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Research Article

Evidence of Dental Lamina Preservation in the Development of Mice First Molars

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ABSTRACT

The aim of this work was to analyse the behaviour of the dental lamina during the development process of first molar teeth. The offspring of female mice were analysed at the 16th day of intra-uterine life and since birth till 17 days old with the aim to evaluate the dental lamina evolution of upper and lower first molars. The animals were sacrificed, and the heads embedded in paraffin in order to get frontal or sagittal sections whose were stained by hematoxylin and eosin method. The results showed a very clear presence of the dental lamina in all periods under analysis, without the occurrence of its disorganization, in other words, it was permanently connecting the developing tooth germ to the oral epithelium until advanced dental eruption.

Introduction

The analysis of dental lamina evolution have brought some misunderstanding once that many have been the descriptions of its destiny and there is also some confusion when it is related to deciduous or permanent teeth. As it can be seen in the literature, while for some authors the dental lamina undergoes a small disintegration process but never disappears, particularly for the deciduous teeth for other authors there is a gradual loss of its structure, rupture of its continuity and invasion by the connective and bony tissues disposed between the tooth germ and the lining oral epithelium with separation and insulation of developing tooth germ [1-4]. The aim of this paper is to verify the fate of the dental lamina, its period of persistence in teeth without successor teeth and also evaluate what happens with the dental lamina of permanent teeth whose have deciduous predecessors as a review of what is described in the literature.

Materials and Methods

Female mice (*Mus musculus*), albinus, 60 days old, were breed with males of the same specie; the vaginal plug identification indicated the day zero of gestational period. Animal cares were taken daily, and they were fed with granulated ration and water *ad libitum*. The animals were sacrificed at the 16th day of gestation and at 0, 3, 5, 8, 9, 10, 15,16 and 17 days of age through ether sulfuric inhalation. All standards of the ethics committee were strictly followed whose approved protocol was the 182003 - Educational Foundation of Barretos. After sacrifice the heads were fixed in 10% formalin, decalcified in 3% trichloroacetic acid and reduced to small pieces containing the upper and lower molar tooth germs. The pieces were embedded in paraffin in such way to provide frontal or sagittal sections whose were stained by hematoxylin and eosin method for examination in light microscopy.

Results

I 16 days of Intra-Uterine Life

The upper and lower molar tooth germs were in the cap stage of development. They showed all the constituent structures, that is dental lamina, inner and outer epithelia, stellate reticulum and the begin of stratum intermedium differentiation of the enamel organ, dental papilla as a cellular mass highly condensed and starting the odontoblast

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differentiation in its surface and the establishment of dental sac structures whose were interrupted at the dental lamina level.

Involving the tooth germ there was bony tissue of the alveolar crypt which was thick and was disposed laterally and partially on its occlusal surface. The dental lamina connected the oral epithelium to enamel organ structures; between the epithelial structures of oral cavity, enamel organ and dental lamina, there was connective tissue (Figure 1).

Figure 1: 16 days old fetus - Upper and lower first molar tooth germs with their own dental laminas connecting the oral epithelium to enamel organ epithelium (arrows). HE - 200 um.

II At Birth

The molar tooth germs appeared well constituted with all structures present. It was possible to see in the upper molar, as well as in the lower molar (Figure 2) differentiated ameloblasts and the other structural elements of enamel organ. The odontoblasts were differentiated in all surface of the dental papilla which was composed by an agglomerated of dense and uniformly disposed cells. At the cusp areas a thin layer of enamel was present between the ameloblast and odontoblast cell layers and a more extensive dentin layer than that of enamel was covering almost all the dental papilla surface. Surrounding the tooth germ was seen a thin layer of connective tissue, the dental sac, which separates it from the developing alveolar bone. The alveolar bone involved the developing tooth germs except on the central part of their occlusal surfaces, where there was connective tissue not only between the epithelium lining the oral cavity and the epithelium of enamel organ but also surrounding the dental lamina which connected both those epithelial tissues (Figure 2).

