helsinki has been working on the key features of dental development. Their web site, <http://bite-it.helsinki.fi/>, includes a wide range of data largely originating in previously published reports and is thus a compilation of the work of the researchers in this field. The web site provide a source for the expressions of growth factors, receptors, signaling molecules, transcription factors, intracellular molecules, extracellular molecules, and plasma membrane molecules during the different stages of tooth development in mice, rats, humans, and other species. The molecular dialogue between oral ectoderm and odontogenic mesenchyme during tooth development is very complicated (Fig. 2.1) (Jernvall and Thesleff 2000). There is a vast knowledge about the signal interchanges that are crucial to control differentiation and morphological changes and spatiotemporal expression of specific genes during teeth formation, yet little is known about the regulation of the signals (i.e., down or up) (Tucker and Sharpe 2004). All of the data show that there is no single gene that is directly connected with ontogenesis or the lack of any specific tooth. Instead, tooth initiation and morphogenesis occur by an orchestration of numerous genetic and epigenetic factors (Jernvall and Thesleff 2000; Koussoulakou et al. 2009). At the same time, most of the developmental defects in teeth usually occur as a result of mutations in genes encoding signaling molecules and transcription factors (Koussoulakou et al. 2009), such as mutations in the *PAX9* gene resulting in partial or total anadontia and mutations in *RUNX2* causing supernumerary teeth (Peters et al. 1998; Ryoo et al. 2010).

2.1 Stages of a Tooth Development

Each tooth passes through four morphological stages: initiation, bud, cap, and cell stages (Fig. 2.2) (Tucker and Sharpe 2004).

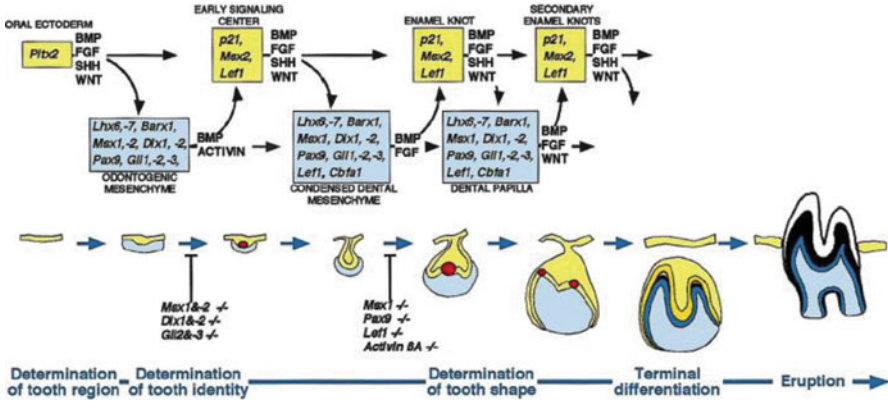


Fig. 2.1 Schematic representation of the signal and transcription factors mediating the reciprocal signaling between epithelium and mesenchyme during advancing tooth development. The molecular cascades are shown *above* and the corresponding morphological stages *below*. The transcription factors and signals considered to be important for particular developmental stages are indicated in the squares and above the arrows, respectively. Note how the same signaling pathways are used reiteratively during advancing tooth development, and how tooth development arrests in the knock-out mouse experiments to the early signaling center or the enamel knot stage. Key: yellow, tooth epithelium; red, enamel knots; blue, tooth mesenchyme (reproduced from Jernvall and Thesleff 2000 with the permission of the publisher)

2.1.1 Initiation

The initiation of tooth begins at the end of the fifth week of human gestation and ED 10 of mouse development. A localized thickening or placodes within the primary epithelial bands, formed after about 37 days of development, initiate tooth development. In a subdivision of the primary epithelial band, the dental lamina, localized proliferative activity leads epithelial outgrowths into the ectomesenchyme. Since the underlying ectomesenchyme is more active than the epithelial cells, these ectomesenchymal cells accumulate the epithelial outgrowths soon afterwards. As those cells fold, the forming structure proceeds as per the following descriptive morphological stages of tooth development: bud, cap, and bell. Folding and growth of the epithelium give the final shape of the tooth crown (Fig. 2.2) (Tucker and Sharpe 2004; Nanci 2008).

