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STAGE-DEPENDENT HISTOMORPHOGENESIS OF THE ALVEOLAR CRYPT DURING PRIMARY TOOTH ERUPTION IN KITTENS

Tooth eruption is a spatially and temporally coordinated process in which alveolar bone resorption and formation are integrated with dental follicle cell differentiation and establishment of the future periodontal complex. Nevertheless, the regional histoarchitectural features of the bony crypt during eruption of primary teeth in diphyodont mammals remain insufficiently characterized. ***Aim of the study.*** *To characterize stage-dependent histomorphogenesis and vestibulolingual asymmetry of the alveolar crypt surrounding mandibular anterior primary tooth germs in kittens and to relate these changes to the direction of crown displacement during eruption.* ***Materials and methods.*** *Post-mortem mandibular material from 10 deceased kittens was examined. Two groups were distinguished using macroscopic developmental criteria: mandibles with a smooth alveolar surface (n=5) and mandibles in which primary tooth crowns protruded above the mandibular surface (n=5). Specimens were fixed in 10% neutral formalin, gently decalcified with Trilon B, processed routinely in paraffin, sectioned, and stained with hematoxylin and eosin or Van Gieson stain. Microscopy and photodocumentation were performed using an Olympus BX41 microscope at ×100 and ×200. The analysis was descriptive and histomorphological.* ***Results.*** *At the follicular stage, a thin periosteal layer generated trabeculae oriented parallel and perpendicular to its surface. Bone maturation was spatially heterogeneous: vestibular trabeculae were predominantly eosinophilic and appeared less advanced in ossification, whereas lingual trabeculae were more basophilic and exhibited greater osteocytic incorporation. Intertrabecular spaces progressively acquired reticular and myeloid tissue. Concomitantly, organized collagen bundles, endosteal structures, and concentric bone lamellae developed. This asymmetry coincided with a change in crown orientation from oral to oblique vestibular.* ***Conclusions.*** *The primary tooth crypt in kittens develops non-uniformly and displays marked vestibulolingual asymmetry of ossification. These findings support a model in which spatially selective alveolar bone remodeling establishes the morphological conditions for directed tooth displacement while the periodontal attachment apparatus is formed.*

Key words: *tooth eruption, primary teeth, dental follicle, alveolar crypt, alveolar bone, osteogenesis, kittens, histomorphology.*

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ЕТАПОЗАЛЕЖНИЙ ГІСТОМОРФОГЕНЕЗ КІСТКОВОЇ ЛУНКИ ПІД ЧАС ПРОРІЗУВАННЯ ТИМЧАСОВИХ ЗУБІВ У КОШЕНЯТ

Прорізування зуба є просторово й часово координованим процесом, у якому резорбція та новоутворення альвеолярної кістки поєднуються з диференціюванням клітин зубного фолікула та формуванням майбутнього пародонтального комплексу. Водночас регіональні гістоархітектонічні особливості кісткової крипти під час прорізування тимчасових зубів у дипгіодонтних ссавців залишаються недостатньо охарактеризованими. ***Метою дослідження було*** *охарактеризувати етапозалежний гістоморфогенез і присінково-язикову асиметрію кісткової лунки навколо зачатків фронтальних тимчасових зубів нижньої щелепи кошенят та зіставити ці зміни з напрямком переміщення коронки під час прорізування.* ***Матеріали та методи.*** *Досліджено постмортальний матеріал нижніх щелеп 10 загиблих кошенят. За макроскопічними ознаками розвитку сформовано дві групи: з гладкою поверхнею альвеолярного відростка (n=5) та з коронками тимчасових зубів, що виступали над поверхнею нижньої щелепи (n=5). Препарати фіксували у 10% нейтральному формаліні, проводили щадну декальцинацію трилоном Б, парафінову проводку, виготовляли тонкі зрізи та забарвлювали гематоксиліном і еозином і за Ван Гізоном. Мікроскопію та фотодокументування виконували за допомогою Olympus BX41 при збільшеннях ×100 і ×200. Аналіз мав описовий гістоморфологічний характер.* ***Результати дослідження.*** *На фолікулярному етапі визначали тонкий шар окістя, від якого формувалися кісткові трабекули, орієнтовані паралельно та перпендикулярно до його поверхні. Виявлено виразну просторову неоднорідність дозрівання кісткової тканини: з присінкового боку трабекули були більш еозинофільними та мали ознаки меншої осифікації, тоді як з язикового боку переважали базофільні, більш зрілі кісткові структури з активнішим включенням остеоцитів. Міжтрабекулярні простори поступово заповнювалися ретикулярною та мієлоїдною тканиною. Паралельно формувалися впорядковані колагенові волокна, ендостальні структури та концентричні кісткові пластинки. Така асиметрія збігалася зі зміною орієнтації коронки від оральної до косої присінкової.* ***Висновки.*** *Формування кісткової лунки тимчасового зуба у кошенят відбувається нерівномірно й характеризується присінково-язиковою асиметрією осифікації. Отримані дані підтримують концепцію, за якою просторово вибіркове ремоделювання альвеолярної кістки створює морфологічні умови для спрямованого*

