Ameloblast: An Enigmatic Cell in Enamel Formation

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Abstract

Formation of teeth is a complex process involving many cells and biochemical interactions. Tooth is made up of both hard and soft tissues derived ectodermally and ectomesenchymally. Enamel is the hardest tissue of the human body, forming the outermost covering of the crown of all deciduous and permanent dentition. It is also the only ectodermally derived structure of the tooth, formed by the ameloblast cells of the enamel organ of the tooth bud. Ameloblasts are unique cells in that they not only help in the synthesis of the organic matrix of enamel but also aid in its removal making way for deposition of minerals during its maturation. Once their functions are over, these cells cease and hence are not seen in fully formed teeth.

Key words:Ameloblasts, enamel, tome's process, amelogenesis, odontoblasts

Introduction

Tooth development is the result of the inductive effect of the neural crest cells on the oral epithelial cells which form the dental lamina from which 20 primary and 32 permanent enamel organs develop. These inturn influence the juxtamesenchyme to form the dental papilla.

The mesenchyme surrounding the enamel organ and dental papilla condense to form the dental sac. The structures of the tooth; enamel, dentin, cementum and pulp are derived from these, enamel from ameloblasts (ectodermal origin), dentin and pulp from dental papilla and cementum from dental sac, both ectomesen-chymal in origin. Thus, together enamel organ, dental papilla and dental sac form the tooth bud which undergoes various complex changes to form the tooth. This article reviews in detail the role of ameloblast cell in formation of enamel, the hardest tissue of the human body. Enamel organ is the ectodermal derivative of dental lamina which gives rise to enamel. It undergoes three morphologic stages; bud, cap and bell stage. During these stages, the cells of enamel organ undergo lot of changes and it is the innermost layer lining the concave portion of the enamel organ that differentiates first to preameloblast and then to ameloblast, the cells of enamel synthesis and maturation.

Epithelial - Mesenchymal Interactions

Epithelial: Mesenchymal interactions are usually defined as tissue interactions that bring about the differentiation of one or both of the involved cell populations. During tooth morphogenesis, the instructions originating from ectomesenchymal cells can induce certain cells derived from the oral ectoderm to undergo terminal differentiation into Enamel forming ameloblasts, characterized by the synthesis and secretion of specific and unique extracellular matrix proteins, the enamel proteins. It has been hypothesised that such instructions reside either in specific ectomesnchymal cell surface molecules or in certain informational molecules that reside in the extracellular matrix Most epithelial - mesenchymal interactions, including those that take place during tooth development, demonstrate at least some reciprocity. The list of suspected extracellular matrix molecules includes ions (potassium, calcium), various genetic types of collagen, glycosylate macromolecules, glycoproteins, glycosamino-glycans, proteoglycans, mRNA, nutrients and neutral protease.

The membrane antigens which are thought to play a role are Major histocompatibility complex, differentation allogenic antigens and theta antigens.

The factors causing maturation and cessation of cell division in the inner dental epithelium reside in the ectomesenchyme of the dental papilla. The shape of the crown results from the interaction between dental papillary

ectomesenchyme and the inner dental epithelium of the dental organ, and this interaction influences the growth pattern of the epithelium so that it folds to produce the outline of the crown pattern. Also the preameloblasts differentiate under the control of the ectomesenchyme into secretory ameloblasts. The messenger system responsible for these epithelial – mesenchymal interactions could involve any or all of the following; shuttling of membrane delimited informational matrix vesicles, cell - to - cell communications involving cytoplasmic processes and low resistance gap junctions, ion transfer, specific extracellular matrix components (e.g. collagen, fibronectin) and molecular diffusion and transfer of informational molecules, such as hormones, specific growth factors etc.

Preameloblast

Preameloblasts are low columnar epithelial cells of inner enamel epithelium approximately 40 microns in height; such cells are immature and are undergoing differentiation. They frequently demonstrate mitotic activity, indicating that they have yet to reach maturity. The nucleus of a typical preameloblast is centrally located and elongated. The cytoplasm stains basophilic with haematoxylin and eosin. With the Electron microscope, the cytoplasmic organelles look rather under-developed, especially the Golgi complex and rough endoplasmic reticulum. Free ribosomes are numerous and a moderate number of mitochondria are scattered throughout the cell. Cytoplasmic filaments are also present. Adjacent cells are attached by desmosomes and a few gap junctions. The preameloblast demonstrates numerous cytoplasmic processes, which extend from the distal cytoplasm into and across the subjacent intercellular matrix i.e. basement membrane complex. These cytoplasmic extensions come into close contact with the ectomesenchymal cells of the dental papilla and are thought to play a role in the epithelial mesenchymal interactions in tooth morphogenesis.

