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Quantitative histological features and ultrastructure of opercula of human teeth showing normal and delayed eruption

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BACKGROUND: Failure of eruption of human permanent molars has been attributed to opercular lesions, although comparisons with specimens from normally erupting teeth are scarce. The aim of this study was to quantitatively analyse opercula associated with normal and delayed tooth eruption.

METHOD: Twenty opercula covering permanent molars delayed in eruption were obtained from 13 patients aged 7.3–18.1 years. Six opercula from normally erupting molars of five 7.3–17.5-year-old subjects served as controls. Specimens were analysed light and electron microscopically and morphometrically.

RESULTS: In addition to features recognized previously, prominent numbers of nerves, high endothelial-like venules and mast cells were observed. Ultrastructurally, large multinucleated cells did not reveal cell boundaries running between the nuclei, and mast cells seemed belonging to the MC_{TC}-type. None of the features differed significantly between opercula from cases of delayed and normal tooth eruption.

CONCLUSIONS: Disturbances of tooth eruption that are attributed to opercular lesions may represent retentions resulting from the failure of the eruption mechanism, rather than impactions because of a physical barrier.

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Introduction

Disturbances of tooth eruption in humans can result from ectopic position of a tooth, local obstacles in the eruption path, or failures in the eruption mechanism (1). Cessation of eruption because of ectopic tooth position or a physical barrier is referred to as impaction. Retention is defined as delayed eruption in the absence of such readily apparent reasons (1).

A particular form of eruption disturbances observed mainly in permanent molars has been attributed to lesions in the opercula of the non-erupting teeth. These lesions are considered to be of odontogenic origin and hamartomatous in nature, although they histopathologically resemble the WHO type of odontogenic fibroma that is regarded as neoplastic in nature (2–7). Designations proposed for the opercular lesions, such as hyperplasia (2, 5, 7, 8) or fibromatosis (8, 9) imply that they constitute a physical obstacle preventing the normal emergence of the subjacent teeth. In order to avoid such a bias, the simple descriptive term operculum associated with delayed tooth eruption (OADTE) is adopted and used throughout this report.

Histopathological components of OADTE observed universally include (1) particularly dense fibrous tissue, (2) rests of odontogenic epithelium and (3) calcifications (3–5, 7–9). The fibrous tissue frequently has been characterized as whorled (3, 5, 7) or nodular (8). Few investigators (2, 3) described hyalinized areas surrounding the odontogenic epithelium, which were interpreted as 'induction phenomenon'. Calcifications were described by some authors as laminated structures (4) and resembling cementum (3, 8, 10), dentin (4, 8, 10) or enamel (8). Certain other authors distinguished small, polarizing type 'A', and large, non-polarizing type 'B', calcifications (5, 7). Another prominent feature of OADTE, less frequently recognized than the above constituents, is multinucleated giant cells that are described as stellate or spindle-shaped (8–10). Hyperplasia of the oral epithelium was observed rarely (8).

Among the investigators of OADTE, only Andrews (11) and Yonemochi et al. (8) seem to have examined opercula of normally erupting teeth. While the former reported an 'increased organization of collagen bundles' around an 'increased number of epithelial rests' in OADTE, many of the control specimens used by the latter appear to have lacked a dental follicle. Thus, a systematic analysis of opercula from non-erupting and erupting teeth seems to be missing, although such an analysis is a prerequisite for associating opercular lesions with eruption disturbance.

Therefore, the aim of this study was to compare opercula obtained from cases of delayed tooth eruption with those from normally erupting teeth, for which light and electron microscopy as well as morphometry were used.

Material and methods

Subjects and tissue processing

With the informed consent of the patients and their parents and with the approval of the local ethical committee, 20 opercula were surgically removed under local anaesthesia from 13 cases exhibiting delayed eruption of one tooth to four teeth. Six opercula obtained from five patients with normal tooth eruption served as controls. The genders and ages of the subjects involved and the locations of the biopsies are summarized in Table 1. Delayed and normal eruption of teeth was ascertained with consecutive panoramic radiographs and, in some cases, also lateral cephalograms taken at intervals of about 1 year (Fig. 1). When, as exemplified by the lower right second molar in Fig. 1a,b, no vertical displacement of a tooth crown could be observed relative to neighbouring and/or contralateral teeth or relative to bony landmarks such as the mandibular canal or zygomatic crest, a delay in eruption was assumed. In contrast, unequivocal vertical movement as revealed by the lower left and both upper second molars (Fig. 1) was indicative of undisturbed emergence.