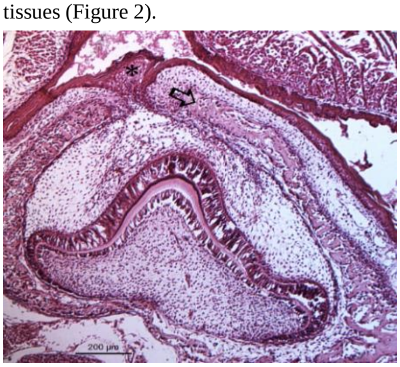

Figure 2: At birth - Tooth germ of lower first molar showing the dental lamina connecting the oral epithelium to the enamel organ epithelium (*). Surrounding the dental lamina can be seen connective tissue and far away young boy tissue (arrow). HE - 200 um.

III 3 Days of Age

The tooth germs showed evolution in its development when compared to the before description and showed the enamel organ with all its structural layers, ameloblasts as columnar cells, completely differentiated in the whole crown surface extension. The dental papilla was composed by similar and uniformly distributed cells and lining by odontoblasts in its surface. The enamel layer was present in all the surface except in the areas where normally it does not occur. The dentin layer was also present in all the papilla surface. Both were thicker in the cusp areas than in cervical regions. The dental sac tissue was thin and involved the tooth germ keeping it apart of the bony tissue of alveolar process. The bony tissue involved all the tooth germ except in the center of its occlusal surface, where there was connective tissue surrounding the dental lamina which connected the dental organ to lining oral epithelium. Bony tissue could appear covering all the tooth germ when the section does not pass through dental lamina (Figure 3), which means it pass outside of the dental lamina.

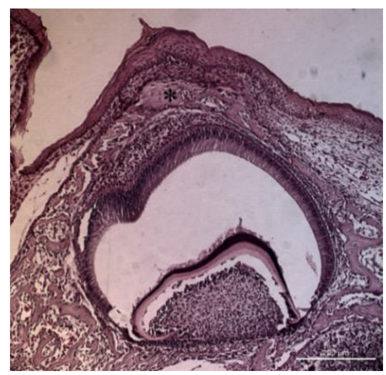

Figure 3: 3 days old animal - Tooth germ showing bony tissue covering all the occlusal surface of the developing tooth (*) because the section did not pass through the dental lamina. HE - 200 um.

IV 5 Days of Age

The tooth germ showed the enamel organ with all its structure elements. The enamel and dentin appeared as thick layers and were disposed covering all the dental crown surface being thinner in the cervical region. The dental sac tissue covered the tooth extensively as well as the bony tissue of alveolar crypt; both these tissues were absent in the central area of the occlusal surface. In this area was present the dental lamina which was very evident and connected the tooth germ to the oral epithelium (Figure 4).

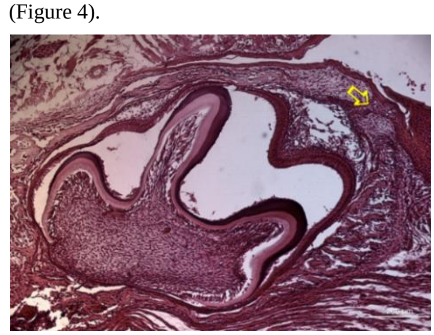

Figure 4: 5 days old animal - In the central area of the occlusal surface of the developing tooth a conspicuous dental lamina is present connecting the enamel organ to the oral epithelium (arrow). HE - 200 um.

V 8 to 10 Days of Age

At 8 days of age the dental lamina was present and connecting oral epithelium to the enamel organ at the region between the molar cusps. The enamel organ was very reduced in its thickness in the region upon the cusps and a thin layer of connective tissue was present between the tooth germ and oral epithelium particularly at the lingual side. The dental organ was well constituted and surrounded by connective tissue and far away from it there was bony tissue of the alveolar crypt. Blood vessels could be seen in the center of the enamel organ under the dental lamina (Figure 5).

