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ARTICLE

Histological structure of the human and rodent periodontium

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ABSTRACT Adult human dental tissue and young rat jaws with developing teeth were investigated. The tissues were fixed by immersion in 4% buffered paraformaldehyde. Decalcified tissues were embedded into paraffin or sectioned on a freezing microtome. Paraffin sections were stained with hematoxylin and eosin. Frozen sections were stained with calcitonin gene-related peptide (CGRP) antibodies using avidin-biotin systems and peroxidase labelling. The histology of the acellular cement and the histology of the periodontal ligament were analyzed and the course of the Sharpey-fibers and the epithelial rests of Malassez were described in human samples. The epithelial sheath of Hertwig in developing rat maxillae and mandibles (postnatal days 1-11) were depicted. The layers of the Hertwig-sheath were described. We observed the numerical increase of CGRP-stained nerve fibers during these postnatal days. The CGRP-stained nerve fibers appeared before the development of the dental root indicating the presence of growth factors which guide the sensory axons. We hypothesize that the epithelial sheath of Hertwig plays essential role in the development of cementoblasts, periodontal fibroblasts and alveolar osteoblasts. Furthermore, the growth factors secreted by the Hertwig-sheath may stimulate the axonal growth, too. **Acta Biol Szeged 59(Suppl.3):345-352 (2015)**

KEY WORDS

dental cement
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Introduction

The periodontium or tooth-bed is a complex structure of hard- and soft tissues supporting the teeth. It consists of the cement covering the tooth, the periodontal ligament, the alveolar bone and the gums or gingiva. The periodontium participates in the fixation of the teeth, participates in the absorption of the root of the deciduous teeth, in the regeneration of the cement and in sensory reflexes which regulate masticatory movements, just to mention the most important functions (Gera 2005). The diseases of the periodontium are manyfold, affecting every structural element and they are treated in clinical subject of periodontology (Gera 2005).

The cement is modified bone tissue, containing cells called cementocytes. The upper part of the cement, close to the tooth neck, does not contain cells: this is the acellular cement. The acellular cement is mainly for the anchorage of the Sharpey-fibers originating from the periodontal membrane. The cellular cement is a layered tissue: according to the layers the collagen fibers of the matrix are arranged circularly and/or longitudinally (Yamamoto et al. 2010). The cellular cement increases in thickness with age: generally it is thickest at the apex of the root. The periodontal ligament/membrane fills the gap between the cement and the alveolar bone. It is rich in cells some of which are undifferentiated stem cells (Volponi et al. 2010). The periodontal membrane is richly vascularized and contains several sensory nerve endings (Hattyasi 1982). The alveolar bone has fine, lamellar structure and a cell-rich periosteum with osteoblasts and osteoclasts (Harokopakis-Hajishengallis 2007). The bone is traversed by minute openings for blood vessels and nerves. The gingiva is a special mucous membrane covered by stratified squamous epithelium which may show parakeratosis. The gingival connective tissue is rich in sensory nerve endings. The periodontal tissues constitute a morphological and functional unit – their diseases often lead to teeth loss (Gera 2005). We illustrate the main morphological-histological components of the periodontium on Figure 1.

The periodontium has a multiple, rich blood supply. Blood vessels originate from the sublingual artery (branch of the lingual artery), from the inferior alveolar artery and the superior alveolar arteries (branches of the maxillary artery), from the descending palatine artery and from the facial artery. Veins are draining into the facial vein, lingual vein and retromandibular vein (Liebgott 2001). The sensory nerves of the periodontium originate from the maxillary- and mandibular divisions of

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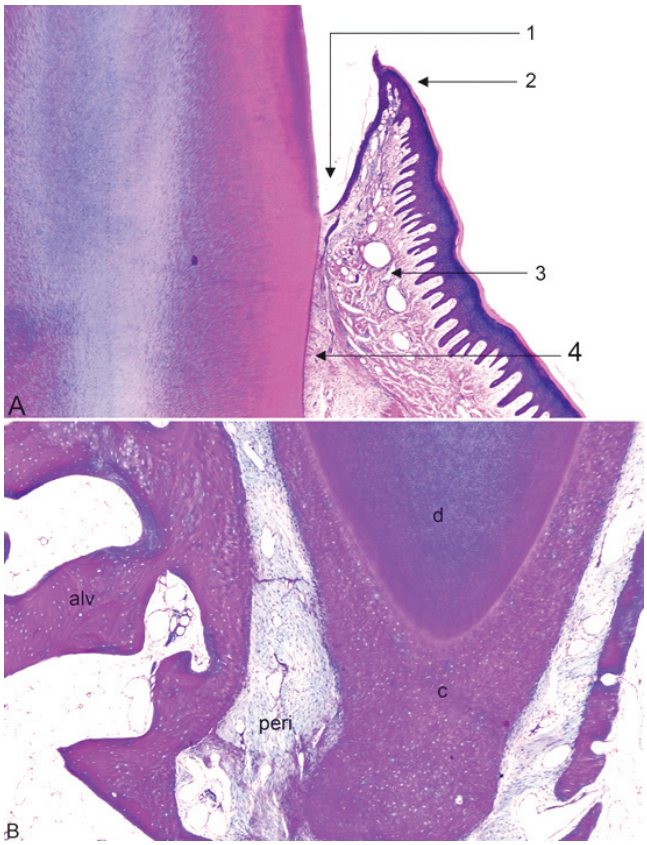

