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The process of mineralisation in the development of human tooth

ABSTRACT

Aim *Tooth development and mineralisation are processes that derive from different tissues interactions, in particular ectodermal and mesenchymal layers. These interactions are responsible for the formation of unique structures with a particular chemical composition. Despite differences, mineralised tissues are similar and they derive by highly concerted extracellular processes that involve matrix proteins, proteases, and mineral ion fluxes that collectively regulate the nucleation, growth and organisation of forming mineral crystals. This review aims at explaining mineralisation, its stages and when damage occurs and alters the hard tissues structure.*

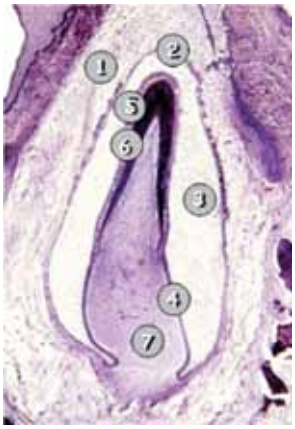
Keywords *Enamel disorders; Germ and tooth development; Tooth mineralisation.*

Introduction

The tooth develops through different chronological stages that can be summarised as follows: growth and differentiation; epithelial proliferation; histological differentiation; organogenesis; mineralisation; eruption; wear and athrophy. At the 6th week of embryonic development, the tooth starts to form. The dental lamina originates from a group of epithelial cells in the oral ectoderm. The underneath mesenchymal layer stimulates the oral ectoderm leading to proliferation of these epithelial cells in the mesenchymal layer, forming the dental lamina. At the 8th week, the buds corresponding to the deciduous teeth appear. They are surrounded by mesenchymal cells from the neural crest.

At the 11th week, the histological differentiation starts its process and the enamel organ develops. This stage is also known as the cap stage. At the end of the 3rd month, in the bell stage, the crown develops and amelogenesis and dentinogenesis start (Fig. 1). At the end of the 5th month, the hard tissues form by mineralisation process. The cap stage and the bell stage are very important cellular signaling centers; in fact, they guide the development process [Simmer et al., 2010]. In addition, during these processes the interface between the epithelium and mesenchyme ultimately differentiates the outer dentin surface. In the developing crown, the outer dentin surface becomes the dentino-enamel junction (DEJ). The cervical limit of the anatomical crown is considered the point where the inner and outer enamel epithelia fuse to form Hertwig's epithelial root sheath (HERS). From then, the interface between epithelium and mesenchyme becomes the outer surface of dentin along the root, which is covered with cementum. In addition, the specific mineralisation process is also controlled by biomolecules that, if stimulated, will inhibit the process, if any injur signal interferes [Atar et. al., 2010]. Knowledge of the mineralisation process is important to understand those diseases that affect the enamel, as they need to be immediately addressed. Enamel integrity is very important because its damage is permanent, as there are no replacing cells.

Speaking about mineralisation, it is fundamental to use the term biomineralisation, when referring to its formation. In particular, there are two kinds of dental hard tissue: enamel, that covers the tooth crown, and dentin, that constitutes the whole body of the tooth. Hard tissue formation involves two main processes: a biological one with cell signaling and a biochemical one with the interaction of biomolecules in crystal apatite formation. Reciprocal cells and matrix interactions play a key role in odontoblasts and ameloblasts differentiation. The biological processes of formation of these two hard tissues are respectively called dentinogenesis



1.	Dental follicle
2.	External epithelium
3.	Stellate reticulum
4.	Internal epithelium
5.	Enamel
6.	Dentin
7.	Mesenchymal cells

FIG 1 Advanced bell stage (courtesy of Edizioni EDRA, Milan:"Manuale di Endodonzia" by Berutti E and Gagliani M).

and amelogenesis. The former occurs always earlier than the latter. In the cap stage, the basement membrane is located between the epithelial cells and the dental papilla. At early stages, the dental papilla cells differentiate and become preodontoblasts. After they have been collected from the mitotic cycle, they become polarising odontoblasts. These cells establish a palisade-like structure, involving the formation of intercellular junctions. When they finally acquire their final polarisation, they become secretory cells.