For ethical reasons, opercula associated with normal eruption were removed only, when there was an orthodontic indication to accelerate the emergence of the subjacent teeth so as to prevent the imminent overelongation of prematurely erupting antagonists. All these patients as well as 10 of the cases showing delayed eruption were followed up after the surgical intervention, although no further radiographs were taken, when the emergence of the teeth was clinically visible.

Table 1 Sources of opercula used

	Delayed eruption	*Normal eruption*
Male (*N*)	9	3
Age range (years)	8.8–18.1	7.3–17.5
Female (*N*)	4	2
Age range (years)	7.3–15.5	7.4–14.4
Upper first permanent molars (*N*)	6	
Lower first permanent molars (*N*)	6	2
Upper second permanent molars (*N*)	3	
Lower second permanent molars (*N*)	5	4

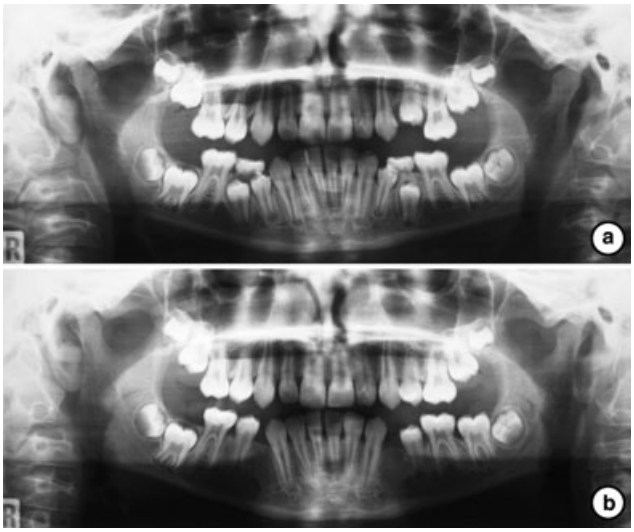

Figure 1 Consecutive panoramic dental radiographs of a boy at the age of 13 years 3 months (a) and 14 years 3 months (b). Note the absence of any eruptive movement of tooth 47 in contrast to teeth 17, 27 and 37. Micrographs of the operculum over tooth 47 are displayed in Fig. 2.

Immediately after excision, the opercula were placed in half-strength Karnovsky's solution and fixed at room temperature for 3–4 days. After an overnight wash in 0.185 M sodium cacodylate buffer (pH 7.4), they were divided buccolingually into five to six slices of 1–1.5 mm thickness. These slices were post-fixed in 1.33% osmium tetroxide buffered with 0.067 M s-collidine (pH 7.4) for 2 h at room temperature, dehydrated in ascending grades of alcohol and embedded in Epon. For the light microscopic examination and morphometric analysis, sections were cut at a thickness of 2 μm using a Reichert Ultracut E microtome (Leica, Glattbrugg, Switzerland) and histo-diamond knives (Diatome, Biel, Switzerland). They were stained with periodic acid-Schiff (PAS) and methylene blue-Azur II or toluidine blue. For the ultrastructural investigation, thin sections of gold-silver interference colour were prepared with the same microtome following reduction in size of the blocks. These sections were contrasted with uranyl acetate and lead citrate and examined with a Philips EM400T transmission electron microscope (TEM; FEI company, Eindhoven, the Netherlands) at 60 kV.