Figure 1. Histological section of an incisor tooth of a Rhesus monkey (from the histological collection of the Department of Anatomy, Histology and Embryology (Faculty of Medicine, University of Szeged, Szeged, Hungary), displaying the elements of the periodontal apparatus. Decalcificated tissue, hematoxylin-eosin staining. A: the gingiva at the neck of the tooth. The enamel was dissolved during the decalcification, therefore, the gingival sulcus (1) is larger, than *in vivo*. The marginal gingiva/gingival crest (2), the lamina propria (3) and the periodontal membrane (4) are visible. B: higher magnification shows the alveolar bone (alv), the periodontal ligament (peri), the cement (c) and the dentine (d) of the apical region of the tooth. Magnification: 10x (A) and 20x (B).

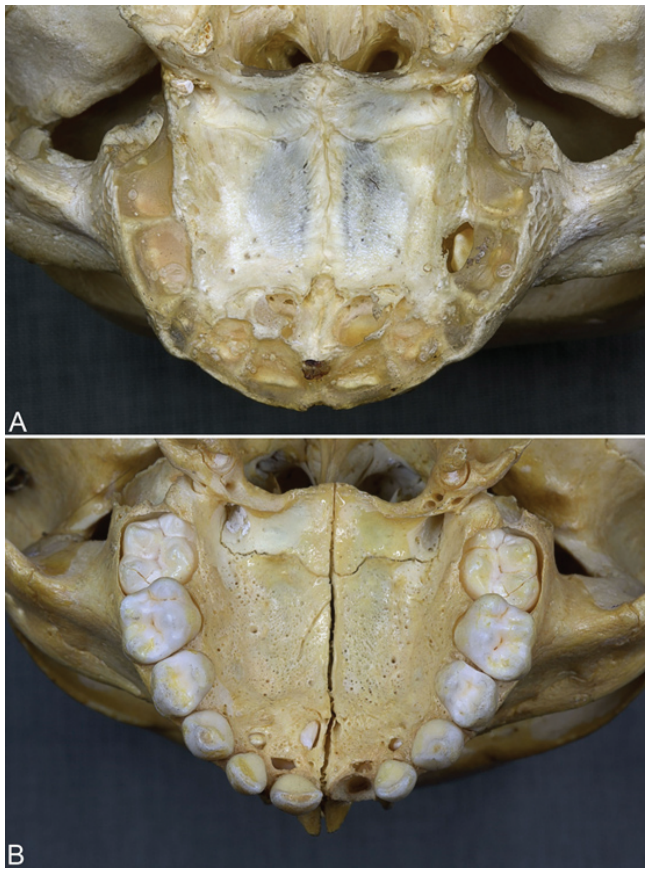

Figure 2. The upper dental arch in newborn (A) and 7 years old boy (B). The alveolar process of the maxilla is not developed in the newborn human; the deciduous teeth are located in the alveolar sockets covered by a dense connective tissue membrane. B: the first permanent molar is just before eruption. Deciduous teeth and alveolar processe are well developed. The palatine sutures are also visible.

the trigeminal nerve. The nerve endings are nociceptors and mechanoreceptors (Hattyasi 1982).

The aim of this work was to describe the anatomical and histological features of the human periodontium, using human skull and teeth samples and histological sections of extracted human teeth. The developmental aspects of the periodontium was investigated in rat pups, on the histological sections of the developing maxillae, mandibles, lower and upper teeth.

