

ORIGINAL ARTICLE

MINERALIZATION OF TEETH ENAMEL AFTER ERUPTION

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ABSTRACT
The aim: The paper was aimed at the study of the processes of mineralization of the enamel of the permanent tooth after its eruption.
Materials and methods: To study the structure of the enamel of permanent teeth has been carried out using light and electron microscopy. The study of the process of the development of the primordia of the permanent teeth involved 10 culled puppies of 30-40 days of age. Microscopic, electron microscopic, immunohistochemical methods of research have been used to study the processes of histogenesis.
Results: The studies show that in the postnatal period, the formation of the crown, externally covered with cuticular epithelium, marks the formation of the primordium of the permanent tooth at the follicle stage. After eruption of a tooth, different parts of its crown have three individual structural and functional barriers to enamel biomineralization. The first one is provided by the cuticular epithelium of the pitted areas of the crown, which ensures filtering of the salivary fluid from the protein deposit in the form of a pellicle. The second barrier is defined on the lateral and cuspidate surfaces of the enamel, where the cuticle is erased or poorly expressed. The third structural and functional barrier of enamel biomineralization is located in the cervical portion of teeth of different classes.
Conclusions: Different areas of the enamel in the tooth crown have specific filtration barriers, which can be distinguished as follows: pit-and-fissure-and-groove, cuspidate-and-approximal, and cervical barriers. The cuticle is poorly expressed or totally absent on the cusps of the tooth crowns in contrast to pitted areas.

KEY WORDS: histogenesis, enamel, biomineralization, cuticular epithelium

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INTRODUCTION

In the medical science, the concept of "morphogenesis" is referred to the process of functioning of stable and capable morphological structures that are formed from embryonic germs. In humans, different classes of teeth are responsible for a certain function in the process of grinding food [1]. Thus, the canines hold food, the incisors cut food into pieces, the premolars coarsely grind it, and the molars masticate it for further digestion in the gastrointestinal tract. To perform these functions on different teeth of both jaws, a corresponding, so called, odontoglyphic enamel pattern of the surface of the crown is formed [2]. The peculiarities of the formation of the topography of the tooth crown are directly related to the process of enamel mineralization, and, therefore, need to be studied in the relationship between them. Biomineralization of the enamel and dentin is a complex, dynamic and gradual process characterized by not only mineralization but also demineralization of stable hard dental tissues [3-9]. Numerous publications are devoted to the process of enamel mineralization in embryogenesis and in the postnatal period of histogenesis, but the variability and mosaicity of the findings of the studies, as well as the lack of comprehensive description of this phenomenon require further in-depth research [10-13].

THE AIM

The paper was aimed at the study of the processes of mineralization of the enamel of the permanent tooth after its eruption.

MATERIALS AND METHODS

To study the structure of the enamel of permanent teeth, the analysis of slices of teeth extracted in 10 patients for orthodontic and surgical indications has been carried out using light and electron microscopy. The slices were prepared according to the conventional technique by longitudinal sawing of teeth fixed in 10% neutral formalin.

The study of the process of the development of the primordia of the permanent teeth involved 10 culled puppies of 30-40 days of age, which in compliance with bioethical requirements were taken from the owners as biological material for further pathomorphological study. After removal of the mandible, the preparations were fixed in 10% neutral formalin. Subsequently, after decalcification with Trilon B, fragments of the jaw were selectively excised. From them, after embedment in paraffin, micropreparations were made for light microscopy, which were stained with hematoxylin and eosin, and for immunohistochemical study, in which the intercellular adhesion at the stages of epithelial cell differentiation is determined by the E-cadherin protein reaction. The findings were analyzed on the Olympus BX 41 microscope. To study the structure of the hard dental tissues, electron microscopy of longitudinal sections of the teeth was also performed, which were prepared according to the conventional technique.

RESULTS

The study of the process of the development of the primordia of the permanent teeth has shown that the crown of the tooth follicle consists of both cuspidate and pitted areas of the enamel. The first are the cusps in the large canines of both jaws. Separate cusps are joined by the crest, forming the "+"- or "Y"-shaped patterns of the molar crown. On the lateral surfaces of the crowns of incisors, canines, premolars and molars, as the corresponding surface topographical prominence becomes less expressed, there are styles in the form of enamel projections that do not reach the contact surface of the crown. Pitted areas of the tooth crown consist of pits, fissures, grooves and furrows. The pits reach the depth of the enamel-dentin border; the fissures do not reach it, but are jointed with it by the enamel tufts. Furrows and grooves depart from fissures or pits in the form of cuticle depressions, forming the perikymata on the surface of the incisors and canines. In the areas of premolars, molars, the grooves are parallel or perpendicular to the fissures and have the form of morphological formations.