Secretory Ameloblast

The secretory ameloblast is an extremely tall columnar epithelial cell approximately 50 microns in height with a highly polarized cytoplasm. It takes its origin from the mitotic activity of a single preameloblast. The secretory ameloblast is a terminally differentiated cell - meaning that it has become so differentiated through cyto, morpho and histo differentiaton that it is incapable of subsequent mitoses. Thus, during amelo-genesis, a steady source of ameloblasts must be provided. That source is the preameloblast population. Due to the sequence of differentiation, the secretory ameloblasts are located more coronally or cuspally in the inner enamel epithelium than are the preameloblasts, which are located cervically. Differentiation occurs initially at the middle area, approximating the crest of the dental papilla and spreads over the slope toward the rim of the bell. Increased cell length, maturation or acquisition of functional competency is accompanied by hypertrophy and reorientation of the organellles. The nucleus is polarized i.e. it shifts position to occupy the base of the cell. The crest cells, those of the incisal edge or tips of the cusps are the first to differentiate, mature, and to deposit enamel; those of the cervical loop are last. Therefore, those of the incisal area or cusp tips possess a longer enamel forming period, whereas the cells of the cervix or base of the cusps have the shorter period of amelogenesis. This accounts for the greater thickness of the enamel in the incisal and cusp tips and thinner enamel over the slope, with minimum thickness at the cervix and the cusp base. As the preameloblasts differentiate under the control of the ectomesenchyme the modifications taking place in the cytoplasm of the future secretory ameloblasts are:

1. The nucleus moves from its more central location to the basal (proximal) aspect of the differentiating cell.
2. The rough endoplasmic reticulum becomes extensively developed in the apical (distal, supranuclear) cytoplasm.
3. The golgi complex, formerly underdeveloped, hypertrophies in the supranuclear cytoplasm, resulting in the formation of multiple golgi complexes.
4. The scattered moderate population of mitochondria, aggregates in the infranuclear or basal cytoplasm.
5. Numerous membrane delimited secretory vesicles (granules) appear in the distal cytoplasm i.e. the cytoplasm nearest the dental papilla and these apparently 'bud' off from the various golgi saccules.
6. The cytoplasm contains numerous microtubules and microfilaments, and these organelles are important to the cytoplasmic shifting of the nucleus, and golgi complex.
7. Microfilaments aggerate both proximally and distally to form terminal webs.
8. The number of cell - to -cell contacts increases, specifically desmosomes and gap junctions. The latter indicates an increase in the cell - to – cell communications between adjacent secretory ameloblasts.
9. The most salient feature of a secretory ameloblast finally appears i.e. the formation of the distally located Tomes' process at the secretory pole of the cell.
10. In addition to secretion of the enamel matrix, some investigators have postulated that the Tomes' process can also function simul-taneously in the resorption / maturation of the enamel matrix. In addition to the structural changes, the secretory ameloblasts also show signs of biochemical differentiation. The enzymes of the citric acid cycle and glycolysis appear. Specific ATPases and phospha-tases are also demonstrable. In general the metabolic activity of the secretory ameloblast is greatly elevated over that of the preameloblasts.

Therefore, as the preameloblasts undergo mitosis and subsequent differentiation the resulting secretory ameloblasts assume the fine structural characteristics associated with a typical protein synthesizing and secreting cell, i.e. the presence of a well developed rough endoplasmic reticulum, an extensive golgi apparatus and numerous membrane delimited secretory granules. The proteins being syntesized and secreted by this cell are the enamel proteins characteristic of the enamal matrix - enamelin and amelogenin, as well as some other organic molecules. Tomes' process is not present at first in the secretory ameloblast. Only after enamel formation is initiated, and the ameloblasts have retreated a short distance occlusally, does Tomes' process form. The first enamel laid down is aprismatic in nature and laid down epitactically on the surface of dentin hydroxyapatite crystals. Prismatic enamel, which forms the bulk of enamel, is made in association with Tomes' process.

Tomes' Process

The tomes' process is a small pyramidal cytoplasmic extension at the distal end of each ameloblast, and it is marked off from the rest of the cell by the terminal bar apparatus. In the electron microscope this shows as a thickening of the cell membrane with associated microfibrils or tonofilaments. The latter passes a short distance into the cell giving the appearance of an incomplete septum between the process and the rest of the ameloblast. With the light microscope the tomes' process seems to be marked off from the rest of the ameloblast by condensations of intercellular substance and this appearance has been described as a terminal bar.