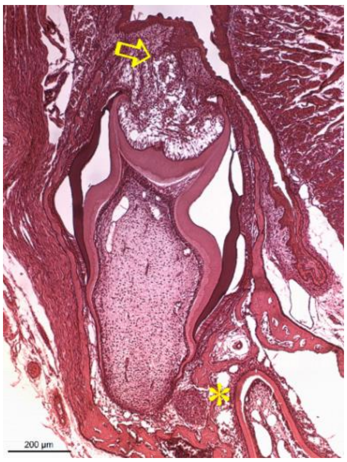

Figure 5: 8 days old animal - The dental lamina is present connecting the oral epithelium to the enamel organ (arrow). The dental organ is surrounded by connective tissue and bone of the alveolar crypt (*). Blood vessels are present in the enamel organ. HE - 200 um.

The 9 days old animals showed dental lamina surrounded by connective tissue and alveolar bone of the alveolar crypt. The distance between inner and outer enamel organ epithelia is bigger in the lateral surfaces than in the cusp regions of the crown (Figure 6). The 10 days old animals showed the dental lamina surrounded by connective and bony tissues. Covering the lower molar lingual cusp was seen the inner and outer enamel organ epithelia fusioned and very near of oral epithelium.

Figure 6: 9 days old animal - Shows the dental lamina connecting the oral epithelium to the enamel organ (arrow) surrounded by connective tissue and bone of the alveolar crypt (*). HE - 200 um.

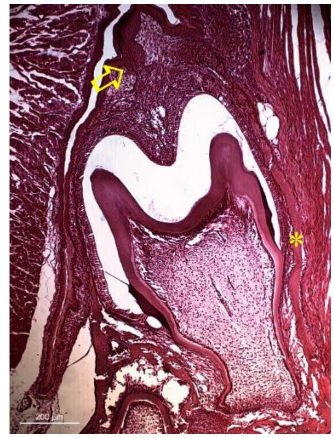

Figure 7: 10 days old animal - The dental lamina is connecting the oral epithelium to the enamel organ epithelium (arrow). The epithelium of the enamel under the dental lamina and between the dental cusps is very large and crossed by many blood vessels. There is no bone tissue upon the lingual cusp, but it is present on the vestibular cusp (*). HE - 200um.

The bone of alveolar crypt at the vestibular side partially covered the dental crown, but at lingual side it is limited to the medium third of the crown. The enamel organ showed many vacuoles and some blood vessels in its structure. The tooth germs presented a thicken layer of dentin, a negative image of enamel tissue in contrast with small areas of acid resistant enamel matrix in cervical third of the crown (Figure 7).

VI 15 Days of Age

At 15 days of age the dental lamina was present and connecting the tooth germ to oral epithelium. Surrounding the epithelial tissue of the dental lamina there was loose connective tissue and, partially covering the tooth germ occlusal surface, bony tissue. The inner (ameloblasts) and outer epithelia of the enamel organ were very closed each other at their lateral surfaces; in its occlusal surface, there was besides the cells of the stellate reticulum many blood vessels deeply situated near to the ameloblastic layer.

Figure 8: 15 days old animal - The dental lamina is present connecting the enamel organ to the oral epithelium and surrounded by loose connective tissue (arrow). The inner and outer epithelia of the enamel organ and the oral epithelium are very near each other, particularly at the lingual surface. In the occlusal surface, besides the stellate reticulum, there is many blood vessels. HE - 200 um.

The enamel organ and oral epithelia were very near each other at the lateral surfaces of the dental organ. There was bony tissue partially covering the occlusal surface of the upper molar but not of the lower molar. In the surface of the alveolar crypt toward the tooth germ there was many osteoclasts (Figure 8). At the lingual side of the developing tooth was seen a cell proliferation which could be related to secondary dental lamina formation for the successional tooth.

VII 16 and 17 Days of Age

At 16 days of age the lower first molar tooth germ has made its appearance into the oral cavity only through the lingual cusp, which mean not by the total crown. In fact, the connection of the enamel organ to the oral lining epithelium was seen in spite of the occurrence of the fusion and rupture of the fused lining oral and enamel organ epithelial but only upon the lingual cusp of the first lower molar.