Material and Methods

Gross anatomy observations were carried out on human skulls and human permanent teeth: the skulls and teeth are properties of the anatomy specimen collection of the Department of Anatomy, Histology and Embryology (Faculty of Medicine, University of Szeged, Szeged, Hungary). The skulls and other bones were prepared by the technicians of the department during the years 1947-1966, according to the prevailing laws and ethical regulations of the Szeged Medical University (Mihály et al. 2014). The bones are stored in the museum of the department (Mihály et al. 2014). The bony structures of the dental alveoli (including the teeth) were photographed with MicroPublisher 5.0 RTV digital camera attached to a Nikon SMZ800 stereomicroscope (Nikon, Japan).

The collection of the histological sections of the extracted human teeth is the property of the Department of Anatomy, Histology and Embryology (Faculty of Medicine, University of Szeged, Szeged, Hungary). The extracted human teeth were obtained from the Dentistry Clinic of the Medical University in years 1982-1990. The ethical permission was

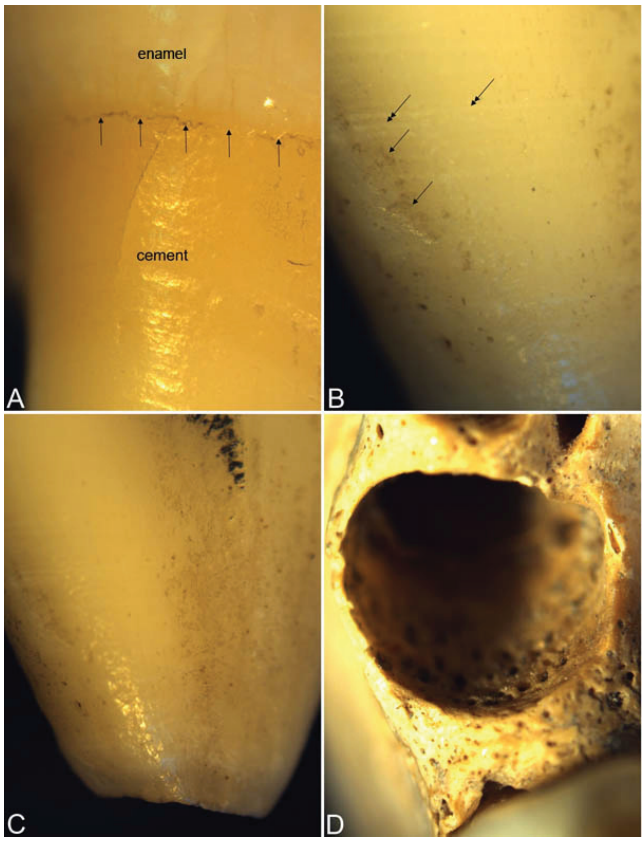

Figure 3. Permanent human tooth and alveolar socket in adult human mandible. A: the enamel and the cement are clearly separated by a serrate line (arrows) at the neck of the tooth. B: transverse ridges (double arrows), and shallow erosions (arrows) on the surface of the root cement of the adult tooth. C: the apical region of the cement is thick and shiny. D: the alveolar bone displays several minute canaliculi for vessels and nerves. Magnification: 3x.

obtained according to the prevailing laws in years 1982-1990. The extracted teeth were treated according to the following methods. The teeth were placed into buffered (pH 7.0) 10% (v/v) formalin solution: the apical foramina were widened in order to facilitate the diffusion of the fixative into the pulp chamber. Following the fixation, the teeth were washed in tap water and put into the decalcification solution, which contained 10% ethylenediamine tetraacetic acid (EDTA) in distilled water and/or 10% (v/v) formic acid supplemented with 10% (v/v) formalin in distilled water. Decalcification was made at room temperature for 5-15 days, which was followed by paraffin embedding (Kiernan 1999). Embedded tissues were sectioned (5-10 μm) and stained with hematoxylin and eosin (Borges Silva et al. 2011). Silver impregnation of the decalcificated teeth was performed according to Romanes (Romanes 1950; Hattyasi 1982). This silver staining method is suitable for the staining of axons, but not for nerve endings (Romanes 1950).