Electron microscopic studies show that the ameloblasts are very closely packed so that their plasma membranes are only separated by a very narrow interval of not more than 100 - 200 Ao in width. The terminal apparatus of the ameloblasts represents a form of junctional complex which is commonly found at the free margins of epithelial cells. At the proximal end is a similar terminal bar apparatus. The Tomes' processes are embedded in the forming enamel. In some sections where the ameloblast layer has been detached from the enamel the Tomes' process can be seen to project from it. The layer of newly formed enamel, when seen in oblique or cross - section, gives the appearance of a honeycomb - like network. The intervals in the network are occupied by the Tomes' process. The cytoplasmic contents of the process are microtubules, microfilaments and membrane delimited secretory granules.

which contain among other things, enamel proteins. The secretory granules are released from the secretory pole of the cell (Tomes' process) by exocytosis.

Maturative Ameloblast

Once the coronal enamel is complete in shape and form, the secretory ameloblasts undergo a type of dedifferentiation to become maturative ameloblasts. These cells function in enamel maturation - a process whereby the immature enamel of the newly made crown is converted to typical mature enamel. The ameloblast is no longer a secretory cell, but rather an absorptive or resorptive cell. The changes which take place in the ameloblasts as a result of change in function are:

1. Disappearance of Tomes' process and replacement by microvilli.
2. Involution of the well developed rough endoplasmic reticulum and golgi complex via autophagosomes.
3. Predominance of lysosomes, vacuoles and mitochondria in distal supranu-clear cyto-plasmic compartment.
4. Increase in the size and number of gap junctions between adjacent cells.
5. Disappearance of the distal terminal web

The cells no longer synthesize and secrete enamel proteins. They produce lysosomal hydrolases which digest the excess organic matrix of enamel to create the space necessary for additional hydroxyapatite crystallization. Thus, as space is created (through protein and water removal) additional calcium phosphate can be added to the existing hydroxyapatite to form the huge hydroxyapatite crystals.

The maturative ameloblast functions in the absorption and uptake of the digested organic matrix and accompanying water. Once internalized in the vacuolar compartment, the matrix proteins are further catabolized. Enamel adjacent to ameloblasts with striated surfaces appears to be more heavily involved in calcium uptake. It has been suggested that the development of the striated border and the occurrence of the enzyme calcium - magnesium ATPase reflects a role in the regulation of the passage of calcium ions to the enamel surface across the cell membrane during maturation. The selective removal of water and organic material and the introduction of inorganic material is a cyclic process which is reflected in the ameloblast morphology. The cell alternates between possessing a ruffled border and a smooth border. The ruffled border ameloblasts are associated with the introduction of inorganic material, the smooth ended with the removal of protein and water. Coincident with these structural changes at the cell surface, facing the formed enamel are changes in function of the distal and proximal junctions between adjacent ameloblasts. Thus, ruffle - ended ameloblasts posses proximal junctions that are leaky and distal junctions that are tight, whereas most smooth – ended ameloblasts have distal junctions that are leaky and proximal ones that are tight. These changes indicate that inorganic material must pass through the ruffle - ended ameloblasts (because their distal junctions are tight) and conversely larger molecules withdrawn from the developing enamel must pass through the leaky distal junctions between smooth - ended cells (because the proximal junctions are tight, be taken up along the lateral surfaces of6these cells). Protein breakdown products may also be absorbed across the ruffled border of ruffle - ended ameloblasts.

Life Cycle of the Ameloblast

The life cycle of the ameloblast may be divided into seven stages, namely:

1. Prepolarization (Morphogenic Stage)
2. Polarization (Organizing Stage)
3. Enamel synthesis and secretion (Formative Stage)
4. Enamel calcification and maturation (Maturative Stage)
5. Enamel protection (Protective Stage)
6. Desmolysis and Attachment (Desmolytic Stage)

Prepolarization (Morphogenic Stage): In the prepolarizing period, the cells constitute the inner enamel epithelium of the cap stage of the dental organ. The prepolarizing cells or preameloblasts are the immature cells capable of engaging in mitotic activity.

Polarization (Organizing Stage): This stage involves the transformation of preameloblasts into secretory ameloblasts capable of synthesizing enamel. The polarizing period is characterised by cell elongation, migration of the organelles and stratification of some of the cytoplasmic constituents as well as formation of the Tomes' process as described earlier. This is seen during the bell stage of tooth bud.