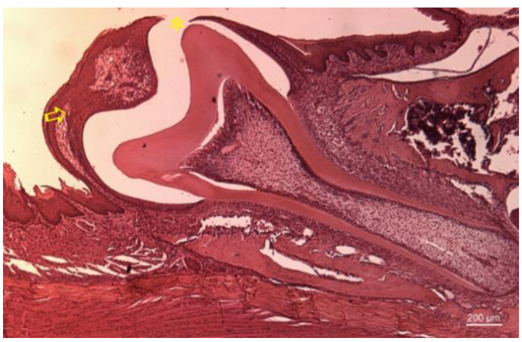

Figure 9: 16 days old animal - The lingual cusp appears in the oral cavity (*). The dental lamina is still connecting the oral epithelium to the enamel organ (arrow) and in areas between the cusps there is epithelial tissue crossed by many blood vessels. HE - 200 um.

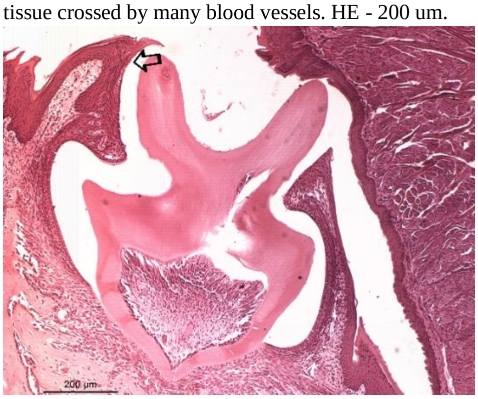

Figure 10: 17 days old animal - The first molar made its eruption into the oral cavity. remaining areas of the enamel organ were seen between the cusps connected to the oral epithelium by the dental lamina and crossed by many blood vessels (arrow). HE - 200 um.

The other areas of the crown showed epithelial tissue of the dental lamina upon it and crossed by many blood vessels (Figure 9). The upper first molar is delayed in its eruption in comparison to the lower first molar. There is no bony tissue in the way of the tooth to the oral cavity. Almost the same aspect could be seen at the 17 days old animals. The upper first molar is now erupted into the oral cavity. The areas of the dental lamina upon the crown of the tooth are crossed by blood vessels whose are located till very near of the ameloblastic layer as identified by frontal (Figure 10) and sagittal sections.

Discussion

The dental lamina is a structure which develops from lining oral epithelium as an indicative of tooth development. From the primary dental lamina, in diphyodont animals, develop ten deciduous tooth germs in the maxilla and ten deciduous tooth germs in the mandible and from extensions at the lingual side of the deciduous teeth, the secondary or successor dental lamina develop the permanent or successional teeth and yet for a backward extension or to distal, the tertiary or accessory dental lamina develop the molar permanent teeth, without predecessor deciduous teeth; the tertiary dental lamina project itself backward without any connection with the oral epithelium and give origin, one after the other, the permanent molar tooth germs [1, 3, 5-13]. The permanent molars do not have deciduous predecessors and the dental lamina grows backward, under the oral epithelium inside of the ectomesenchyme [10].

In the monophyodont animals, as the rodents, the incisors teeth have continuous growth and the molars teeth have a limited growth and, as happens in the human beings they do not have permanent successors. Thus, it is acceptable to utilize mouse molars as a model for tooth formation and development studies. These teeth, as was stated by Cohn, "have attributes which make them more suitable for dental investigation than are rat molars" and they have been used in several kinds of investigation as to verify the effect of many substances on tooth development, for comparative studies of its development '*in vivo*" and "*in vitro*" and also for identification of cellular interaction and induction during morphological development and dental histogenesis [14-20].

The dental lamina, as classical description, is the primordium of the ectodermal portion of the teeth which grows into the underlying ectomesenchyme and in its deep extremity at different points and times develop the tooth germs [6, 9, 21]. After tooth germ differentiation and the beginning of dental tissues and supporting structures formation bony tissue develops surrounding the dental organs which will constitute the bony crypt which will form the dental socket [7]. Concerning evolution and fate of primary dental lamina does not have an agreement on the reports found in the literature.

The connection of lower incisor tooth to the oral epithelium is degenerated in 14 weeks old or 16 weeks old human fetus when the tooth germ is in the bell stage of development [22, 23]. This observation is not in accordance with the report that upper and lower incisor teeth are in a differentiation process, also in the bell stage of development and connected to lining oral epithelium by dental lamina in the 14 weeks old fetus or 20 weeks old fetus being already, the tooth germ in advanced bell stage [3, 8]. The upper canine tooth germ in the human fetus 19 weeks old is at initial bell stage of development and connected to primary dental lamina but in the same tooth germs, in another level of section, the dental lamina shows an initial disorganization allowing to teeth going to be involved by the maxillary connective tissue [3, 4].