2.1.2 Bud Stage

This stage occurs between the 7th and 9th weeks of human gestation and ED 11–13.5 in mice embryos. It is represented by the first epithelial invagination into the oral ectomesenchyme. The internal part of the tooth bud contains star-like shaped, glycosaminoglycan synthesizing stellate reticulum cells. Some cells within the stellate reticulum in mice have been identified as putative stem cells (Bluteau et al. 2008). Odontogenic

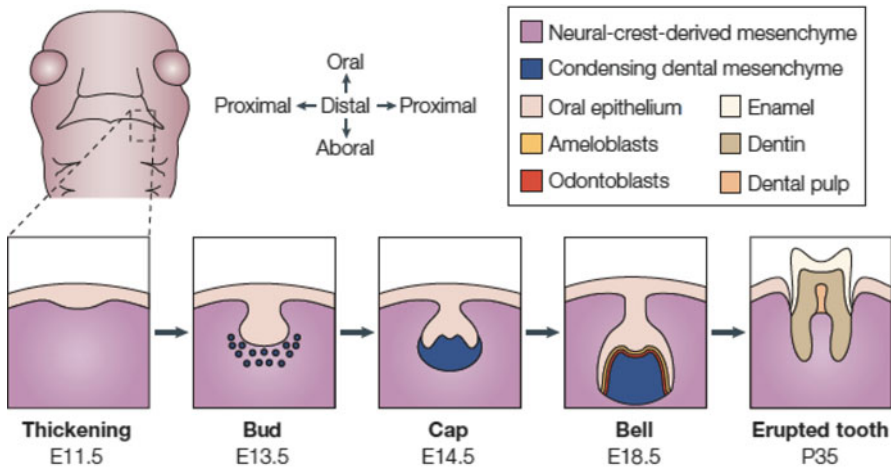


Fig. 2.2 Stages of tooth development. A schematic frontal view of an embryo head at ED 11.5 is shown with a *dashed box* to indicate the site where the lower (mandibular) molars will form. Below, the stages of tooth development are laid out from the first signs of thickening at ED 11.5 to eruption of the tooth at around 5 weeks after birth. The tooth germ is formed from the oral epithelium and NC-derived mesenchyme. At the bell stage of development, the ameloblasts and odontoblasts form in adjacent layers at the site of interaction between the epithelium and mesenchyme. These layers produce the enamel and dentin of the fully formed tooth (reproduced from Tucker and Sharpe 2004 with the permission of the publisher)

potential is switched from the epithelium to ectomesenchyme during the bud stage. While many ectodermal organs such as exocrine glands, hair follicles, beaks, and teeth share morphological similarity in the bud stage, different ectodermal organs become specific from the beginning of bud-to-cap transition (Jernvall et al. 2000).

2.1.3 Cap Stage

The tooth bud transforms into a cap by differential proliferation and in-folding of the epithelium (Koussoulakou et al. 2009). As the epithelial bud cells proliferate, ectomesenchymal cells condense and morphological differences between tooth germs begin during the cap stage. At ED 12, the histologically distinct epithelial mass, called the enamel knot, is induced by WNT and BMP4. Enamel knot cells do not show cell division and after their transient organizing role is complete, they go apoptosis at the end of the bell stage (ED 16) (Jernvall et al. 1998). Histo-differentiation begins late in the cap stage and in the next bell stage the cells of the crown *ameloblasts* and *odontoblasts* are differentiated. A single layer of columnar cells, which border the dental papilla and reside inside the cap, is called *inner dental epithelium* (IDE). The outer part of the cap is covered by the *outer dental epithelium* (ODE) (Marson et al. 2008). While the cap-shaped epithelial growth is widely referred to as *enamel organ*, the condensed ectomesenchymal cells are referred as *dental papilla*. The *dental follicle*

covers the outside of these two substances. The enamel organ, dental papilla, and dental follicle constitute the tooth germ. The dental papilla is separated from the enamel organ by a basal lamina and is located between IDE and undifferentiated mesenchymal cells of the papilla.