Ameloblasts produce extracellular structures in the form of enamel prisms that are capable of biomineralization. They are preserved at an early stage of the dental follicle, forming parenchymal extracellular elements of the enamel. Due to the latter, there is s-shaped arrangement of the enamel in certain areas of the tooth crown, which ensures its amortization.

We also found that at the late stage of the dental follicle, separate bundles of enamel prisms are formed, which are separated by the pitted areas of the crown: pits, fissures, furrows, grooves, and on the approximal surfaces by the lamellae. Each cusp of molars on the surface is separated by fissures, which, depending on their number, flow into the central α or additional β and γ pits. In premolars there is a reduction of pits and partial preservation of fissures and additional grooves in the areas of the styles. In the canines and incisors on the approximal surfaces of the styles, areas of enamel in the form of superficial perikymata are prominent.

Therefore, the formation of pitted areas of the crown of different classes of teeth is due to the remnants of the cuticular epithelium of the pulp of the enamel organ, which further forms the structural components of the enamel, ptomoting its remineralization. At the same time, in the sites of the expected pit, this epithelium reaches the dentinal layer, and in the areas of fissures it is adjacent to the partially formed layer of prism enamel. On the approximal surfaces, the cuticular epithelium is preserved, resembling numerous parallel grooves in the form of perikymata.

Cuspidate areas of the tooth crown at the follicle stage are characterized by focal reproduction of ameloblasts and the formation of enamel prisms. At the same time, due to the different orientation of the odontoblast processes in the areas of the cusps, bundles of enamel prisms have a circular course, resembling the shape of springs. Styles that form bundles of enamel prisms are located in the form of lamellae of bridge-like structures. On the approximal surfaces there are s-shaped bundles of enamel prisms. From the point of view of mechanics, all three types of formed bundles of enamel prisms perform the cushioning function of enamel when pressure is applied to the tooth crown during chewing.

This statement is confirmed by the analysis of the surface of the crowns of permanent teeth after eruption (both premolars and molars) with a complex odonoglyphic pattern using histochemical staining with PAS-Alcian blue (Fig. 1). In this way, the pits and fissures are dark purple. Dotted, sometimes clustered, additional grooves are adjacent to the major fissures, which separate the cusps. The cusps and crests that connect them are light blue or whitish. In our opinion, the dark purple color of the pits and fissures indicates the presence of residues of salivary fluid filtrate, which includes acidic glycosaminoglycans. Admittedly, cusps and crests filter salivary fluid to a lesser extent. In comparison with the latter, the lateral surfaces - styles occupy an intermediate position in the course of remineralization during saliva filtration and are stained in blue color.

On the transverse slices of the tooth (Fig. 2) the pit consists of a duplication of the cuticular epithelium. Two layers are noted:

- external, in which the scales are located around the perimeter of the pit;
- internal, formed by unstructured layers of enamel, between which lime crystals are spotted.

On the longitudinal slice, the pit has a zigzag structure. Notably, in the superficial layer of enamel, along the pit, the PAS-positive area, which further branches off and reaches the enamel-dentin border is noted (Fig. 3). Zones of necrosis are observed in the thickened areas of branching. Calcium salts, which have the form of semicircular light masses, are layered around the latter in separate clusters.

Biomineralization of perikymata occurs somewhat differently than in the pits. Perikymata are a single layer of epitheliocytes connected by contacts. The most pronounced structure of the grooves in the form of perikymata is presented in cervical portion of incisors and canines.

On the longitudinal section, the epitheliocytes are layered in the form of fish scales. The scaly shape of the cuticle is ensured by the presence of layers of squamous epithelium during embryogenesis (Fig. 4).

Electron microscopically, it has been found that enamel prisms consist of heads and tails. The heads are tightly adjacent to each other and have the form of parallel fastened rhombuses. The tails of the enamel prisms of the first layer are joined with the heads of the next layer resembling clasps on clothes in the form of puzzles (Fig. 5). Behind the layer of enamel prisms there is a layer of atrophied ameloblasts. It is represented by the thin, long and parallel strips. The latter are separated by equal thin fissures.

Fig. 1. Complex odonoglyphic pattern of the molar crown. PAS-Alcian blue stain. 7×magnification.