Morphometric analysis

Linear measurements were taken in two histological sections, each, selected from three tissue blocks per specimen. The thickness of the epithelium over connective tissue papillae, the height of the papillae, as well as the thicknesses of the lamina propria and subjacent dental follicle were measured at a magnification of ×40 using a conventional light microscope equipped with an eye-piece micrometer. Similarly, the diameters of nerve fiber bundles were recorded at a magnification of ×100. As the lamina propria blended gradually with the dental

Table 2 Histometric measurements as well as volume densities (V_V) and numerical densities (N_V) of connective tissue components in opercula associated with delayed and normal tooth eruption

	Delayed eruption		*Normal eruption*		
Layer/component	N^a	*Mean (range)*	N^a	*Mean (range)*	*Significance*[b]
Epithelium					
Thickness (μm)	18	174 (102–286)	6	159 (106–186)	$P > 0.10$
Lamina propria (Lp)					
Thickness (μm)	19	836 (443–1242)	6	746 (505–902)	$P > 0.10$
Papilla height (μm)	19	303 (183–418)	6	353 (227–459)	$P < 0.05$
Dense connective tissue V_V (%)	20	79.1 (73.1–86.9)	6	82.1 (77.3–88.4)	$P > 0.05$
Odontogenic epithelium V_V (%)	20	4.1 (0.0–13.4)	6	3.3 (1.6–3.3)	$P > 0.10$[c]
Blood vessels					
V_V (%)	20	2.3 (0.0–5.6)	6	2.9 (1.0–4.4)	$P > 0.10$[c]
N_A (N/mm^2)	20	17 (1–46)	6	22 (13–32)	$P > 0.10$
Nerve fiber bundles					
V_V (%)	20	1.7 (0.0–3.4)	6	1.5 (0.9–2.8)	$P > 0.10$
N_A (N/mm^2)	20	12 (0–22)	6	13 (5–22)	$P > 0.10$
Giant cells					
V_V (%)	20	0.5 (0.0–1.3)	6	0.2 (0.0–0.5)	$P > 0.10$
N_A (N/mm^2)	20	3 (0–8)	6	2 (0–3)	$P > 0.10$
Dental follicle (Df)					
Thickness (μm)	19	1951 (494–3263)	6	1359 (0–1982)	$P > 0.05$
Dense connective tissue V_V (%)	20	56.8 (43.2–73.1)	5	54.3 (47.1–65.5)	$P > 0.10$[d]
Odontogenic epithelium V_V (%)	20	1.8 (0.0–6.0)	5	1.6 (0.0–4.9)	$P > 0.10$[d]
Blood vessels					
V_V (%)	20	0.3 (0.0–0.8)	5	0.5 (0.1–0.9)	$P > 0.10$[d]
N_A (N/mm^2)	20	2 (0–10)	5	4 (2–6)	$P > 0.10$[b]
Nerve fiber bundles					
V_V (%)	20	0.3 (0.0–0.9)	5	0.1 (0.0–0.3)	$P > 0.05$[d]
N_A (N/mm^2)	20	2 (0–8)	5	1 (0–2)	$P > 0.10$[d]
Giant cells					
V_V (%)	20	0.5 (0.0–1.7)	5	0.3 (0.1–0.6)	$P > 0.10$
N_A (N/mm^2)	20	4 (0–11)	5	2 (0–3)	$P > 0.10$
Nerve fiber bundles (Lp + Df)					
Thickness (μm)	20	76.7 (20–1220)	6	59.6 (30–140)	$P > 0.10$

[a]Number of specimens available.
[b]Significance of differences between delayed and normal eruption as obtained with Mann–Whitney test.
[c]Significantly ($P < 0.05$) different, when categorized data were tested with chi-square test.
[d]Significantly ($P < 0.05$) varying between top, middle, and deep region of dental follicle; differences between delayed and normal eruption insignificant ($P > 0.1$) in any region.

follicle, the boundary between the two layers was assumed to be where the dense arrangement of the collagenous connective tissue fibers appeared to brake up.

Three histological sections, each, derived from three tissue blocks per specimen were analysed stereologically. The volume proportion and numerical frequency data of selected connective tissue components were determined using a Wild M501 sampling microscope (Leica) equipped with a viewing screen and coherent double lattice stereological test grids. Particles and test points were counted at a magnification of ×200. This allowed to place one test field per sampling site over the lamina propria, whereas for the analysis of the thicker dental follicle, as many contiguous test grids as possible were arranged vertically and counts obtained from the various depths recorded separately. The volume (V_V) and numerical (N_A) densities thus determined were transformed into percentages and number of profiles/mm^2, respectively. The connective tissue components that were analysed comprised (1) areas of dense connective tissue with compact arrangement of collagen fibers, (2) odontogenic epithelium, (3) blood vessels, (4) nerve fiber bundles and (5) multinucleated giant cells (Table 2).