2.1.4 Bell Stage

Terminal differentiation of ameloblasts from IDE and odontoblasts from mesenchymal cells of dental papilla, and the formation of two principal hard tissues of the tooth, enamel, and dentin, are initiated during the bell stage. Ameloblast and odontoblast differentiation is regulated by interactions between the epithelium and mesenchyme (D'Souza 2002; Nanci 2008). While dental papilla is the origin of the future dental pulp, dental follicles give rise to cementoblasts, osteoblasts, and fibroblasts. In conclusion, NC cells give rise to dentin-producing cells, odontoblasts; cementoblasts, which produce root dentin covering; osteoblasts, which participate in the formation of dental alveoli; and fibroblasts, which synthesize collagen for periodontal ligaments (Fig. 2.3).

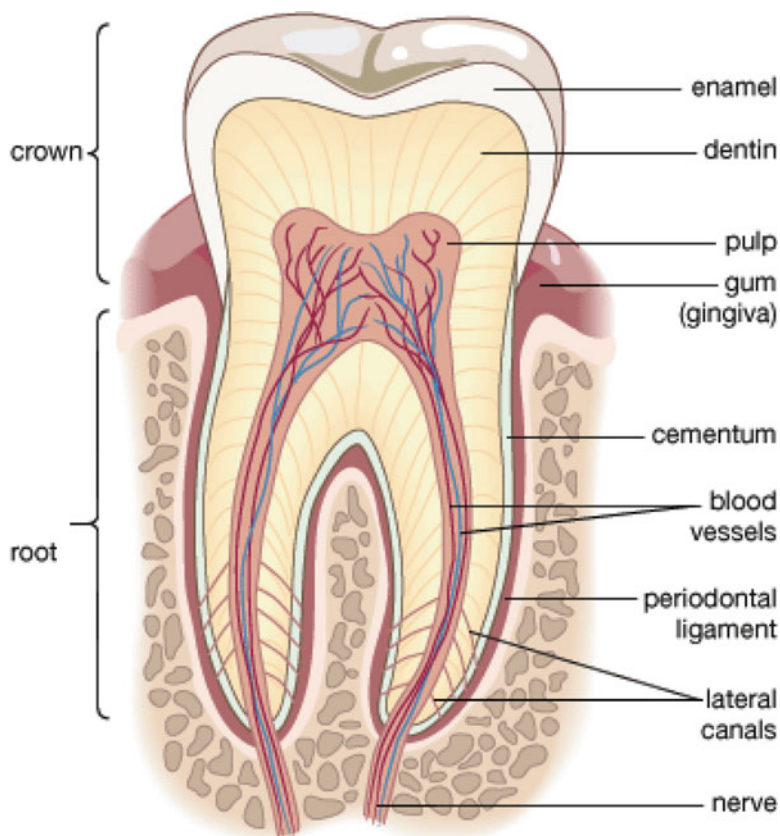
2.2 Odontoblast Differentiation

Although the main function of odontoblasts is dentin production, initiation of innate immune responses and suspected pain transmission are also roles attributed to them (Allard et al. 2006; Farges et al. 2009).

Odontoblast's terminal differentiation occurs according to tooth-specific patterns. In the Swiss mouse molar, the terminal differentiation of odontoblasts starts at the tip of the principal cusps and progressively continues through the apical parts. The specific temporo-spatial pattern of odontoblast terminal differentiation has been characterized by Ruch et al. in the following steps: (1) pre-odontoblasts withdraw from the cell cycle. (2) After the last cell division, the daughter cell that is in contact with the basement membrane elongates and polarizes. (3) These cells start to synthesize first predentin and then dentin components (Ruch et al. 1976, 1982, 1983, 1995; Ruch 1985). All these steps are completed within 6 h in the mouse (Lesot et al. 2001).