Developing teeth were studied in rat pups: 1, 3, 5, 9, 10 and 11 days old rats were used. The rats were deeply anesthetized with diethylether, decapitated and their heads were put into 4% phophate-buffered (pH 7.4) paraformaldehyde. The heads were fixed by immersion at 4 °C for 10 days. The paraformaldehyde solution has been changed three times during the 10 days. The mandibles and maxillae with soft tissues and teeth were separated from the skull and decalcificated. The decalcificating solution contained 1.4% (v/v) EDTA and 1% (v/v) dimethyl sulfoxide (DMSO) in distilled water (Sanderson et al. 1995). Decalcification was done for 5-10 days. The tissues were sectioned on a freezing microtome (25-30 μm) and the sections were used for immunohistochemistry. Other samples were embedded into paraffin; thin sections (5 μm) were cut and stained with hematoxylin-eosin and Mallory s trichrome stain for collagen fibers (Kiernan 1999). Immunohistochemistry sections were stained with rabbit anti-calcitonin-gene-related protein (CGRP) diluted to 1:10 000. The secondary antibody (goat anti-rabbit; 1:400 dilution) was biotinylated. The detection system was based on peroxidase (streptavidin-peroxidase; 1:2000 dilution). The peroxidase activity was localized with a diaminobenzidine-tetrahydrochloride (DAB-HCl) and hydrogen peroxide (H_2O_2) substrate solution containing nickel ammonium sulfate (Károly et al. 2015). Nerve fibers containing CGRP were appearing in black color. Controls of immunohistochemistry included incubations omitting the primary antibody. Detailed description of the CGRP immunostaining method is given in our previous article (Károly et al. 2015). Chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Results

Alveolar processes of the maxilla and mandible in human

The alveolar process of the maxilla is not seen on newborn skulls – compared to the maxillae in 5-6 years old child. The alveoli are visible with the teeth inside. The alveoli are covered with a dense connective tissue membrane (Fig. 2). The bony surfaces of the mature alveoli present microscopic openings and canaliculi (cca. 0.1 mm wide) which are present inside, on the interalveolar septa and on the gingival surface of the alveolar bone (Fig. 3). Histology pictures display characteristic lamellar bone structure (Fig. 4).

The histology of the human and rodent periodontal ligament

The surface of the extracted teeth displays remnants of soft tissue: the periodontal ligament. This tissue is rich in

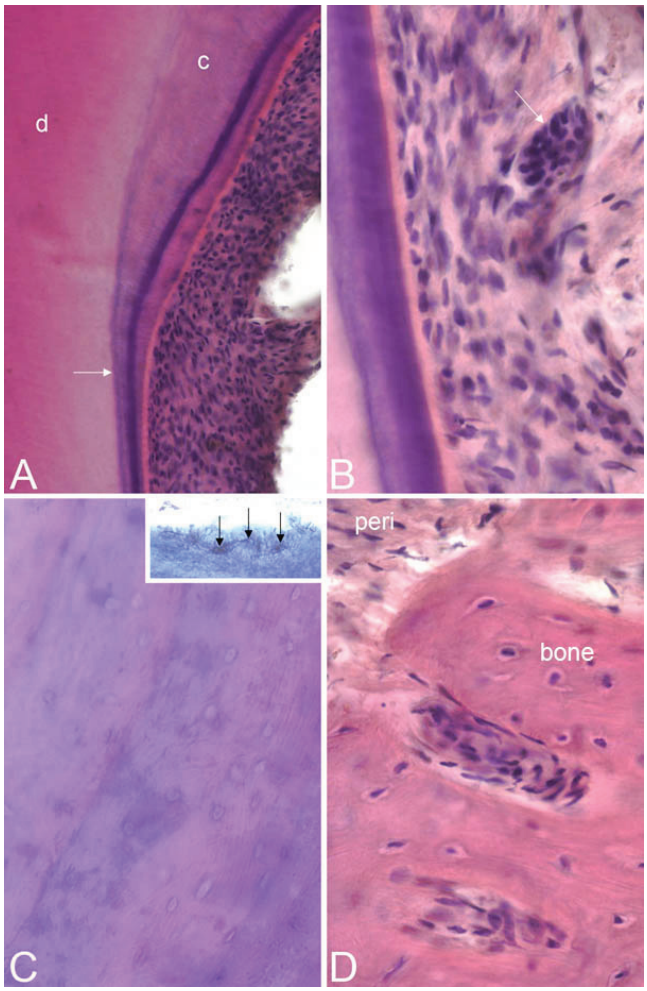

Figure 4. Histology of the human periodontium of extracted teeth (decalcification, hematoxylin-eosin staining). A: the border of the acellular and cellular cement is pointed by an arrow (c: cement; d: dentine; magnification: 10x). B: the periodontal membrane contains the epithelial rests of Malassez (arrow). C: the cellular cement displays layers with slightly different basophilia. Inset: cementocytes (arrows). Inset picture was taken from a Romanes-stained human tooth. D: the alveolar bone (bone) displays lamellar structure with osteocytes (peri: apposition of the periodontal membrane and the alveolar periosteum). Magnification on B, C, D: 20x.