Statistical analysis

The statistical analysis was carried out with the program SYSTAT 10 (SPSS, Chicago, IL, USA). For tabulation, data from individual specimens were pooled and mean values and SD were calculated for opercula associated with normal and delayed tooth eruption. Measurements of thicknesses and the proportion of dense connective tissue revealed more or less symmetric, normal distributions and were compared statistically using two-way (normal vs. delayed eruption and specimens) ANOVA with repeated measures (blocks and sections). All other parameters that characterized relatively rare components and, therefore, exhibited markedly right-tailed distributions, were tested in two steps. The raw data were first transformed into the categories 'component absent', 'rare', and 'frequent' and the categorized data were analysed using cross-tabulation and Pearson chi-squared test. As these analyses did not reveal significant variations within specimens, the raw data were subsequently averaged across sections and blocks. The

resultant mean values were used for comparisons with the aid of Mann–Whitney test.

Results

In all the cases that were available for follow-up examinations, the involved teeth erupted spontaneously after removal of the opercula. Within a period of a few months to about 1 year, they reached the occlusal plane or at least emerged into the oral cavity. Even in a case in which the root growth of two lower first molars, lacking any appreciable eruptive movement, had resulted in sharp distal bends of the roots, the two teeth emerged spontaneously following the surgical exposure.

The microscopic evaluation of the opercula revealed that the composition of the connective tissue differed considerably between the lamina propria and the dental follicle (Fig. 2a; Table 2). However, the constituents of the two layers appeared similar, both in OADTE and specimens from normally erupting teeth. Strands or islands of odontogenic epithelium were observed frequently (Fig. 2a,c; Table 2). Sometimes they were arranged in a perforated band that ran perpendicular to the opercular surface and traversed the entire dental follicle (Fig. 2a).

Blood vessels and bundles of nerve fibers were abundant, particularly in the lamina propria (Table 2). These two constituents often formed clusters surrounded by whirls of collagen fibers (Figs 2b and 3a). Individual nerve fiber bundles that were identified based on the presence of myelinated fibers were also encapsulated by collagenous sheaths (Figs 2b and 3a). Electron microscopically, thin unmyelinated fibers were evident in addition to the myelinated fibers that exhibited a diameter of 1.5–3.5 μm. Apart from local widening and disruptions of myelin sheaths, they appeared perfectly normal. Vascular profiles included numerous high endothelial-like venules (HELV) revealing a distinct, often undulating basal membrane subjacent to the high endothelial cells and relatively large cuboidal pericytes (Fig. 2b). Ultrastructurally, the basal membrane consisted of multiple layers of irregular basal lamina complex (Fig. 3b).

Inflammatory cell infiltrates were not observed. But cells resembling mast cells, which contained numerous metachromatic cytoplasmic granules, were seen in the vicinity of blood vessels and nerves (Fig. 3a). In the TEM, these membrane-bound granules contained electron-dense, amorphous or fine granular material, but lacked membranous scroll formations (Fig. 3b). Stellate or spindle-shaped large multinucleated cells were interspersed among the collagen fibers (Fig. 2a, inset). Ultrastructurally, the cytoplasm of these cells was contiguous and did not reveal any cell boundaries between the nuclei (Fig. 2d).

Occasionally, specimens associated with both delayed and normal tooth eruption contained calcified bodies embedded in the connective tissue matrix. Two types of them were identified. The first was relatively large, round or irregular in shape, and surrounded by a sheath of collagen fibers and cuboidal cells (Fig. 3c). The mineralized core usually appeared amorphous and did not reveal the typical appearance of normal dental hard substances (Fig. 3c). The second type of calcification was much smaller than the first one and more or less spheroid in shape. Electron microscopically, it displayed a very electron-dense, irregularly outlined core and several regular peripheral lamellae of variable electron density (Fig. 3d). These calcifications were surrounded by elongated, flat lining cells and did not reveal any inflammatory or foreign-body reaction in the neighbourhood (Fig. 3d).