The final differentiation of odontoblasts seems to be dependent on specific cell membrane/cytoskeleton interactions with basement membrane constituents and other extracellular components. At this stage, the cells show little polarity. The nucleus to cytoplasmic ratio is high, and each nucleus contains one or two nucleoli. In the cytoplasm, numerous free ribosomes can be seen and the rough endoplasmic reticulum (RER) is scarce with short cisternae and only at the beginning of its development. The Golgi apparatus is already formed but at an initial functional stage. Few lysosomal structures are observed. Intercellular junctions are formed at this stage at some distance from the site where extracellular material has accumulated between the cell processes.

At the end of odontoblast polarisation, the junctional complexes have caused the establishment of individual tissue compartments; it means that the predentin extracellular space has become isolated from the pulp extracellular space.

Regarding the biochemical process, a study on the mineral characteristic of dentin is necessary. The mineral of dentin is basically hydroxyapatite, CaO1(OH)2(PO4)6. The mineral crystals in dentin are very close to the collagenous matrix, in that they are largely arranged with their c-axes parallel to the collagen fibers [Margolis et al., 2014]. Induction of mineral crystals during mineralisation of calcified tissues such as dentin is caused by heterogeneous nucleation. This term means that there are macromolecules in the organic matrix that display a specific stereochemical arrangement of reactive groups, possessing an electrical charge or other properties that lower the energy barrier, in order to form a solid phase of calcium phosphate mineral from a solution that would otherwise be stable. The stereochemical geometry and the charge distribution are supposed to mimic certain crystal planes of the crystal to be nucleated. Mineral nuclei formed at these nucleation sites would then grow and fuse in order to form mineral crystals. Because of their unique chemical characteristics, the non-collagenous dentin components, such as phosphoproteins, have come into focus as being responsible for the induction and regulation of mineral formation. Because of their pronounced anionic character, non-collagenous proteins (NCPs) such as phosphophoryn (PP-H) and proteoglycans (PGs) have affinity for Ca2+ ions, suggesting that they may function as hydroxyapatite nucleators *in vivo*. Dentin NCPs can nucleate apatite at physiological ionic conditions. Available evidence shows that, in order to nucleate a mineral phase, the polyanionic macromolecules need to be immobilised by some solid support; they are inductive in quite minute amounts. Free in solution, however, they exert their well-known inhibitory function, but this requires relatively high molecular concentrations. It is likely that the use of polyanionic macromolecules is a general motif for biomineralisation in nature [Margolis et al., 2014].Linde et al. [Linde, 1993; Linde, 1995, Linde et al., 1989] in his numerous reviews, stated that polyanionic NCPs such as PP-H and PG may be responsible for the induction and regulation of mineralisation during dentinogenesis. PP-H and one of the PG pools obviously bypass predentin and they are directly transported to the site of mineral formation, thus giving circumstantial evidence for some function for these molecules in mineral formation. γ-carboxyglutamate-proteins can be localised in odontoblast processes, thus suggesting a direct intracellular transport. The PP-H or PG, first released into the matrix, could promote the formation of the initial mineral crystals, whereas the additional accumulation of NCPs could participate in the regulation of the extent of crystal formation. The strong affinity of calcium ions with PP-H with the ions that are highly mobile on the surface of the molecule, may cause a facilitated calcium ion diffusion that would ensure a rapid formation of calcium phosphate mineral in hydroxyapatite. Different biological and biochemical process regulates the enamel formation and maturation. Enamel is formed by the cellular activity of ameloblasts. Those cells are the result of the terminal differentiation of the inner epithelia, including the expression of tissue-specific gene products. The ameloblasts produce two major classes of proteins: hydrophobic proteins known as amelogenins and non-amelogenin proteins such as anionic enamel proteins (enamelins, tuft proteins, tuftelin), enamel proteases, proteoglycans and/or sulfated glycoproteins [Smith, 1998]. As stated above, amelogenesis starts later than dentinogenesis [Simmer, 2012; Kana et al., 2013]. The enamel proteins production and secretion are subordinated to the relative gene-expression in ameloblasts. For example, the instructive signal which controls amelogenin transcription occurs prior to or during early cap stage. Furthermore, the inducer for tuftelin transcription is possibly different from that required for amelogenin, since tuftelin and amelogenin are sequentially expressed and tuftelin is expressed at the bud. These results support the hypothesis that multiple, sequential regulatory signals, provided by the dental papillae mesenchyme, control the biochemical differentiation of inner enamel epithelia into ameloblasts [Jeremias et al., 2013]. Ameloblasts begin their life cycle as a layer of low columnar, proliferative cells sitting on a basement membrane. In this moment, there is little specificity in the system so that any uncommitted epithelial cell can be useful to become an ameloblast