Enamel synthesis and secretion (Formative Stage): Concomitant with the elaboration of mantle dentin and the activities of the distal surface of the ameloblast, short processes are produced, which invade the matrix of the developing mantle dentin. Similarly, the elongating processes of the odontoblasts grow into the troughs of the undulating ameloblast surface. It is at this time that the young ameloblasts synthesize and secrete the foundational enamel which is the component of the DEJ. This enamel is aprismatic (rodless). The overlying enamel layers, however are composed of rods (prismatic). Subsequent activity involves the synthesis and secretion of lateral pools of enamel matrix in the intercellular area extending from the terminal bar to the free surface.

The secretory activity of the ameloblasts follows a circadian cycle. There are slow and fast phases of secretory activity. There is a hypothesis concerning the development of the cross - striations of the enamel prisms. During the slow phases of the circadian cycles of the secretory activity of the ameloblasts, there is a reduction in the rate at which the surface of the ameloblast moves relative to the formative front. This movement is largely confined to those surrfaces of the Tomes' process in relation to the circumdepression crystallites i.e. in the interpit enamel. A change in the rate of the secretion of the ameloblasts would lead to a change in the balance of growth in pit floor as against interpit sites.

The relative positions of the prism boundaries with respect to the secretory territories of individual ameloblasts (considered to be hexagonal in shape) is a function of time during the 24 hour secretory cycle. At the fast phase of the cycle, the boundary is close to the hexagon; at the slow phase; it is more towards the centre of the hexagon. This is equivalent to a relative shift in the ratio of pit - floor - to interpit - wall - secreted material.

Increments of enamel of 4 microns thick are deposited daily. Accordingly, each ameloblast produces an enamel rod composed of 4 microns thick. The bulk of the head of each rod is formed by one ameloblast, whereas three others contribute to the tail of each rod. Therefore, each rod is formed by 4 ameloblasts and each ameloblast contributes to 4 different rods.

The ultimate number of adjuncts is equal to the number of days of activity. The crest ameloblasts of the cusp and incisal areas may produce rods of hundreds of increments. On the other hand, the cervical cells may be active only a few days or weeks and consequently produce very short rods consisting of only a few increments and these are usually less than 4microns thick.

Enamel calcification and maturation (Maturative Stage): Mineralization of enamel occurs in two almost uninterrupted phases: primary and secondary calcification of the enamel matrix. That occuring immediately following matrix production is known as the initial or primary phase and it involves the nucleation of apatite crystals. It is in this primary phase of mineralization that the first 25microns or more of the total apatite is deposited. The secondary or maturation phase is that in which an additional 72-75 microns is acquired so that the enamel posseses its full complement of the apatite.

Enamel protection (Protective Stage) & Desmolysis and Attachment (Desmolytic Stage): Subsequent to the formation of the total thickness of enamel, the Tomes' process disappears and the ameloblast synthesizes and secretes the surface layer of enamel which is aprismatic as was the original or foundation

enamel at the DEJ. Having deposited the appropriate amount of enamel, the ameloblasts finally complete the crown by forming a nonmineralizing thin basement lamina like organic membrane - the primary enamel cuticle. Following the elaboration of the primary cuticle, the cytomorphologic features of the ameloblasts are greatly altered. These ameloblasts known as postameloblasts are drastically reduced in height, having decreased the quantitative aspects of the golgi components and rough endoplasmic reticulum systems. Mitochondria dominate the organelle population of the distal portion of the cell. Dilated tubular channels, dense bundles of tonofilaments and large intercellular spaces packed with microvilli are present. Numerous hemidesmo-somes abut the primary cuticle.

The post ameloblasts and the overlying stratum intermedium or other remanants of the enamel organ are referred to as the reduced enamel epithelium. The reduced enamel epithelium which covers the entire surface is not of a smooth contour but is thrown into folds (similar to epithelial ridges). Between these are connective tissue extensions resembling papillae. One function that has been attributed to the reduced enamel epithelium is that of protection of the crown from the connective tissue as it grows towards the oral cavity in the eruption process. It has been shown that crowns which aren't shielded from the action of the connective tissue are subject to coronal anomalies due to resorption of enamel or deposition of cementum . This is known as the enamel protective stage of the ameloblast.

Another possible function of the reduced enamel epithelium is that of removing the connective tissue in the path of the erupting tooth. It has been suggested that the reduced enamel epithelial cells secrete enzymes which lyse the connective tissue. This lysis is referred to as the desmolytic stage of the ameloblast. On this basis, it has been stated that untimely atrophy of this protective structure may result in failure of the tooth to erupt.