The only statistically significant ($P < 0.05$) difference, found between OADTE and specimens from normally erupting teeth, applied to the height of the connective tissue papillae of the lamina propria (Table 2). In the dental follicle, the proportions of dense connective tissue, odontogenic epithelium, blood vessels and nerve fiber bundles decreased significantly ($P < 0.05$) with increasing depth. However, data from the successive sublayers did not vary significantly ($P > 0.1$) between opercula associated with normal and delayed eruption (Table 2).

Discussion

The results of this study indicate that opercula associated with normal and delayed eruption are similar with respect to the type and quantity of their connective tissue components. Among the constituents assessed quantitatively, odontogenic epithelium (2–9, 11) and giant cells (8–10) have been repeatedly noted by several investigators of OADTE. Calcifications resembling enamel, dentin, or cementum have also been regularly reported as constituents of the dental follicle covering non-erupting teeth (3–5, 7, 8, 10). In contrast, the neural elements of opercula, whether associated with delayed or normal eruption, seem to have been judged normal. In comparison with published micrographs of healthy human gingiva (12), the densities of opercular nerve fiber bundles appeared to be markedly higher. The fact that this has gone unnoticed previously could be due to the absence of post-fixation with osmium tetroxide that considerably enhances the visibility of myelinated nerve fibers. The role of the rich nerve supply of opercula is unknown. As the size and arrangement of some of the small calcified bodies observed in this and a previous (7) study resembled the appearance of nerve fiber bundles, these mineralized structures could possibly be due to calcification of neural elements. However, the TEM examination did not reveal any abnormal nerve fiber bundles or changes in neural structures, which could not be accounted for by artefacts of aldehyde fixation (13).

Similar to nerves, blood vessels seemed more abundant in the opercula than in the biopsies of normal human masticatory mucosa. Furthermore, a large proportion of vascular profiles resembled HEV. Apart from being encountered regularly in lymphoid tissue, this type of vessel also occurs in chronically inflamed gingiva and is known to constitute the vascular site of lymphocyte emigration (14). However, no neutrophilic and/or round