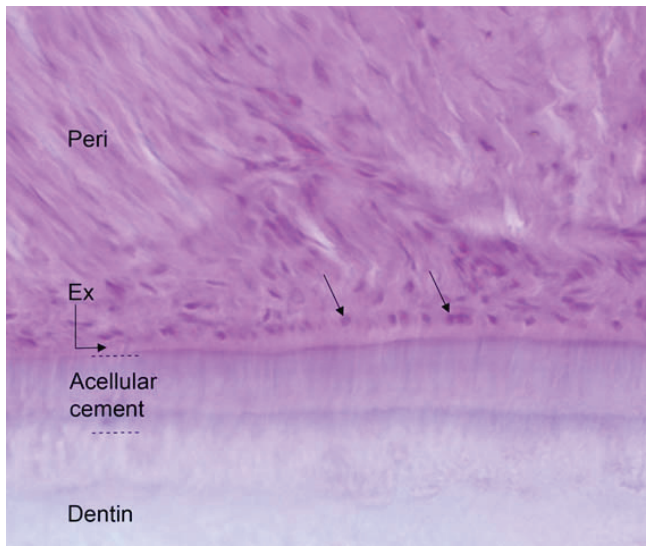

Figure 5. Higher magnification of the periodontal membrane (peri) and acellular cement (acellular cement). The fibers and cells of the periodontal membrane are visible. A single layer of cells (presumably cementoblats) are localed close to the surface of the cement (oblique arrows). The right-angled arrow points to a thin layer of eosinophilic collagen fibers attached to the cement (Ex). These are the Sharpey-fibers. The cement is covered by a thin basophilic layer and perpendicular to this, thin basophilic extensions showing the ordered internal structure of the matrix of the cement. Dentin: dentine layer. Hematoxylin-eosin staining, magnification: 40x.

cells resembling to spinocellular connective tissue. The hematoxylin-eosin staining did not reveal the exact nature of the cells, but some important features were observed. The cells of the connective tissue form a continuous layer on the surface of the cement: we suppose that most of these cells are cementoblasts (Fig. 5). The other cells could be fibroblasts/fibrocytes, eosinophils and macrophages although clear definition was not possible. The fibers of the connective tissue are arranged in parallel bundles, which are perpendicular or oblique to the surface of the cement (Fig. 5). We observed cell groups containing 20-30 cells located close to each other in the periodontal membrane: the cells resembled to epithelial cells and formed oval nests (Fig. 4). We identified them as the epithelial rests of Malassez (Tadokoro et al. 2008). Using the Romanes-technique we identified nerve fibers in the periodontal ligament: the Romanes-technique is not suitable for nerve endings, therefore only axon bundles and single axons were observed (Fig. 6). The nerves enter from the alveolar bone through the small bony canaliculi.

The structure of the human dental cement

The enamel-cement border is clear on the tooth: it is a serrate line on the neck of the tooth. The cement surface is shiny, yellowish with transverse lines visible under the stereomicroscope (Fig. 3). The root cement often displays shallow surface erosions (Fig. 3). Close to the root apex it becomes whitish, showing its thickening at the apex.

Histological investigation of the extracted human teeth revealed the clear border between the acellular- and cellular regions of the cement. Hematoxylin-eosin staining shows a thin, homogenous eosinophilic surface layer (2-4 μm), which contains the Sharpey-fibers (Fig. 4). In the acellular cement, a strongly basophilic layer follows, which is followed by a less basophilic layer: this layer displays the Sharpey-fibers running perpendicularly to the surface, and ending 30-50 μm