Brief descriptions follow:

1. Shortly after the cells of the IDE (pre-ameloblasts) at the sites of the future cuspal tips stop dividing and assume a columnar shape (pre-ameloblasts), the most peripheral cells of the dental papilla enlarge and become organized along the basement membrane (BM). Those cells now become pre-odontoblasts that align as a tall single layer at the periphery of dental papilla adjacent to IDE. Between pre-odontoblasts and pre-ameloblasts, BM exists as the tooth's epithelial-mesenchymal interface. IDE controls those stages and BM plays a major role as a reservoir of paracrine molecules for the continuous reciprocal epithelial-mesenchymal



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Fig. 2.3 The main structures of the tooth (<http://www.britannica.com/EBchecked/media/112882/Cross-section-of-an-adult-human-molar>)

interactions (Ruch et al. 1982; Timpl and Brown 1996). Since isolated dental papillae never give rise to differentiated odontoblasts alone, the specific signals from BM are necessary to initiate odontoblast differentiation (Karcher-Djuricic et al. 1978). Type IV collagen, fibronectin, tenascin, laminin, nidogen, hyaluronic acid, and heparan sulfate are the main components of BM (Lesot et al. 1981). Of note, in mature pulp, BMs are located at the cell-connective tissue interfaces of endothelial cells and Schwann cells (Okiji 2002).

2. From dental lamina formation to the appearance of the first postmitotic odontoblasts, 14 or 15 cell cycles may occur. After a significant lengthening of the duration of the pre-odontoblast cell cycle from approximately 10–14 h, the last cell division occurs and it is asymmetric. The mitotic spindle is oriented perpendicular to the BM during the last cell division, and at the end of the division, only the daughter cell that is in contact with the BM will give a terminally differentiated odontoblast (Fig. 2.4) (Ruch et al. 1982).

- Initiation of odontogenesis by the preodontoblasts (PO)
- Progressive addition of collagens, glycoproteins, and proteoglycans to the BL gives rise to stage specific basement membranes (BM)
- BMx might control the orientation of the spindle of PO. The daughter cells become potential mature preodontoblasts (MPO). Only the MPO in relation with the BM will overtly differentiate
- MPO controls the turn-over of proteoglycans synthesized by the preameloblasts (PA): BMx becomes BMy
- GAG modifications might increase the adenylate cyclase activity of MPO which become post-mitotic (tetraploid?) odontoblasts (PMO). These cells no longer synthesize collagen type III; fibronectin is redistributed. BMy becomes BMz
- BMz modifies the activity of the cytoskeleton: PMO become polarized odontoblasts (PoO). PoO present amplification of collagen type I and type I trimer syntheses
- The predentin (PD) secreted by functional odontoblasts (FO) maintain their functional state. The BL disappears, PA become postmitotic ameloblasts (PMA)

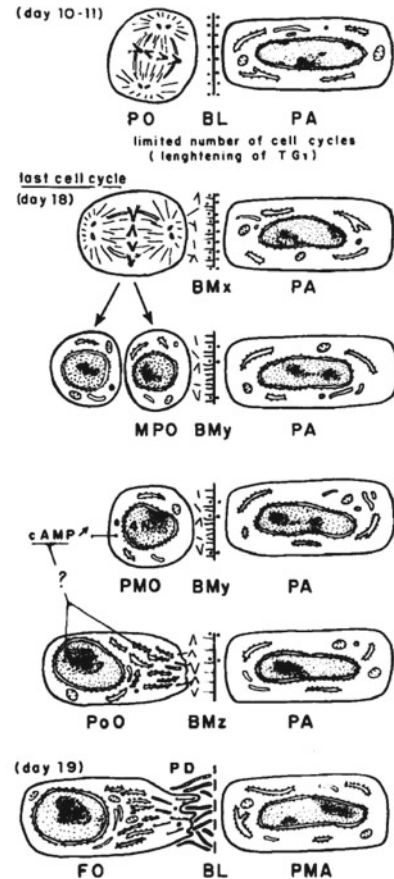


Fig. 2.4 Hypothesis concerning odontoblast differentiation (reproduced from Ruch et al. 1982 with the permission of the publisher)

3. This cell rapidly polarizes as the nucleus takes up an eccentric basal position and shows active protein synthesizing properties (Ruch et al. 1982; Ruch 1985, 1998).