in combination with appropriate tooth mesenchyme. The cells have their rudimentary Golgi apparatus positioned at the pole of the nucleus on the side away from the basement membrane. Following induction of their differentiation by underlying odontoblasts and/or predentin, the preameloblasts undergo dramatic changes in volume derived from increases in cell height and sagittal width. Ameloblasts secrete small amounts of enamel proteins from their functional apical surfaces as they differentiate. Ameloblasts undergo major reorganisation in cell size and morphological characteristics once the final enamel layer is formed. They first undergo post-secretory transition and revert to a height similar to what they showed as inner dental epithelial cells but with slightly broader width dimensions in the supranuclear compartment. In addition, as much as 25% of the total ameloblast cell population dies during post-secretory transition and another 25% disappears slowly as the enamel matures. Hence, while there is a direct 1:1 ratio between the number of ameloblasts and the number of enamel rods that are created during appositional growth of the enamel layer, there is initially a 1:1.3 and eventually a 1:2 ratio between the number of ameloblasts and enamel rods that are undergoing maturation [Jeremias et al., 2013].

As this happens, row organisation is lost with ameloblasts taking on a more polygonal shape when seen in tangential section. Ameloblasts also undergo major reorganisational changes in their secretory functions during post-secretory transition into maturation. These include:

1. downregulation (but not complete cessation) of secretory activity for enamel matrix proteins;
2. upregulation of secretory activity for proteins that are targeted to the functional apex where they reform the 'inner basal lamina' on the surface of immature enamel;
3. upregulation of synthetic activity for certain enzymes which coat the ruffle-ended apical and degradative enzymes associated with membranes;
4. perhaps upregulation of secretory activity for enamel proteinases or molecules that activate them.

Fully mature enamel represents a signal for ameloblasts to stop modulating and they undergo regression. Enamel biomineralisation differs from that of bone and dentin: for example, it starts its maturation immediately after ameloblasts lay down the enamel matrix and this matrix is transient, unlike the bone and the dentin. Dental enamel forms by the deposition of characteristic, non crystalline, mineral ribbons by a mineralisation front apparatus closely associated with the secretory surfaces of the ameloblast plasma membrane. The shape and orientation of enamel mineral ribbons is established at the mineralisation front and is not due to stereospecific inhibition of mineral deposition on selected crystal faces by acidic enamel proteins. The hierarchical organization of enamel ribbons into rod and inter rod enamel is established by the topographical re-configuration of the mineralisation front that occurs with formation of the Tomes process. The mineralisation front apparatus is the key to enamel formation, and significant advances in our understanding of amelogenesis will be achieved by gaining a better understanding of molecular events occurring at the enamel mineralisation front [Margolis et al., 2014; Linde, 1995; Linde and Goldberg, 1993; Linde, 1989; Simmer, 2012].

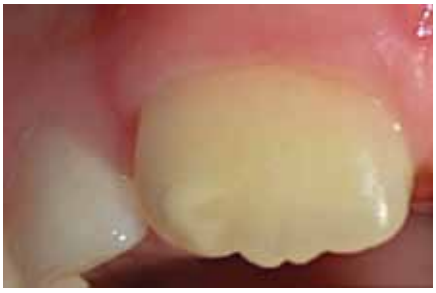


FIG. 2 Clinical appearence of hypo-mineralisation on incisor vestibular surface.