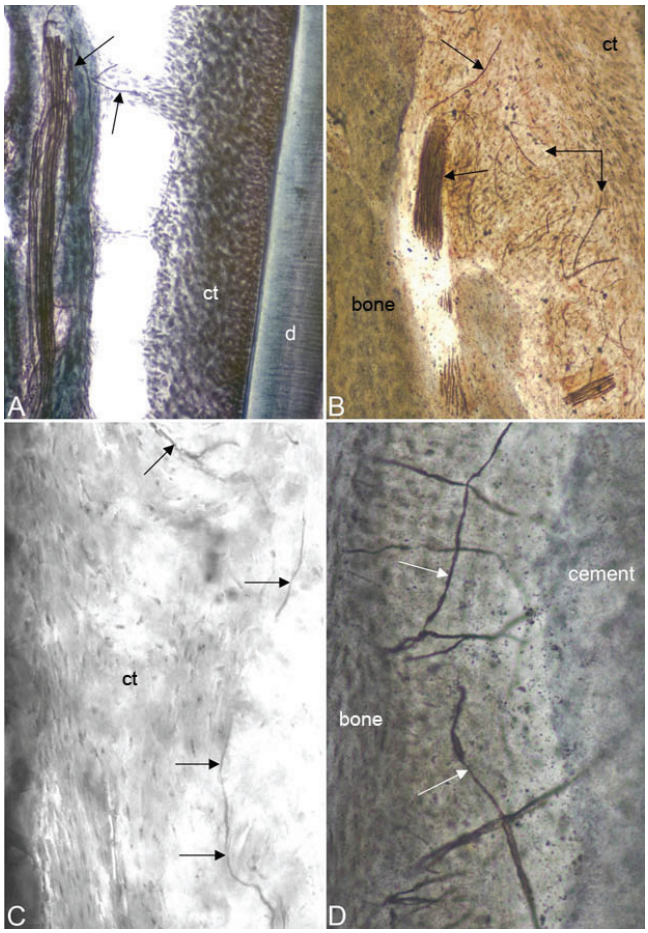

Figure 6. Innervation of the periodontium in human (C) and rat (A, B, D), Romanes staining. The axon bundles run in the bone (bone), and enter the periodontal membrane (ct) from there. The arrows point to single axons running in the connective tissue of the periodontal ligament (d: dentine). Magnification is 20x in A, B; 40x in C, D.

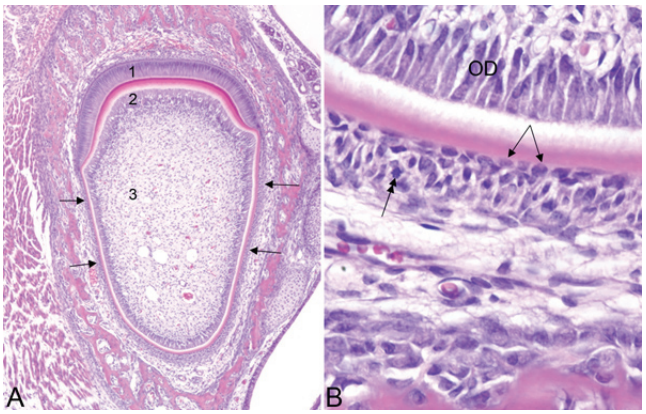

Figure 7. Developing tooth primordium in the maxilla of 2-day-old rat. Ameloblasts (1), odontoblasts (2) and the developing pulp (3) are seen on A. The arrows point to the sheath of Hertwig. Small amount of enamel and dentine are deposited. Magnification: 10x. B: higher magnification (40x) of the future root region. Odontoblasts (OD) and secreted dentine are visible. Outside of the dentine, a single layer of cementoblasts is visible (arrows). Outside of this cell layer, the Hertwig-sheath cells form an epithelium-like layer, with some mitotic figures (double arrow). Outside of the Hertwig-sheath richly vascularized periodontal connective tissue can be observed. Hematoxylin-eosin staining.

deep in the acellular cement (Fig. 4). In the cellular cement, the superficial basophilic layer becomes thinner, and the cementocytes appear under surface (Fig. 4). The cells possess short processes and are located in characteristic lacunae (Fig. 4). The cellular cement displays layers, which are visible because they differ from their basophilia. The thickness of the layers is between 20-50 μm (Fig. 4). The cellular cement contains elongated canaliculi rarely, resembling the Volkmann-canals in the bone: these may contain small blood vessels (not shown).

Periodontal structure in developing rat molars

Observations were made on the developing alveolar bone and periodontal membrane. The tooth primordium is embedded into the developing maxilla. In one-day-old rats, the alveolar bone is spongy, its surface is covered by osteoblasts and occasional oscteoclasts are observed, too (Fig. 7). Adamantoblasts and odontoblasts form a closed vesicle-like structure, inside which the future dental pulp tissue is located. The secreted dentine and enamel are first visible in 2 day-old-rats, and become thicker in later days (Fig. 7). The space between the tooth primordium and the alveolar bone is filled by a cell-rich connective tissue, containing blood vessels. The amount of collagen fibers was increasing with time during the 11 days long investigation. This was demonstrated with the Mallory-trichrome staining (not shown). The ameloblast layer covers the future crown; at the neck of the primordium it turns into a thinner, multi-layered epithelium-like structure, which covers the rest (the future root) of the primordium (Fig. 7). This structure was termed the epithelial root sheath of Hertwig (Hertwig 1847). The Hertwig-sheath was attached to the dentine, covering it completely (Fig. 7). The periodontal membrane is outside of it: whilst the Hertwig-sheath is a solid layer, the periodontal membrane is a loose connective tissue. The Hertwig-sheath does not contain blood vessels, whilst the periodontal ligament is richly vascularized (Fig. 7).