During the eruption process, the reduced enamel epithelium fuses with that of the oral epithelium and thickens selectively over the erupting tooth tip. The fused epithelia overlying the emerging tips are sloughed, forming a passageway for the tooth, throughout the depths of the epithelia. The remaining reduced enamel epithelium covering the crown is now called the junctional or attachment epithelium. The noncellular organic layer forming the junctional attachment between the attachment epithelium and the enamel surface is called the primary epithelial attachment. The reduced enamel epithelium forms a band around the tooth and is known as the attached epithelial cuff. This is then referred to as the attachment stage in the life cycle of the ameloblast.

In due time, the attached epithelial cuff which is exclusively composed of reduced enamel epithelium, is gradually replaced by the epithelium originating from the oral epithelium of the gingiva. The transformation of the reduced enamel epithelium into junctional epithelium takes place both during and after tooth eruption. It progresses apically from the occlusal region. During the course of this transformation the primary epithelial attachment, becomes the secondary epithelial attachment. This transformation takes place through the following steps - The reduced cuboidal ameloblasts change their shape to become elongated cells oriented parallel to the enamel surface. At the same time, their cytoplasm becomes reorganized, the number of mitochondria diminishes, more end-oplasmic reticulum and golgi fields appear, and more cytoplasmic filaments are formed. As a result of these alterations in form and structure, the cuboidal reduced ameloblasts are transformed into elongated flat junctional epithelial cells. These cells are however incapable of division. Their transformation into junctional epithelium serves their final function of ensuring that the bonding mechanism between epithelium and enamel is maintained during tooth eruption. Soon after, they are exfoliated at the sulcus bottom. The complex of internal basl lamina and hemidesmosomes arising between the altered ameloblasts or future daughter cells of the stratum intermedium is called the secondary epithelial attachment.

Clinical Considerations

Ameloblasts are metabolically sensitive cells and disturbances may occur during the development of enamel. In deciduous teeth and in first permanent molars an accentuated incremental line appears between the enamel which is formed before birth and that which is formed after birth. This line is known as the Neonatal line and is associated with the disturbance in enamel formation, produced at birth, due to a change from a more to less favourable environment. A broadening and prominence of the incremental lines of Retzius may also be seen, indicating metabolic disturbances which affect the rhythmic alteration of the periods of enamel matrix formation and of rest, wherein the rest periods are prolonged. The effects of disturbance in the development of enamel may be encountered clinically on examination. These disturbances can affect either formation of the enamel matrix or its calcification. Disturbance of matrix formation results in hyperplasia; disturbance of calcification, in a hypocalcification of enamel. In the latter the enamel persists as enamel matrix and is soft and acid insoluble in routine preparations after formalin fixation.

Hypoplasia as well as hypocalcification may be caused by systemic, local or hereditary factors. Since the formation of enamel extends over a longer period (of time) and the disturbance is in most cases of shorter duration, the defect is limited to a circumscribed area of the affected teeth. A single narrow zone of hypoplasia may be indicative of a disturbance of enamel formation during a short period, in which only those ameloblasts that, at that time had just started enamel formation were affected. Multiple hypoplasia develops if enamel formation is interrupted on more than one occasion. The possible etiological factors include exanthematous fevers, nutritional deficiencies such as those of vitamins A, C and D, congenital syphilis, chemical ingestions (eg. fluoride in excess of 1ppm in drinking water) and hypocalcemia. When a single tooth shows a hypoplastic defect, the most likely cause is sepsis, usually of a deciduous tooth which affects the formation of the underlying permanent sucessor. These teeth are frequently referred to as Turner's teeth and the condition is called Turner's hypoplasia. A similar type of hypoplasia may follow trauma to a deciduous tooth which is, as a result driven into the alveolus, thereby disturbing the permanent tooth bud. A generalized disturbance of the ameloblasts results in the hereditary type of enamel hypoplasia (Ameloge-nesis imperfecta) which is transmitted as a mendelian dominant character, wherein the entire enamel of all the teeth (deciduous and permanent) is affected.

Conclusion

Ameloblasts are the cells that form enamel in the human teeth. They are ectodermally derived, highly sensitive cells which differentiate prenatally and persist till the development of the last permanent tooth enamel postnatally. Any hereditary or environmental disturbance to this cell during its junctional stages causes defect in the developing enamel and hence to the esthetics and functioning of the corresponding tooth. The molecular and ultrastructural studies have thrown much light on this perplexing cell and made the understanding of this cell more clear.

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