переміщення зуба та одночасного формування тканин пародонтального комплексу.
Ключові слова: *прорізування зубів, тимчасові зуби, зубний фолікул, кісткова лунка, альвеолярна кістка, остеогенез, кошенята, гістоморфологія.*

Tooth eruption is not a simple consequence of root elongation or passive displacement. It is a multistage developmental event that transfers a tooth from its intraosseous position to functional occlusion through tightly coordinated remodeling of the surrounding tissues. Current evidence identifies the intact dental follicle and competent osteoclast function as indispensable components of this process. The prevailing model therefore emphasizes regional asymmetry: bone is resorbed along the coronal eruption pathway while bone formation at the basal or apical aspect of the crypt contributes to intraosseous displacement [1, 2].

The dental follicle is a neural crest-derived ectomesenchymal compartment surrounding the enamel organ and dental papilla. Its progenitor populations contribute to alveolar osteoblasts, periodontal ligament fibroblasts, and cementoblasts, while also regulating the local recruitment and differentiation of osteoclast precursors [2–5]. This dual competence provides a biological basis for the spatial polarity of eruption-associated remodeling. Coronal follicular regions favor osteoclastogenesis through mediators that include colony-stimulating factor 1 and the RANK–RANKL–osteoprotegerin axis, whereas basal and lateral regions retain pronounced osteogenic potential. RUNX2, NELL-1, Wnt, bone morphogenetic protein, and PTHrP–PTH1R signaling participate in the temporal control of these cell fates [3, 4, 6, 7].

Recent lineage-tracing and single-cell studies have refined this concept. PTHrP-positive follicular cells populate distinct periodontal lineages during root and crypt formation, and their fate depends on precisely timed epithelial–mesenchymal signaling [5, 8]. In particular, the Hedgehog–Foxf axis must be dynamically restricted to permit differentiation of follicle-derived alveolar osteoblasts [8]. Contemporary developmental data also indicate that macrophage-lineage cells are not merely inflammatory bystanders: they enter dental tissues early, expand before eruption, and influence odontogenesis, osteogenesis, and tissue homeostasis [9]. These molecular advances reinforce the view that eruption emerges from a coordinated tissue ecosystem rather than from a single propulsive structure.

Despite these advances, molecular maps do not fully describe the three-dimensional histoarchitecture through which eruption is accomplished. Most mechanistic work has used mouse or rat molars and incisors, although follicular behavior differs substantially by tooth type and eruption pattern [10]. Histological descriptions of primary dentition in other diphyodont mammals remain comparatively limited. Such comparative observations cannot be transferred directly to humans, but they can reveal spatial relationships between periosteal osteogenesis, trabecular maturation, marrow formation, and tooth-germ orientation that are difficult to reconstruct from isolated-cell systems.

A further terminological distinction is relevant. Before eruption and complete periodontal attachment, the developing tooth occupies a bony crypt rather than a mature functional socket. Characterizing how that crypt acquires region-specific trabecular and lamellar organization may clarify the tissue-level substrate on which contemporary molecular pathways operate. The present investigation therefore re-examines a staged kitten model using a histomorphological framework centered on regional bone maturation and the direction of primary tooth displacement.