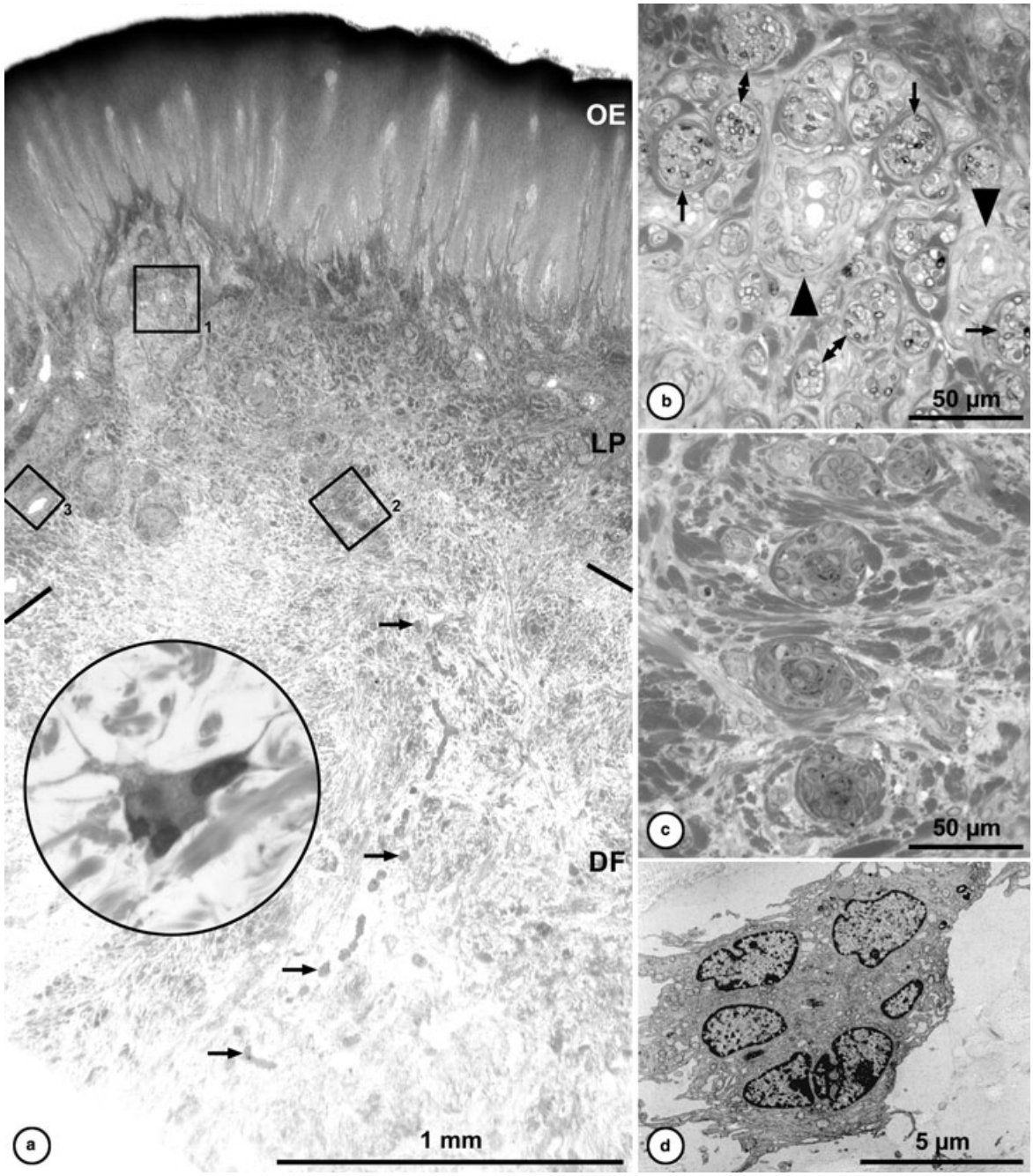

Figure 2 Operculum of tooth 47 taken from the case presented in Fig. 1. (a) Overview light micrograph of the keratinized oral epithelium (OE), the lamina propria (LP), the dental follicle (DF) and remnants of odontogenic epithelium (arrows). The approximate border between LP and DF is marked by bars at the left and right panel margins. The demarcated areas numbered 1, 2 and 3 are enlarged in b, c, and Fig. 3a, respectively. The inset shows a multinucleated giant cell in the dental follicle. (b) Magnified view of rectangle 1 in (a) showing high endothelial-like venules (arrowheads) and bundles of nerve fibers (arrows). (c) Detail of odontogenic epithelium in area 2 demarcated in (a). (d) Transmission electron micrograph of a multinucleated giant cell that does not reveal any cell boundaries between the nuclei. (a–c) periodic acid-Schiff (PAS)/methylene blue-Azur II; original magnifications (a) ×20, inset ×500, (b–c) ×320, (d) ×1200.

cell infiltrates were observed in opercula, irrespective of whether they were associated with normal or delayed eruption.

Rather than neutrophils, lymphocytes and plasma cells, numerous mast cells were consistently observed in the vicinity of HELV. Based on the ultrastructure, the cytoplasmic granules of mast cells appeared to be similar to those of the MC_{TC} type that is increased in number in certain fibrotic diseases. But they may also have particular role in tissue remodeling and angiogenesis (15).