Odontoblasts are post-mitotic cells and their differentiation has many properties in common with the events seen in asymmetric cell division of stem cells (Horvitz and Herskowitz 1992). For stem cells to self-renew, it has been suggested that an intrinsically asymmetric cell division whereby stem cells segregate cell fate determinants into only one of the two daughter cells by two different mechanisms occurs (Fig. 2.5) (Knoblich 2008).

In the niche-dependent type of asymmetric cell division, the mitotic spindle of dividing stem cells orients perpendicularly to the niche surface; hence it ensures that only one daughter cell can maintain contact with the stem cell niche and retain the ability to self-renew (Li and Xie 2005). Alternatively, regulators of self-renewal are localized asymmetrically during mitosis by an intrinsic mechanism, so that those

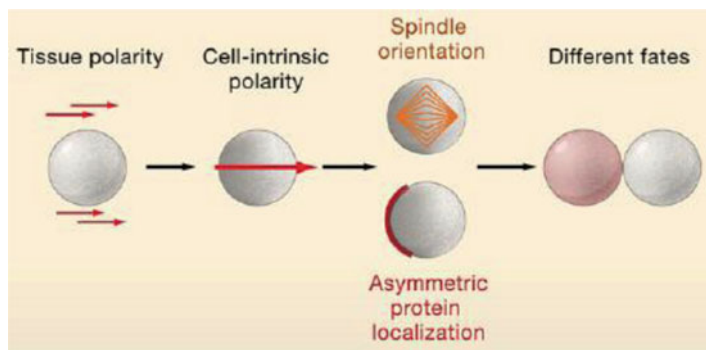


Fig. 2.5 An asymmetric cell division is defined as any division that gives rise to two sister cells that have different fates—a feature that can be recognized by differences in size, morphology, gene expression pattern, or the number of subsequent cell divisions undergone by the two daughter cells (reproduced from Knoblich 2008 with the permission of publisher)

regulators are inherited by only one of the two daughter cells (Yu et al. 2006a). In this sense, the mitotic spindle orientation during primary odontoblast differentiation may become a starting point to chase the stem cells in dental pulp. Obviously there is an asymmetrical division, in which mitotic spindle orientation occurs after withdrawal of the pre-odontoblast from the cell cycle. During the last division, the mitotic spindle, originally parallel to the BM, realigns to be perpendicular to BM and only the cell in contact with the BM is able to enter into the terminal differentiation process and become a fully differentiated odontoblast (Osman and Ruch 1976). The other daughter cell, which is not in contact with the BM, becomes a part of Höhl's cell layer. In 1896, the German scientist Höhl gave his name to those cells and Höhl cells have been assumed to differentiate into odontoblastoid cells when original odontoblasts have died (Höhl 1896). In odontoblast terminal differentiation, BM may act as a niche and after asymmetrical division of the pre-odontoblast, the Höhl cell would continue as a "stem cell" of the dental papilla and reside in the subodontoblastic layer or migrate into deeper pulp, while the other would differentiate into odontoblast.

2.3 Dental Papilla and Follicle

Simultaneous to odontoblast differentiation, IDE differentiates to ameloblasts that secrete enamel just before the first mantle layer of dentin is formed by odontoblasts. It has been thought that the proteins or growth factors secreted by ameloblasts have some effects on the terminal differentiation of odontoblasts, possibly by interacting with components of BM (Nanci 2008). When dentin has formed, the enamel producing cells assemble as a layer. Then, ameloblasts move away from the dentin leaving secreted enamel behind. Differentiating

odontoblasts need signals from differentiating ameloblasts and vice versa, meaning that tooth development needs reciprocal, complex epithelial-mesenchymal interactions (Ruch et al. 1976).

When the first calcified matrix appears at the tip of the principal cusp, the dental papilla is referred as the tooth pulp. The cells of the pulp in this stage are undifferentiated mesenchymal cells and a few collagen fibrils are seen in the extracellular matrix (Nanci 2008). The blood vessels in the dental papilla form clusters, whose position coincides with the root formation positioning. Unfortunately, there is no detailed information about angiogenesis during tooth development. On the other hand, the first nerve fibrils, as well as the vessels, approach the developing tooth during the bud-to-cap transition stage. Nerve fibrils penetrate the papilla when dentinogenesis begins. It has been assumed that the initial innervations is involved in the sensory innervation of future periodontal ligament and pulp (Nanci 2008).