The dental lamina being disintegrated in the 19 cm fetus which means, approximately, 19 weeks old fetus, and the tooth germ was in bell stage of development [12, 13]. This observation is not in agreement with that which shows the lower first molar tooth germ in advanced bell stage in an 18-week-old fetus and the dental lamina connecting the enamel organ to oral epithelium [8]. Otherwise, in accordance with other observations, in the 13,5 weeks old fetus the upper deciduous molar tooth germs were connected to dental lamina and thus stay in the 19 weeks old fetus, when starts its disorganization process, however as the teeth keep developing and arise the bell stage or advanced bell stage the dental lamina disintegrates by mesenchyme tissue invasion and is digested by macrophages of the dental sac [3, 5, 9, 12, 21].

This becomes more evident because it passes to totally involve the tooth germ in humans or almost in mice and the dental lamina which was until then connecting the tooth germ (enamel organ) to lining oral epithelium becomes fenestrated, disintegrated or is disrupted and the tooth germ lose contact with the oral epithelium at the begin of an incomplete way because does not reach the whole dental lamina thickness [1-3, 6, 7, 10, 11, 21]. Dental lamina remnants can persist as pearls or epithelial isles in the maxilla as wells as in the mandible besides the gingival whose have the potential to product, sometime later, several kinds of cysts or odontogenic tumors [3, 6, 7, 9, 12].

In accordance with other descriptions, in the occlusal surface of developing teeth occurs, at first, the crypt bony tissue formation respecting the dental lamina presence, thus, temporally stays an area without bone formation. Later the bone of the alveolar process completely involves the dental follicle to constitute the bony crypt which promotes dental lamina erosion finishing to eliminate it and the developing tooth germ becomes included in the bony tissue and consequently isolated from the lining oral epithelium where did it come from [2, 3, 4, 14]. As remnant of dental lamina, some epithelial cells together with connective tissue stays inside the bony tissue formed in such way and constitute the gubernacular cord, which is related to dental eruption upon which plays an important role [2, 3, 8].

Thus, the enamel organ is connected to dental lamina through a large connection which undergoes a gradual constriction and is almost severed at 18th day of intra-uterine life in mouse; finally, the dental lamina becomes with spaces and is resorbed; the dental sac which surround the tooth, sever the connection between enamel organ and oral epithelium [14]. Remnants of dental lamina, sometimes, persist as epithelial islands and can differentiate in cysts, enamel mass or supranumerary teeth [1, 7, 8]. The tooth germ during its initial formation and calcification stages stays loosely situated in a connective tissue bed, the dental sac, and in its great part inside of a bony crypt. In developing deciduous teeth, the crypt does not involve their occlusal surfaces, and thus leave, in fact, large openings on them. The permanent teeth, however, are more completely surrounded by the bony crypt. Epithelial bands pass through an opening in their crypts to connect the tooth germs to dental lamina.

The permanent tooth germs without deciduous predecessors also do not stay completely included in bony crypt which bound only a lateral position of tooth germ little covering the occlusal surface, observation which is reinforced by the analysis of the figure 206 of Schour book [1]. The permanent tooth germ develops at the same bony crypt of deciduous tooth until when its crown is almost complete when a total separation occurs between both through a lamina of alveolar bone and then the permanent tooth comes to its own crypt. This crypt does not move with the occlusal movement of deciduous teeth and thus the permanent teeth become to a lingual and apical position of its deciduous predecessor [1]. The permanent teeth are completely included in a bony crypt, being the gubernaculum, in the occlusal surface the unique communication with the outer medium. So active odontoclasts are present at the crypt roof interne wall [1, 9].