Mineralisation defects

In 1992, a working group of the FDI (World Dental Federation) presented an aepidemiological index of defects relating to the development of the enamel: the DDE index (Developmental Defects in dental enamel). A development problem has been defined as a disease on the matrix of hard tissues and their mineralisation during odontogenesis. The literature suggests that the most frequent developmental defects are hypoplasia and hypomineralisation [FDI Commission on Oral Health Research & Epidemiology, 1992; Fagrell, 2011] (Fig. 2). The aetiology can be either local or systemic. Local defects affect a specific tooth or a group of adjacent teeth, general defects affect instead all the teeth that are mineralised during the insult. The nature of local causative agent can be physical (traumas, radiations) or infective (caries). The general causative agents can be nutritional disorders (calcium levels), systemic pathologies, toxic factors (fluorine) and genetics (amelogenesis) (Table 1). The duration of the insult will determine type and extent of damage [Fagrell, 2011].

Development alterations

Enamel hypoplasia is a quantitative defect and it is the consequence of an altered formation of the enamel matrix. It is caused by a reduction in the thickness of the enamel surface. It can show as:

- single or multiple, deep, spread or organised in horizontal rows dimples;
- single or multiple, narrow or wide, highlighting the absence of enamel, partial or complete furrows on a considerable portion of dental crown. The reduction of thickness of the enamel has rounded and smooth edges and no visible demarcation line. It is however possible to observe the areas of opacity [Fagrell, 2011; Koch and Poulsen, 2001].

The opacity or hypomineralisation of the enamel

Causative agent	Distribution	Example
Mechanic	Local	Trauma to a deciduous tooth can cause sequelae to the respective permanent tooth
Radiations	Local	Radiation therapies can affect both conservative therapy and mineralisation
Infections	Local	The acidic environment of infection / inflammation such as a sequel of osteitis or trauma may disturb the mineralisation
Nutritional disorders	General	Nutritional disorders and/or metabolism disorders of calcium with changes in the levels of Ca- or pH changes in the blood can affect enamel mineralisation
Systemic diseases	General	High fever and infections are examples of a variety of diseases that lead to an increased risk of enamel defects
Toxic factors	General	Fluorine and tetraciciline
Genetics	General	Amelogenesis imperfecta

TABLE 1 Most common enamel alterations [Fagrell, 2011].

Type of alteration	Development alterations	Bacterial alterations	Nonbacterial alterations
Timing	During development	After development Bacterial factors	After development Chemical, physical and mechanical causes
Clinical Appereance	Hypoplasia Hypomineralisation Fluorosis Trauma	Caries	Friction Erosions Abrasions

TABLE 2 Aetiology of enamel and dentin alterations.

comes from its incomplete mineralisation and it is a qualitative defect. The opacity is distinct in diffused and limited lesions. Limited injuries have precise boundaries with the adjacent normal enamel and colours are white, yellow or brown. The opacity can have a linear, irregular or contiguous distribution, but boundaries are not accurate. Mineralisation is incomplete under the enamel surface that remains intact during eruption; it appears as a change of color and transparency of the glaze, whose surface may break due to a trauma produced by masticatory forces, leaving sharp edges.