The CGRP-immunoreactive nerve fibers are present in the the periodontal membrane already in 1-day-old rats. The nerves enter from the alveolar bone and surround the entire tooth primordium (Fig. 8). The thinner fibers displayed fine varicosities. The density of the nerves increases during the development (days 1-11). We did not perform counting or densitometry of CGRP-containing axons in this study.

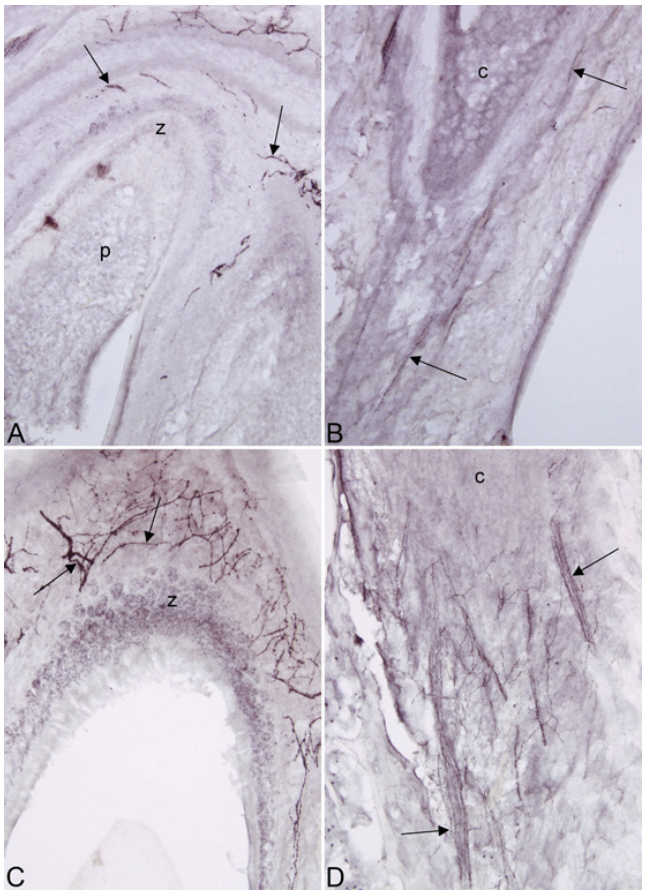

Figure 8. The CGRP-like immunoreactivity of the developing rat incisors. Arrows point to CGRP-stained axons. A, B: 1-day-old rat; C, D: 11-day-old rat (p: pulp; c: cement; z: enamel). Note the increase of immunoreactive axon number in 11-day-old animals. Magnification: 20x.

Discussion

Our results are in accordance with literature data of the periodontal apparatus (Liebgott 2001; Gera 2005). We discuss the most important issues, which were described in our experiments.

Basophilia of the cement

The acellular and the cellular cements contain layers which are seen with conventional stains (*e.g.*, hematoxylin-eosin stain). The thin basophilic layer on the surface of the acellular cement is due to the accumulation of glycosaminoglycans and proteoglycans (Kiernan 1999). These molecules are necessary for the fixation of the collagen fibers which come from the periodontal ligament and penetrate the cement as Sharpey-fibers (Yamamoto et al. 2010). The cellular cement also contains layers; these indicate the growth of the cement – the thin basophil staining between the layers may indicate the „glue" function of these matrix molecules (Yamamoto et al. 2010). These matrix components ensure the stability of the cement: the cement is fixed to the dentin and outside to the periodontal ligament, and therefore, it has a very important role in the fixation of the tooth.

The periodontal membrane

The periodontal membrane (or periodontal ligament) is a complicated connective tissue system, containing blood vessels, nerves, and connecting the alveolar bone to the radix of the tooth. The connective tissue contains organized and ordered collagen fiber bundles, which penetrate the bony tissues (cement and alveolar bone) and contribute to the fixation of the teeth (gomphosis). The periodontal membrane is rich in cells: we find fibroblasts/fibrocytes, cementoblasts, cementoclasts, osteoblasts, osteoclasts and a few immune cells. Literature data exist on the presence of stem cells in the periodontal ligament (Volponi et al 2010). Their functional role is a matter of recent investigations (Washio et al. 2010; Saito et al. 2015; Zhu and Liang 2015).