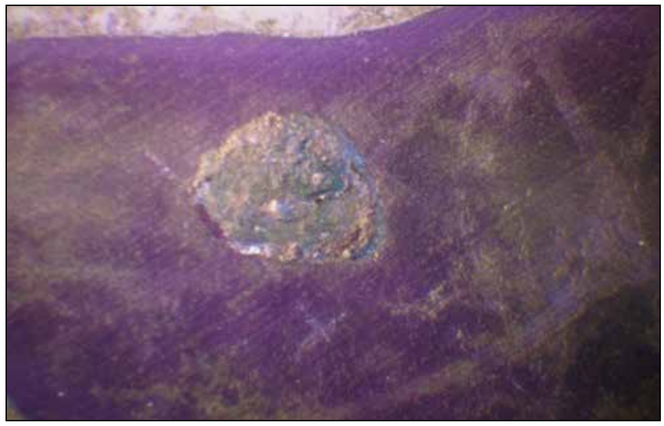

Fig. 2. Lime crystals on the transverse slices of the tooth; section is made at the level of the pit's bottom. PAS-Alcian blue stain. 56×magnification.

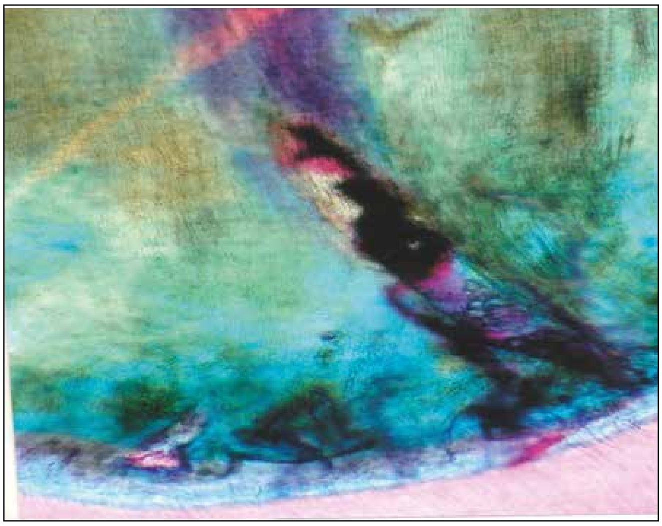

Fig. 3. The pit with a zigzag structure on the longitudinal slice of the tooth. PAS-Alcian blue stain. 56×magnification.

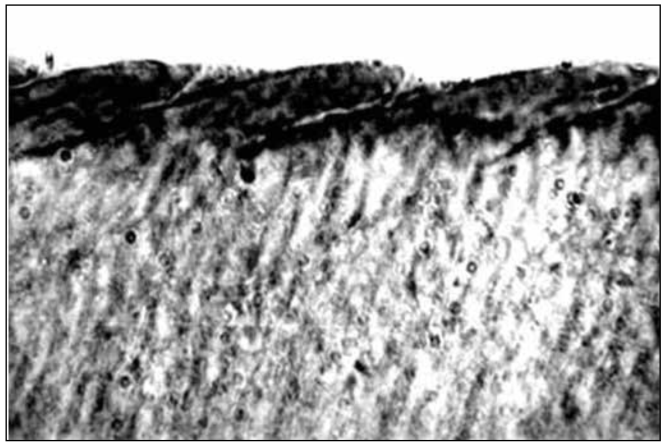

Fig. 4. Layering of epitheliocytes in the form of fish scales on the longitudinal section of the tooth. SEM. 1750×magnification.

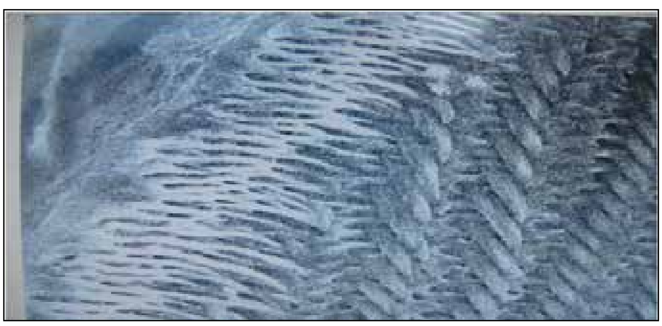

Fig. 5. Electronogram of the enamel prisms of the tooth crown. SEM. 13000×magnification.