During the eruptive process the roof of the crypt is resorbed to allow tooth passage [1]. The bony crypt where the first molar tooth germ is included is resorbed in its roof between 5th to 10th day after birth in mouse and shows a large opening by which the crown of the tooth may pass through [9, 14]. In humans the bony crypt of deciduous canine was open at 19 weeks old fetus and partially recovered by bone in molar at 20 weeks old fetus; in fact, the bony crypt of deciduous teeth never completely involves the tooth germs and are always open once that never occurs bony tissue in the incisal surface of the tooth or on molar occlusal surface during its formation and so only peripheric bony walls are found [1, 3]. These observations are corroborated by the results found in this as well as in a before paper [17] and showed that the alveolar bone only involves the whole tooth germ when the histological section level does not pass through the occlusal surface center of developing tooth and thus through the dental lamina [17].

The permanent molars do not have either predecessor or successional teeth thus is perfectly acceptable the information that they never are completely involved by bony tissue, that is, always will persist an area on their occlusal surfaces which shows connective tissue surrounding a central area of epithelial cells which is the dental lamina. This observation corroborates the description of absence of bony formation on occlusal surface of deciduous teeth and permanent molars, situation which could be inferred from the figures 203, 205 and 206 of the Schour book, of the figure 11-2 of Bhaskar book, of the figures 18 to 27 of Balducci-Roslindo thesis and from what is observed in this paper showing the upper and lower molars never covered by bony tissue in their whole occlusal surfaces [1, 4, 6].

Against this information is the observation that after cellular thin out of dental lamina occurs its complete elimination by total envelopment of tooth germ by bony tissue of the crypt and the sever between development tooth germ and lining oral epithelium from where it came from [2-4, 10]. Forms in such way the roof of the bony crypt which is considered essential to protect the future cusp regions of the teeth because soon after the roof of bony crypt formation start the odontoblasts and ameloblasts differentiation events and the begin of dentine and enamel formation. However, the persistence of thin trabecules of bone in the roof of the crypt is very short and very soon will appear osteoclasts to resorb them. The appearance of osteoclasts is preceded by the presence of mononuclear cells (monocytes) from where they come from and realize the resorption of the occlusal portion of the alveolar crypt first to allow to the crown of the tooth grow through dentine and enamel apposition during crown stage and after to establish an eruptive route to allow tooth eruption, coinciding to root stage of development [2, 3, 6, 9, 11].

Significant changes occur in the tissues overlying the eruptive phase of the teeth like loss of the connective tissue between reduced enamel epithelium and the oral epithelium [7, 11, 24]. When the permanent tooth develops in the same bone crypt of its deciduous predecessor the bone involves both tooth germs but does not form a completely closed structure upon them [6, 7]. With the deciduous tooth eruption, the permanent tooth germ becomes to an apical position of the deciduous tooth and even that it become almost totally involved and isolated in its own bony crypt the dental follicle is continuous with the lamina propria of oral mucosa [7].

The tissue architecture on the way of the successor tooth eruption is different from that found in the way of deciduous tooth. The fibrocellular follicle which surround a successional tooth maintains its connection with the lamina propria of the oral mucosa through a band of fibrous tissue containing remnants of the dental lamina, the gubernacular cord, which could have a function as a guide for permanent tooth eruption; the gubernacular cord is inside of the gubernacular canal, which is quickly increased by local osteoclastic activity, determining the traject of tooth eruption [6, 9-11]. The dental lamina tissue may have the responsibility to avoid bony sealing of the crypt around the successional teeth [7].

On the other hand, the permanent presence of dental lamina in all the observation periods analysed in this paper, at any moment showed either a thin out of its cells at 16 days of intra-uterine life embryos or that it was going to occur a complete sever of developing tooth germ from the oral epithelium where did it come from by bony encapsulation, as stated by Cohn [14]. Does not corroborate the report that at the bell stage the dental follicle totally involves the tooth germ including its occlusal extremity with the occurrence of dental lamina disintegration between dental organ and oral epithelium; it does not support also, the information that the bone of the alveolar process completely surrounds the dental follicle to constitute the bony crypt [2, 4].

Conclusion

Based on the results found in this paper it was concluded that the dental lamina of mice first molars is a structure which appears at the begin of tooth germ formation, persists for all time of its development and only disappears with the total eruption of the dental crown of the tooth into the oral cavity.

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