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Tooth Enamel, the Result of the Relationship between Matrix Proteins and Hydroxyapatite Crystals

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Abstract: Enamel, a structure of epithelial origin, represents a protective tooth cover. The cells responsible for the formation of enamel, ameloblasts, are lost at the time of tooth eruption, so that enamel becomes an acellular structure that can no longer regenerate. In order to compensate for this particular phenomenon, enamel has acquired a complex structural organization and a high mineralization degree, in its mature state. This reflects the particular life cycle of ameloblasts and the unique physico-chemical characteristics of matrix proteins, which regulate the formation of the extremely long crystals of enamel. These characteristics differentiate enamel from all the other tissues of the organism.

Keywords: Enamel; Ameloblasts; Matrix proteins; Crystals.

Enamel

Enamel is translucent, the tooth having a color varying from light yellow to gray-white. This can be explained by the different enamel thickness, which is maximum at the level of occlusal surfaces, of approximately 2.5 mm, and minimum at the level of the cervical line. This variation in thickness determines the tooth color, because the subjacent yellow dentin is seen through the thinner enamel areas.

Completely formed enamel represents the tissue with the highest known mineralization, consisting of 96% mineral salts, represented by hydroxyapatite crystals and 4% organic matter and water. At the level of hydroxyapatite crystals, different ions (fluoride, strontium, magnesium or lead) can be incorporated, if they are present during the formation of enamel. The susceptibility of these crystals to be dissolved by acids represents the chemical substrate for the appearance of dental caries [1].

Because of its highly mineral content, enamel is extremely strong and can resist to the mechanical forces exerted during functioning. However, this hardness makes it easy to break. This is why a stronger dentin substrate is required in order to maintain the integrity of enamel. If this supporting layer formed by dentin is destroyed by caries or inadequate tooth care, enamel remains without support and may easily fracture. The junction between enamel and dentin is established as these two hard tissues start to form. Scanning electron microscopy shows the fact that the junction is irregular, having an undulating appearance, an arrangement which probably increases the adhesion between dentin and enamel. This irregular surface is more pronounced in coronal dentin, where occlusal forces are higher.

Due to the peculiar character of enamel, of a highly mineralized matrix, its structure is difficult to study in histological preparations.

When conventional demineralized sections are examined, in the areas previously occupied by mature enamel, only empty spaces can be seen, because mineral substances are dissolved and organic matter is washed.

In developing decalcified enamel sections, sufficient organic matter is retained for some details of its structure to be noted.

The use of an electron microscope, with thin sections and high processing power, has allowed to overcome many study difficulties for enamel.

Tooth enamel is considered to be formed by adamantine prisms and interprismatic substance. Both structures are represented by an organic matrix in which mineral salts are deposited. Adamantine prisms are the morphological units of enamel. They were described for the first time as being hexagonal in cross section and the term "enamel prism" was frequently used. Subsequently, enamel prisms were proved not to fit into a strictly geometric shape.

An adamantine prism has a shape similar to a cylinder and is formed by hydroxyapatite crystals with most of their long axis oriented parallel to the long axis of the prism, especially for crystals found in the central portion of the prism.

The interprismatic substance surrounds the prisms and joins them together. At this level, crystals are oriented in various directions.

The limit between the prism and the interprismatic substance is marked by a narrow space that contains organic matter known as the prism membrane or sheath. At cervical level, the prism sheath is absent, so that crystals in the interprismatic substance are confluent with those forming the prism. In longitudinal sections of enamel prisms, the lateral portion of the prism crystals are continuous with the interprismatic substance, located in the cervical area, until the crystals are positioned almost perpendicular to the prism. In cross section, adamantine prisms have been compared to a "key hole" or a "tennis racket". These prisms are 2 μm long, 4 μm wide and 8 μm high. Because the analogy to the key hole does not adequately cover some variations in the structural arrangement of the components of enamel and does not correspond to the enamel formation pattern, this terminology is about to be abandoned.

Currently, the most adequate terminology for the description of the enamel pattern is cylindrical rods, joined by the interrod substance. The notion of rod will replace in the future that of prism [2].