The root is formed via *Hertwig's epithelial root sheet*, which consists of epithelial cells of the IDE and ODE. This sheath extends around the dental pulp and is almost closed except for the little opening, *apical foramen*, in the apical portion of the root. As root formation proceeds, epithelial cells influence the differentiation of odontoblasts from the ectomesenchymal cells at the periphery of the dental papilla as well as cementoblasts from follicle mesenchyme. This leads to the deposition of root dentin and cementum, respectively.

Although this describes the formation of a single root, multi-rooted teeth are formed in the same manner (D'Souza 2002; Nanci 2008). While roots are forming, the supporting tissues of the tooth from the dental follicle also develop. The dental follicle gives rise to various components of the periodontium, namely the periodontal ligament fibroblasts, the alveolar bone of the tooth socket, and the cementum. These structures also play a role during tooth eruption, which marks the end phase of odontogenesis (D'Souza 2002).

2.4 Formation of Permanent Dentition

Permanent dentition has the same pattern as primary dentition and the tooth germs of permanent incisors, canines, and premolars also arise from the dental lamina. However, permanent molars have no deciduous predecessors. Their dental lamina forms posteriorly beneath the lining of the epithelium of oral mucosa into the ectomesenchyme. Albeit at different times, permanent molars form in essentially the same manner of deciduous teeth. While primary dentition takes place between the sixth and eighth weeks of embryonic development, permanent dentition occurs between the 20th week in utero and tenth month after birth, and the permanent first molars between the 20th week in utero and the third molar in the fifth year of life (Nanci 2008).

In conclusion, the development of a tooth can be summarized in Fig. 2.6 (Nanci 2008).

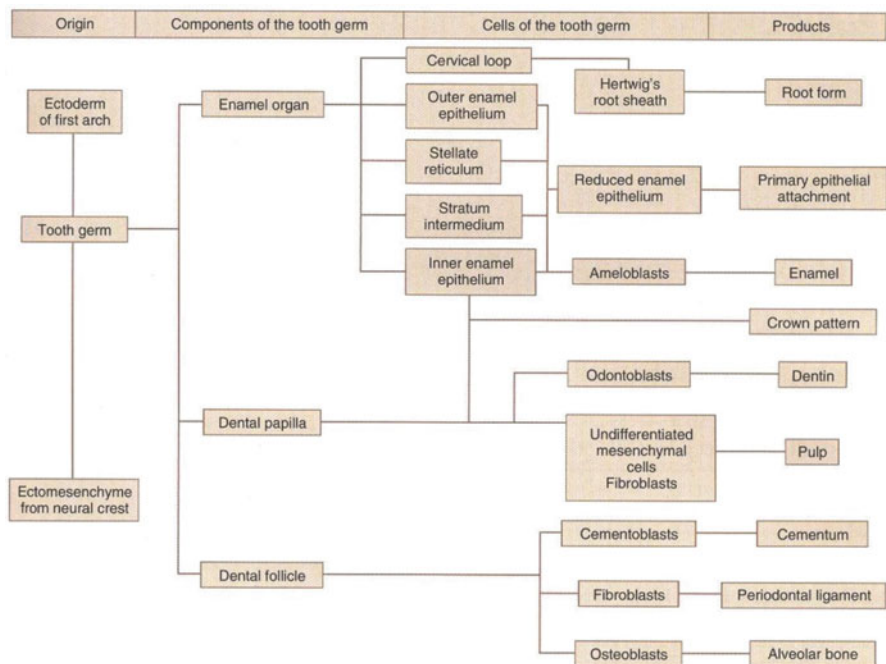


Fig. 2.6 Summary of tooth formation (reproduced from Nanci 2008 with the permission of the publisher)

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Dental Pulp Stem Cells

Yildirim, S.

2013, X, 83 p. 76 illus., 73 illus. in color., Softcover

ISBN: 978-1-4614-5686-5