The purpose of the study was to characterize stage-dependent histomorphogenesis and vestibulolingual asymmetry of the mandibular alveolar crypt surrounding anterior primary tooth germs in kittens and to assess how these spatial patterns coincided with changes in crown orientation during eruption.

Materials and methods. Study design and material. This descriptive histomorphological study used post-mortem mandibular specimens obtained from 10 deceased kittens. No intervention was performed on living animals as part of the investigation. Exact chronological age and sex were not available in the original specimen records; consequently, developmental stage was defined macroscopically rather than chronologically.

The material was divided into two equal groups. Group 1 comprised five mandibles with a smooth alveolar surface and no clinically protruding primary crowns, corresponding to a pre-eruptive stage. Group 2 comprised five mandibles in which the crowns of primary teeth protruded above the mandibular surface, corresponding to a later eruptive stage. The anterior mandibular region was selected because it occupied a compact area while permitting consistent preparation of the developing primary tooth germs and surrounding bony crypts.

After removal of the mandible, specimens were fixed in 10% neutral formalin. Gentle decalcification was performed with Trilon B (disodium ethylenediaminetetraacetate). Targeted blocks containing the anterior tooth germs and adjacent alveolar tissues

were excised, dehydrated, cleared, embedded in paraffin, and sectioned on a microtome. Deparaffinized sections were stained with hematoxylin and eosin for general tissue architecture and with Van Gieson stain for collagen-fiber organization. Microscopic examination and photodocumentation were performed with an Olympus BX41 light microscope at original magnifications of ×100 and ×200.

The analysis focused on: (1) orientation of the primary tooth germ; (2) periosteal architecture; (3) direction, staining characteristics, thickness, and cellular composition of the developing trabeculae; (4) maturation of osteoblasts and osteocytes; (5) organization of intertrabecular reticular and hematopoietic tissue; and (6) lamellar and collagen-fiber arrangement in the forming alveolar crypt. Because the study was qualitative and the number of specimens was limited, no inferential statistical analysis was applied.

Results of the study and their discussion. At the follicular stage, the central structure was the developing tooth crown, bordered by mature secretory ameloblasts and the dental papilla. The papilla consisted of mesenchymal tissue at different stages of differentiation. Secretory ameloblasts were columnar, becoming partly flattened toward the apical crown region. In the developing root region, a loop-like configuration consistent with Hertwig's epithelial root sheath was observed. The reduced enamel epithelium surrounding the crown became progressively attenuated where it was compressed by adjacent trabeculae.

The tooth germ changed from a predominantly oral orientation to an oblique vestibular trajectory. On the vestibular aspect of the canine crown, a projection beneath the non-keratinized stratified squamous epithelium corresponded topographically to the future emergence site. Immediately below the epithelium, a thin periosteal layer containing collagenous fibers and fibroblasts at different stages of differentiation was present. Within recessed areas of this periosteum, osteogenic cell condensations and early trabecular processes arose, establishing the initial outline of the bony crypt (Fig. 1).

Mineralization and subsequent ossification were not uniform around the follicle. Trabeculae adjacent to the epithelium on the vestibular side retained pronounced eosinophilia and appeared less advanced in ossification than those on the lingual side. This regional difference coincided with the vestibular redirection of the crown and suggested that the developing tooth encountered a less mature bony environment along its prospective path of displacement. The vestibular trabeculae remained relatively delicate and were bordered by osteoblasts and early marrow elements (Fig. 2).

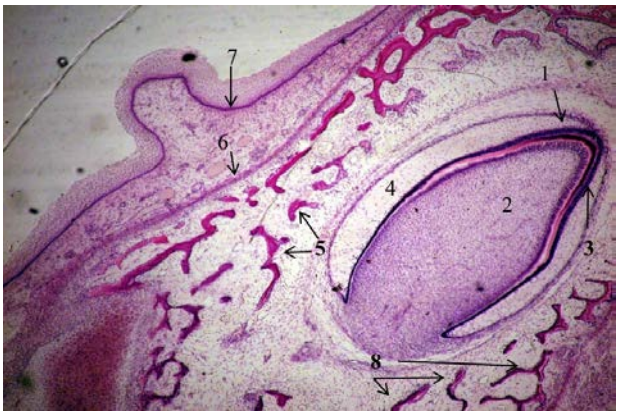

Fig. 1. Formation of the alveolar crypt at the follicular stage.