In human teeth, enamel rods are disposed in groups arranged circumferentially around the long axis of the tooth. In general, rods are perpendicular to the dentin surface, with a slight inclination towards the cusp. In the proximity of the cusp tip, they have a more vertical direction, and in cervical enamel, mainly horizontal. So, the trajectory is slightly undulating, so that any section of the rods will only include short segments of these.

Other two patterns that complicate enamel structure overlap with this arrangement:

- Each rod, as it directs to the surface, follows an irregular pathway, bending to the right and the left, in the transverse plane of the tooth (except for cervical enamel, where rods have a straight direction) and upwards and downwards, in vertical plane.
- In the two internal thirds of the enamel layer, adjacent rod groups intersect and thus have dissimilar local orientations, but a similar general orientation.

These complex relationships det4ermine some of the structural characteristics found in enamel.

Sometimes the prism orientation can be misinterpreted because the crystalline nature of enamel results in optical interference as the light passes through the section, their shape being difficult to interpret [3].

Forming enamel does not express a distinct non-mineralized pre-enamel layer, similar to the osteoid and the predentin layer.

The organic enamel matrix is formed by:

- non-collagenous proteins
- enzymes
- enamel proteins.
    - 90% are represented by a heterogeneous group of proteins with low molecular weight, known as amelogenins.
    - 10% are non-amelogenins, like enamelin and ameloblastin. [1]

Amelogenins

They accumulate during the secretory stage. They regulate the growth in thickness and width of enamel. They are hydrophobic proteins rich in proline, histidine and glutamine. The genes responsible for the transcription of amelogenin are found on chromosomes X and Y. Due to the fact that these two genes are not 100% homologous, there is a sexual heterogeneity. The functional significance of this sexual polymorphism is not yet completely understood. On the other hand, the amelogenin gene contains at least 7 exons, which can be combined in various ways in order to produce messenger RNA, which might include all the seven exons or just some of them. The functional significance of the alternately combined amelogenin forms is not known. Amelogenins undergo a minor extracellular (short duration) or extensive (long duration) processing by proteolytic enzymes, in fragments with low molecular weight, of which the thyrosine rich amelogenin polypeptide (TRAP) and the leucine rich amelogenin polypeptide (LRAP) are significant, as they form the raw material for the primary organic matrix of maturing enamel [4].

Non-amelogenins

Ameloblastin, enamelin and tuftelin are the most studied members of this family. A sulfated protein has also been described. Non-amelogenins (except for tuftelin) are considered to undergo rapid extracellular processing and do not accumulate in enamel for long time periods.

Ameloblastin undergoes rapid degradation. Intact molecules appear in the proximity of the forming enamel surface. In deeper areas, fragmented forms are mainly found. They promote the formation of minerals and the elongation of crystals.

Enamelin undergoes mild degradation in the secretory stage, which decreases in deep areas, where the molecule binds hydroxyapatite.

Tuftelin is believed to be specifically located at the junction between enamel and dentin and to participate in the establishment of this junction. The status of tuftelin is not clear, as it has recently been shown to be present in some tissues and it has a different distribution from that of amelogenin and other non-amelogenins [5].

The fact that non-amelogenins represent minor components in the formation of enamel does not automatically involve the fact that they are produced in small amounts, but this is rather due to their short half life (they do not accumulate in time).

Dentin phosphoprotein/dentin sialoprotein are expressed transiently [6].

At least two general classes of proteinases are involved in the extracellular processing and the degradation of enamel proteins. A group of enzymes of the family of matrix metalloproteinases (MMP) including MMP-20 (enamelysin) seem to be involved in the short term processing of newly secreted matrix proteins, while another group of the family of serine-proteinases including serine-proteinase 1 of the enamel matrix seem to function as general digestive enzymes, in particular during the maturation stage.[1]

The extracellular matrix of developing tooth enamel is at this point quite well defined in terms of its major protein components.