Judging from the published nomenclature of OADTE (2, 5–10), these lesions have been thought of mechanical obstacles that hinder normal tooth eruption. This view is not substantiated by the present morphometric data,

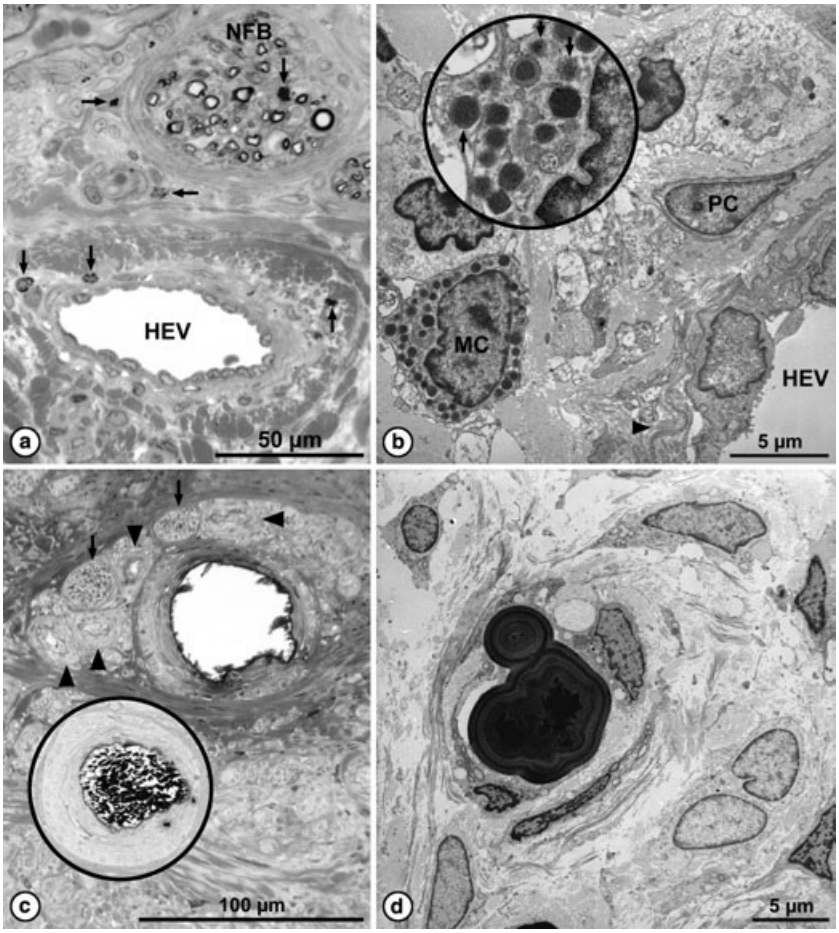

Figure 3 (a) Magnified view of area 3 demarcated in Fig. 2a showing mast cells (MC, arrows) in the vicinity of a high endothelial-like venule (HEV) and a nerve fiber bundle [NFB; periodic acid-Schiff (PAS)/methylene blue-Azur II; original magnification ×320]. (b) Transmission electron micrograph of a MC and part of a HEV revealing the basal lamina complex (arrowhead) between high endothelial cells and a pericyte (PC; original magnification ×1200). From the inset, the fine-granular content of some cytoplasmic granules (arrows) of the MC is evident (original magnification ×7000). (c) Light micrograph of a large calcified body surrounded by a sheath of collagen fibers and cuboidal cells, HEV (arrowheads), and NFB (arrows; PAS/methylene blue-Azur II; original magnification ×200). The same calcification stained using van Kossa's method and counterstained with neutral red is shown in the inset. (d) Transmission electron micrograph of two confluent small, laminated calcifications surrounded by flat lining cells (original magnification ×1200).

which suggest that the proportions of dense connective tissue, odontogenic epithelium, nerves, blood vessels and giant cells are similar in OADTE and specimens covering normally erupting teeth. Thus, none of these constituents conceivably can be responsible for a delay in tooth emergence. Calcifications, too, were similar in opercula of teeth with normal and delayed eruption so that they obviously would not constitute a physical barrier. This is plausible, because bone and mineralized tissues of deciduous teeth normally do not hinder or prevent the emergence of the permanent successors. The increased thickness of OADTE could be due to an early cessation or complete absence of eruptive vertical tooth movement that results in a tooth position similar to that during crown formation (Fig. 1). Consequently OADTE reflects the original thickness of the dental follicle and oral mucosa at the onset of the eruptive migration, rather than being hyperplastic. Thus, the disturbance of tooth eruption associated with opercular lesions may constitute a retention because of a failure in the eruption mechanism rather than an impaction.

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