1 – crown of the tooth follicle; 2 – dental papilla; 3 – secretory ameloblasts; 4 – remnants of the stellate reticulum of the enamel organ; 5 – eosinophilic trabeculae of developing lamellar bone; 6 – periosteum; 7 – non-keratinized stratified squamous epithelium; 8 – basophilic trabeculae of developing bone. Hematoxylin and eosin, ×100

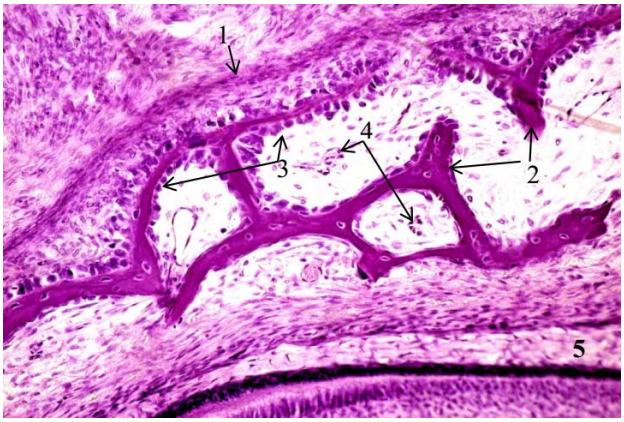

Fig. 2. Eosinophilic trabecular structures on the vestibular aspect of the developing crypt.

1 – periosteum; 2 – mineralized trabeculae; 3 – osteoblasts; 4 – hematopoietic marrow cells; 5 – remnants of the stellate reticulum of the enamel organ. Hematoxylin and eosin, ×200

On the oral/lingual aspect, progression toward lamellar bone was more conspicuous. Trabeculae increased in thickness, and osteocytes became incorporated within the mineralizing matrix. The greater number of embedded osteocytes was accompanied by more pronounced basophilia of the tissue, reflecting a different stage of matrix maturation from that seen vestibularly. Osteoblasts remained aligned along the trabecular surfaces, indicating continuing appositional growth (Fig. 3).

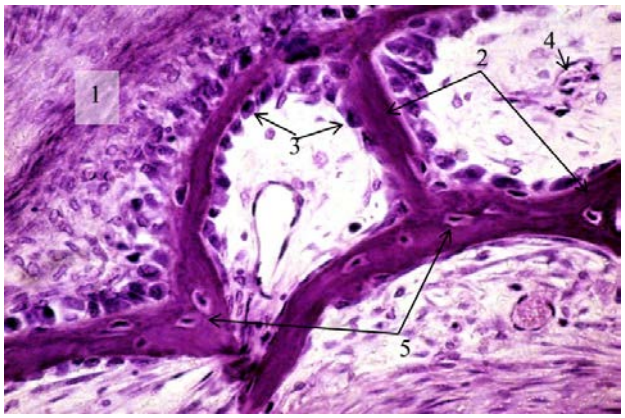

Fig. 3. Ossification of trabecular bone on the oral aspect of the developing crypt.

1 – periosteum; 2 – ossified trabeculae; 3 – osteoblasts; 4 – hematopoietic marrow cells; 5 – osteocytes. Hematoxylin and eosin, ×200

Intertrabecular lacunar spaces underwent parallel maturation. A pale reticular framework appeared between the ossifying trabeculae and contained developing myeloid and other hematopoietic cells. This transition indicated that crypt formation included not only deposition of mineralized matrix but also establishment of a functional marrow compartment. The spatial association of osteoblasts, osteocytes, and hematopoietic elements was especially apparent in areas of more advanced lingual ossification (Fig. 4).