At the time of the eruption, enamel thickness is normal. The enamel, at a correct degree of mineralisation, is translucent and has an index of refraction of 1.62; if there is a development defect or even a carious lesion, the enamel is more porous and has a lower index of refraction. This condition is clinically visible with a different color that varies from white to yellow, rather than brown. The color is a symptom of change of refractive index caused by an increased porosity of the enamel. It may happen that in severe cases, the surface could collapse and then look like a zone of loss of substance, which may appear similar to hypoplasia, but in hypoplasia there are smooth margins, while in this condition borders are irregular. A particular type of hypomineralisation is the MIH (molar-incisor-hypomineralisation). This type of enamel defect is located primarily in the first molars and incisors in permanent dentition. A number of different denominations have been used for the condition with hypomineralised permanent first molars (i.e. idiopathic hypomineralisation, morbus S, cheese molars). MIH is defined as a general and chronological hypomineralisation of systemic origin of the permanent first molars and incisors. One or more of the molars may be affected, each with different degrees of severity. The permanent incisors may also be affected. Even though MIH is defined as a chronological and general disturbance the number of permanent first molars and the degree of hypomineralisation vary extensively. Enamel in teeth affected by MIH exhibits disorganised enamel prisms, a porous structure and loosely packed crystallites. When the clinical and histological appearance of MIH is compared with normal enamel in a polarising microscope analysis, yellow/ brown enamel opacities appear more porous than lighter opacities [Fagrell et al., 2013; Fagrell et al., 2011; Xie et al., 2008; Jedeon et al., 2013; Jeremias et al., 2013; Crombie et al., 2013; Elfrink et al., 2012; Kuscu et al., 2013] (Fig. 3).

Fluorosis is a qualitative defect of the enamel caused by a long-term consumption of fluoride during teeth formation. The threshold dose for the development of a mild fluorosis of permanent teeth was estimated 40-100(m) g/day of fluoride per kg of weight. It was also found that, individually, there are thresholds below

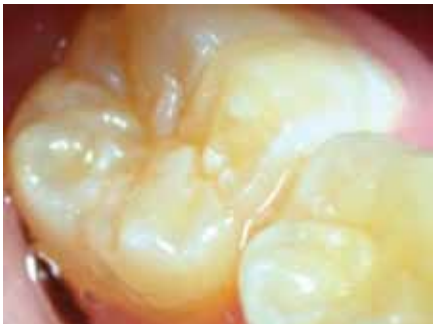


FIG. 3 Hypo-mineralisation on a molar occlusal surface.

which fluorosis cannot develop. The defects of enamel caused by fluorosis vary from little opacity to white, yellow or brownish ones.

Dental trauma can lead to sequelae that result in alterations of the enamel. The most affected teeth are the upper central incisors (30%), both deciduous and permanent. During thier initial development, permanent incisors lie towards the palate and near the apex of the deciduous tooth. It follows that lesions of primary teeth can cause permanent damage to the underlying permanent tooth. The alterations are consequences of developmental disorders that occur on the follicle, whose tear will determine dysfunctions in the development of the enamel.

Bacterial alteration, caries

Tooth decay is an infectious disease caused by bacteria whose diagnosis is based on clinical registration of detectable abnormalities, ranging from small mineral losses to the complete destruction of the tooth. It is a dynamic physical-chemical process that involves the dissolution and precipitation of minerals.

Nonbacterial alterations

- **Friction** Loss of the substance of the teeth due to mutual contact for physiological and/or pathological causes. The physiological causes mainly involving the edge of the incisors, followed by the occlusal surfaces of molars. The pathological causes may be the result of abnormal occlusion, bruxism or abnormalities of the tooth structure. Once the dentin is exposed it acquires a brown coloration.
- **Abrasion** Abnormal loss of tooth surface that derives from the forces of friction between the teeth and the external objects, and between the contact teeth in the presence of an abrasive substance. The most common example is the toothbrush abrasion. It is therefore the pathological wear of tooth substance due to the action of rubbing by a foreign body, regardless of occlusion.
- **Abfraction** It is caused by an abnormal occlusal loading and it predisposes cervical enamel to mechanical or chemical wear. At the level of the cervical region, tensile and compression stress may produce micro-fractures of the enamel.
- **Erosion** Wear or loss of the tooth surface due to a chemical-mechanical action. It is a loss of mineralised tooth tissue caused by chemical processes that do not involve bacteria. These processes are caused by acidic substances of exogenous origin (acidic foods and beverages) or by substances of endogenous origin as stomach acids.

Conclusion

This study aims at explaining the complex chemical-biological mechanism that leads to the development of dental germs and teeth. It is impossible to pinpoint the exact moment when the process drifts or the aetiological causes of important diseases, such as the MIH, without a solid understanding of this long and fascinating process.

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