Probably related to undifferentiated cells, the human periodontal ligament contains the epithelial cell rests of Malassez (Becktor et al. 2007). We also observed these cells in the periodontal membrane of the extracted teeth. The cells are separated from the connective tissue by means of lamina basalis, and the cells are connected by intercellular junctions characteristic of the covering epithelia (Tadokoro et al. 2008). Experimental data prove that these cells secrete interleukins regulating the functional activity of periodontal cells (Cerri et al. 2009). Following injuries to the periodontal membrane, the cells secrete ameloblast-proteins, which indicates that these cells are less differentiated (Nishio et al. 2010). Other data indicated that these cells synthesize cytokeratins, neuropeptides and extracellular matrix proteins (Rincon et al. 2006). Maybe, the cell rests of Malassez play some role in the regeneration of the periodontal apparatus: they regulate the connective tissue matrix, cementoblast differentiation and mineralization (Rincon et al. 2006).

The nerves of the periodontal ligament are mainly sensory branches of the trigeminal nerve: these are mechanoreceptors (mainly Ruffini-endings) and nociceptors (Hattyasi 1982; Wakisaka et al. 2000). The CGRP is regularly used to stain these sensory nerves and nerve endings (Kosaras et al. 2009). Few postganglionic sympathetic axons are present, too (Hattyasi 1982). The nerve endings mediate brainstem reflexes, detecting the stretching of the tissue during mastication (Wakisaka et al. 2000; Umemura et al. 2010). The sympathetic axons probably mediate vasoregulation effects (Hattyasi 1982). The nociceptors participate in inflammation and pain sensation transmission (Wakisaka et al. 2000). The

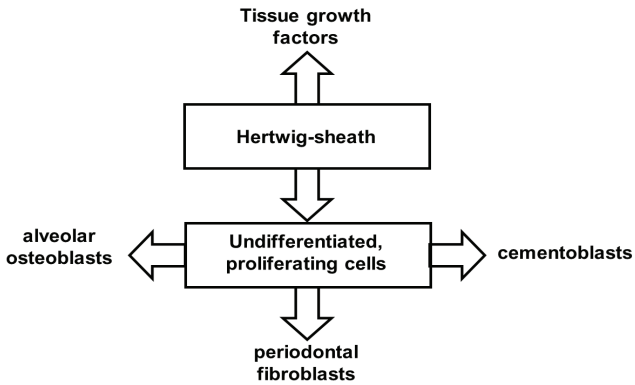

Figure 9. Summarizing diagram of the functional role of the Hertwig-sheath in the developing tooth.

CGRP immunohistochemistry proved the early appearance of the nociceptors: pointing to a growth regulatory (trophic) function of the thin axons during the development (Higuchi et al. 2008).

Developmental aspects of the parodontal apparatus: the epithelial sheath of Hertwig

The development of the parodontal apparatus is strongly related to the function: the mastication. In human, the alveolar processes (in maxilla) are not present at birth. As the teeth develop and erupt, the alveolar process grows, too. In rats, the eruption of the teeth happens during the first month: the incisors erupt first, then the molars in order of their position. The teeth develop in the bony socket of the alveolus and at the time of the eruption, they are mineralized. The periodontal apparatus develops earlier: the periodontal membrane is present already on the first day of life in rats, although the composition and the innervation are not fully developed. The innervation of the periodontal membrane is developing faster than the cement. In newborn rats, the cement is not developed, instead, the root is covered by a cell-rich sheath, which is known as the epithelial root sheath of Hertwig (Hertwig 1847). This sheath is the continuation of the ameloblast layer: the ameloblast stops at region of the tooth neck, where a stratified epithelial rooth sheath, the Hertwig-sheath follows. This sheath contains mitotic, proliferating cells, which cells probably contribute to the generation of the cementoblasts and the cement (Kumakami-Sakano et al. 2014). The Hertwig-sheath cells are probably differentiating from the neuromesenchyme of the tooth bud (Luan et al. 2009). On the other hand, this sheath also produces fibroblasts and osteoblasts, thereby contributing to the development of the alveolar bone and periodontal membrane (Kumakami-Sakano et al. 2014). We think that the Hertwig-sheath cells secrete growth factors which regulate the developmental processes of the periodontium, including the ingrowth of sensory axons (Kumakami-Sakano et al. 2014). It is tempting to speculate that the cell rests of Malassez are the remains of the epithelial sheath of Hertwig. The functional role of the Hertwig-sheath as a cell generator during tooth development is depicted on Figure 9.

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