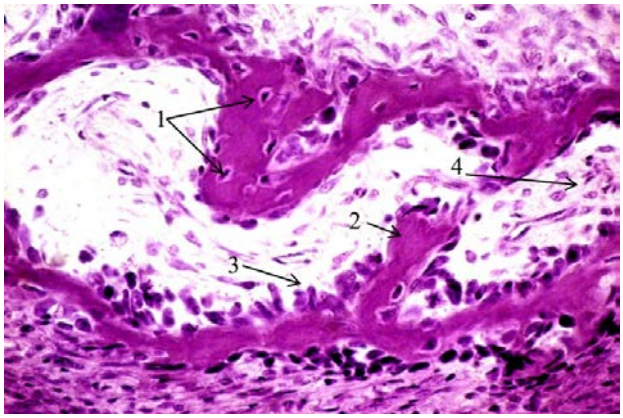

Fig. 4. Developing hematopoietic tissue within the intertrabecular spaces.

1 – osteocytes; 2 – ossified trabeculae; 3 – osteoblasts; 4 – hematopoietic marrow cells within a pale reticular stroma. Hematoxylin and eosin, ×200

The formation of red marrow and the conversion of woven trabeculae toward lamellar bone proceeded more slowly on the vestibular side than on the lingual side. Van Gieson staining demonstrated collagen bundles with parallel and oblique courses within the developing crypt. The periosteum appeared as a narrow layer of parallel collagen fibers, while deeper bundles intersected the forming trabeculae and bounded lacunar spaces. This architecture documented a transition from a fibrous osteogenic scaffold to organized bone plates (Fig. 5).

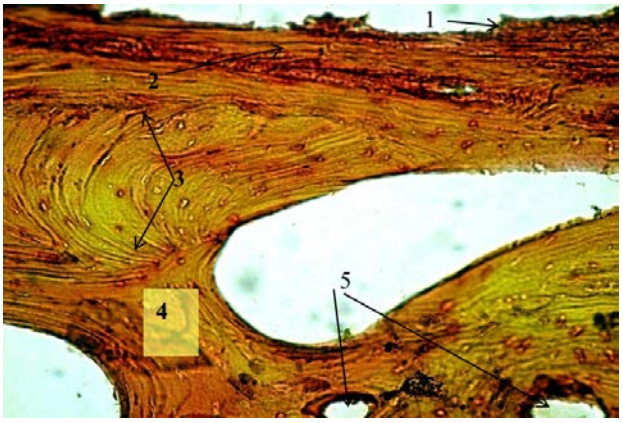

Fig. 5. Formation of lamellar bone at the follicular stage.

1 – periosteum; 2 – parallel collagen-fiber course; 3 – oblique collagen-fiber course; 4 – trabeculae; 5 – lacunae. Van Gieson stain, ×100

Near the periosteum, collagen fibers surrounded individual bone plates and adopted an oblique orientation relative to the periosteal surface. Elongated cells were occasionally visible between the fiber bundles. At higher magnification, cells of the osteoblastic lineage displayed different degrees of differentiation. Flattened osteocyte-like cells occupied concentric layers around developing endosteal surfaces, whereas rounded osteoblasts remained closer to active matrix-forming fronts. Eosinophilic lamellae formed concentric arrangements around microvascular spaces, and cellular processes traversed adjacent plates (Fig. 6).

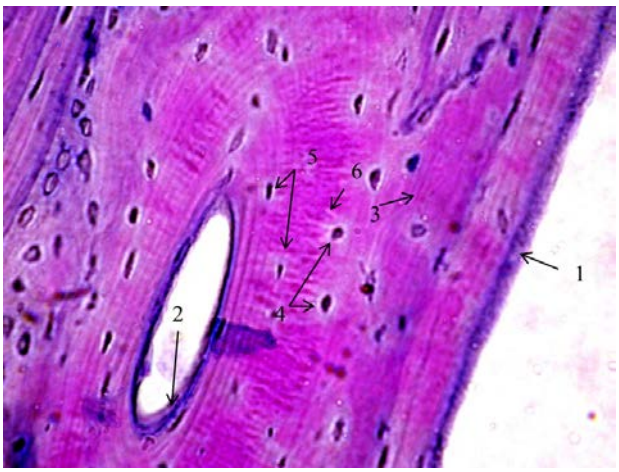

Fig. 6. Lamellar bone architecture adjacent to the periosteum.