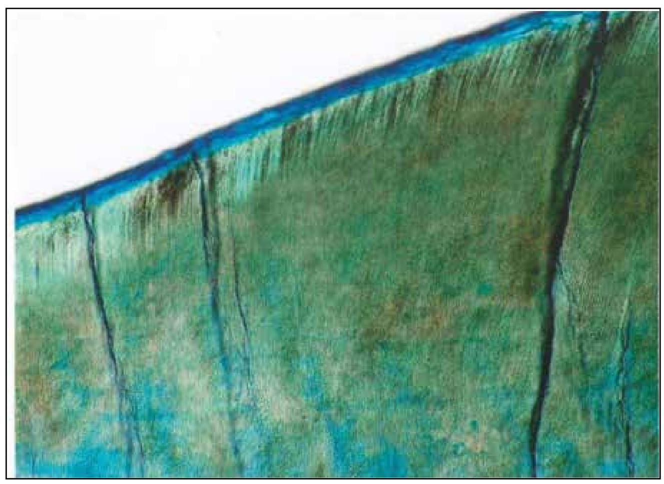

Fig. 6. Cervical enamel on the thin native section of the tooth. PAS-Alcian blue stain. 32×magnification.

The layer of atrophied ameloblasts promoted filtration of salivary fluid, which ensures biomineralization of the cuspidate areas of the tooth crown. This statement is confirmed by the presence of protein deposit on the enamel surface.

Thus, different parts of the tooth crown have three separate structural and functional barriers to enamel biomineralization.

The first barrier is provided by the cuticular epithelium of the pitted areas of the crown, which filters the salivary fluid from the protein deposit in the form of a pellicle.

The second barrier is defined on the lateral and cuspidate surfaces of the enamel, where the cuticle is erased or poorly expressed. The filtration is carried out through perpendicularly located atrophied ameloblasts of the cortical layer of enamel.

The third structural and functional barrier of enamel biomineralization is located in the cervical portion of teeth of different classes. It is here that the gingival sulcus, filled with its liquor, comes into contact with the enamel and, partially, with the cement.

On the thin native sections, the cervical enamel has a triangular shape and is bounded by the cuticle and cementum (Fig. 6). It is divided into segments of different thickness by the lamellae.

The lamellae are perpendicularly located between the cuticle and dentin or cementum. When histochemically stained with PAS-Alcian blue, the cuticle turns blue and light stripes of the Nasmyth's membrane are located beneath it.

There are cases when whitish lime crystals can be noted inside some flaked lamellae.

The interprism substance is represented by the thin fibrous structures. It is in it that the process of biomineralization begins, which extends to the tails of the enamel prisms. A capsule is formed in the heads of the latter due to biomineralization.

Enamel prisms, formed by secretory ameloblasts in the form of bundles on the enamel surface, form a characteristic cortical shiny enamel layer. The presence of soluble calcium salts in salivary fluid promotes their penetration into the enamel layer covered with a layer of atrophied ameloblasts, forming a homogeneously mineralized shiny cortical substance, which is determined clinically.

DISCUSSION

Thus, during the formation of the primordium of the tooth at the follicle stage, a tooth crown is formed, which is externally covered with cuticular epithelium. By certain invaginations of a cuticular epithelium the specific topography of a tooth crown is formed. Various cuspidate and pitted areas are formed, on the one hand, by the cuticular epithelium of the pulp of the enamel organ, and on the other hand by the enamel projections formed by ameloblasts. The cuspidate areas of the crown are formed due to the formation of enamel prisms by ameloblasts. Enamel, a special tissue that after eruption of the tooth is capable of biomineralization with salivary fluid. Different areas of the enamel in the crown of the tooth have specific filtration barriers, which can be distinguished as follows:

1) pit-and-fissure-and-groove barrier;
2) cuspidate-and-approximal barrier;
3) cervical barrier, which is formed during tooth eruption.

Notably, in contrast to the pitted areas where the cuticle is present, on the cusps it is poorly expressed or erased. Therefore, under natural conditions in the enamel, due to the presence of a cortical shiny layer, there are different types of biomineralization, which provides different degrees of mineralization of cuspidate and pitted areas of the enamel.

CONCLUSIONS

Different areas of enamel in the crown of the tooth have specific filtration barriers, which can be distinguished as follows: pit-fissure-groove, cuspidate-approximal and cervical barriers. The cuticle is poorly expressed or totally absent on the cusps of the tooth crowns in contrast to pitted areas. Therefore, after eruption, in permanent teeth the various degree of biomineralization of the cuspidate and pitted areas of enamel is noted.

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Conflict of interest:
The Authors declare no conflict of interest.

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