Even if the matrix formed by amelogenins might provide some physical support, enamel proteins seem to play a major structural role, as it is the case of bone collagen, dentin and cellular cement. The three-dimensional organization seen in enamel seems to result from the direct ordering of extremely long crystals [7].

From a morphological point of view, the forming organic enamel matrix seems uniform in decalcified histological preparations; however, immunohistochemical analyses have shown that enamel proteins are partially mixed along the enamel layer. Ameloblastin is concentrated close to the cell surface at the secretion site, while the great majority of degradation fragments are found in deeper enamel. The areas where ameloblastin is immunodetected in the highest concentration correspond in fact to the position in enamel where rod and interrod crystals increase in length (enamel growth areas) [8].

Amelogenins and ameloblastin are secreted together and are contained in the same secretory granules. Amelogenins are believed to form from supramolecular aggregates called nanospheres

which surround the crystals along their long axis and are visible in section under electron microscopy, as granular matter between the crystals. Non-amelogenins are thought to promote and guide the formation of enamel crystals, while amelogenins regulate the increase in thickness and width of crystals. Amelogenins prevent the fusion of crystals during their formation and should be removed in order to allow growth [9].

The early secretion of amelogenin, when odontoblasts have not yet completely differentiated, suggests the fact that this protein is multifunctional. When enamel mineralization is under way, amelogenin might regulate the increase in thickness and width of crystals [10].

Tuftelin, by its early presence, several days before the start of mineralization, has led to the idea that it might play a role in cell signaling and subsequently in mineral deposition. Thus, the functioning of certain enamel proteins might show certain similarities to that of non-collagenous bone and cement proteins, such as bone sialoprotein and osteopontin, which have cell and matrix activities. The complete or partial absence of amelogenins esentially results in the formation of hypoplastic enamel. However, no structured enamel layer is formed in the absence of ameloblastin.

New imaging techniques, including atomic force microscopy, near field scanning, molecular imaging by cryoelectron microscopy and nuclear magnetic resonance are important, offering information for the development of biomimetic approaches in the treatment of abnormal enamel.

The pathway by which mineral ions are introduced in forming enamel is particularly interesting, as it prolongs the secretory and maturation stages in the formation of enamel, the latter requiring a massive increase in mineral inflow [11].

The enamel layer is created and maintained by the enamel organ. The pathway by which calcium passes from blood vessels through the enamel organ to reach enamel seems to involve both intercellular and transcellular pathways. Several years ago, in the secretory stage of ameloblasts, a fine tubular network which opens at enamel level was described. It was then speculated that this might play a role in the control of calcium ions, in a similar way to the endoplasmic reticulum. Recent biochemical findings have demonstrated that calcium ions are directed through ameloblasts to high capacity deposits associated with the endoplasmic reticulum, avoiding in this way the cytotoxic effects of excessive calcium in the cellular cytoplasm.

Even if amelogenesis is correctly described as a two stage process, which involves the secretion of partially mineralized enamel and its subsequent maturation, microradiographic studies on thin cross sections, with computerized improvement, indicate the fact that enamel mineralization might involve several stages. These stages determine the creation of an enamel layer with the highest mineralization at its surface, the degree of mineralization decreasing towards the junction between enamel and dentin [2].

Enamel is formed by tightly packed hydroxyapatite crystals, 60-70 nm wide and 25-30 nm thick. The length of crystals extends over the entire thickness of the enamel layer. The calcium phosphate cell unit has a hexagonal symmetry.

In brief, the amelogenesis process involves cells that secrete enamel proteins, which are mineralized immediately in almost 30% to form the entire enamel thickness. This enamel subsequently matures by the addition of a significant amount of mineral salts, concomitantly with the removal of enamel proteins and water [13, 14]. These complex processes are under cellular control and associated cells undergo significant morphological changes during amelogenesis, which reflect their ongoing physiological activity.

Tooth enamel is a tissue that is incapable of regeneration. With age, enamel becomes increasingly worn out in the areas where masticatory forces act. Other characteristics of old enamel include discoloration, low permeability and changes in the surface layer. The apparent reduction in the incidence of caries is related to these changes [15].

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