1 – periosteum; 2 – endosteal lining of a lacunar space; 3 – eosinophilic fibrous matrix; 4 – rounded osteoblasts; 5 – elongated osteocytes; 6 – osteoblastic cell processes. Hematoxylin and eosin, ×200

The principal observation was a reproducible spatial inequality in maturation of the bony crypt. Vestibular trabeculae were less advanced, whereas lingual trabeculae displayed greater basophilia, osteocytic incorporation, lamellar organization, and marrow maturation. This vestibulolingual gradient accompanied a change in crown orientation toward an oblique vestibular direction. The findings therefore extend the well-established coronal–apical model of eruption-associated remodeling by indicating that, in the examined anterior primary teeth, regional maturation may also vary across the vestibulolingual axis.

The result should be interpreted as a tissue-level association rather than proof of a single mechanical mechanism. Contemporary models regard eruption as an emergent outcome of coronal bone clearance, basal bone formation, follicular signaling, root and periodontal development, and local tissue resistance [1, 3, 6]. In this context, delayed vestibular ossification could reduce resistance along the prospective eruption trajectory, while more advanced lingual bone formation could stabilize the crypt and contribute to directional bias. Both processes may operate concurrently. The present sections cannot determine their relative mechanical contribution, but they provide a morphological substrate for that hypothesis.

The observed periosteal and trabecular architecture is compatible with the current understanding of the dental follicle as a source and regulator of periodontal cell lineages. Follicular progenitors can adopt osteoblastic, fibroblastic, and cementoblastic fates, and their differentiation is regionally regulated [2–6, 11]. In particular, recent work has shown that alveolar crypt osteoblasts arise substantially from PTHrP-positive follicular cells and that controlled Hedgehog–Foxf signaling is required for their proper differentiation [8]. The simultaneous presence of osteogenic condensations, surface osteoblasts, incorporated osteocytes, and increasingly ordered collagen bundles in the kitten crypt reflects successive tissue states expected during this lineage transition.

The coexistence of reticular marrow tissue and developing myeloid cells is also relevant. Osteoclasts derive from the monocyte–macrophage lineage, and eruption depends on coordinated osteoclastogenesis in the coronal region [1, 9, 12]. Very recent developmental evidence further demonstrates that macrophage populations participate in normal tooth patterning and osteogenesis rather than functioning exclusively in inflammation [9]. However, hematoxylin and eosin staining alone cannot establish lineage identity or functional state. Accordingly, the cells observed between trabeculae should be regarded as hematopoietic elements; confirmation of osteoclast or macrophage subpopulations would require tartrate-resistant acid phosphatase staining, immunohistochemistry, or molecular profiling.

The histochemical contrast between eosinophilic and basophilic trabeculae was useful for mapping relative maturation, but it is not a direct quantitative measure of mineral density. Van Gieson staining strengthened the interpretation by demonstrating reorganization of the collagen scaffold and emergence of lamellar architecture. Future studies should integrate undecalcified histology, micro-computed tomography, quantitative histomorphometry, and markers of bone formation and resorption. Candidate panels include RUNX2, SP7/osterix, alkaline phosphatase, osteocalcin, RANKL, osteoprotegerin, CSF1, PTHrP/PTH1R, and macrophage/osteoclast markers.

Comparative interpretation requires particular caution. Tooth-type-specific differences in follicular osteogenic capacity and RANKL/osteoprotegerin expression have been demonstrated even within rodent dentitions [10]. The kitten anterior dentition therefore offers a complementary diphyodont mammalian model but cannot be assumed to reproduce human primary tooth eruption. Its value lies in the preservation of the spatial relationship among epithelium, periosteum, follicle, trabeculae, marrow, and the changing axis of the tooth germ–relationships that are necessarily simplified in isolated-cell systems.

Conclusions. During primary tooth eruption in kittens, the alveolar crypt developed through coordinated periosteal osteogenesis, trabecular thickening, osteoblast-to-osteocyte maturation, collagen reorganization, and establishment of marrow spaces. These events were regionally asynchronous: ossification and lamellar maturation were more advanced lingually than vestibularly. The asymmetry coincided with vestibular redirection of the developing crown and may contribute to the spatial guidance of eruption. Tooth movement and formation of the periodontal supporting apparatus should therefore be considered interdependent components of a single morphogenetic process.

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