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Immunohistochemical localization of epithelial rests of Malassez in human periodontal membrane

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SUMMARY The aim of the present study was to describe the localization and extension of epithelial rests of Malassez (ERM) in the periodontal membrane (PDM) in normal human third molars.

The material consisted of 24 normally developed human third molars surgically removed from patients with an age range from 15 to 27 years (six females and six males). The root lengths were developed from close to half-length to complete apex closure. The extracted teeth were fixed in 10 per cent neutral-buffered formalin, decalcified in ethylene diamine tetra acetic acid EDTA, paraffin embedded and cut sagittaly in 5 μm serial sections. Immunohistochemistry was performed using polyclonal rabbit anti-bovine cytokeratin (wide-spectrum screening, WSS) and the EnVision+ dual link system. The results were based on the visual comparison of WSS in the tissue sections using a light microscope.

It was demonstrated that the ERM cells were distributed in the PDM in a network-shaped manner along the root surface and in the furcation region. The distribution of ERM was more prominent in teeth with incomplete root formation.

The ectodermal tissue layer might influence not only the morphology of the tooth but also tooth eruption. The reaction of this epithelial layer in connection with ankylosis and orthodontic tooth movement may be of future interest.

Introduction

Tooth development is regulated by a series of reciprocal interactions between the surface epithelium and the underlying neural crest-derived dental ectomesenchyme. During initiation, the surface epithelium buds into the ectomesenchyme and secretes growth factors that induce the expression of specific genes in the dental ectomesenchyme in a highly co-ordinated pattern, and morphogenesis is initiated (Pispa and Thesleff, 2003). Crown morphogenesis progresses through a series of stages known as bud, cap, and bell (Pispa and Thesleff, 2003). When crown formation is complete, the outer and inner dental epithelium fuses to form Hertwig's epithelial root sheath (HERS).

HERS is believed to send an inductive message, possibly by secreting enamel proteins to the facing ectomesenchymal pulp cells. These cells differentiate into odontoblasts and produce a layer of pre-dentine. The next event taking place is formation of cementum on the root surface. The specific trigger factors responsible for cementum formation are unknown. However, one theory is that HERS cells transform into cementoblasts (Ten Cate, 2003). Thus, not only crown development but also development of the root and the periodontal membrane (PDM) are influenced by these epithelial and ectomesenchymal interactions. Epithelial abnormalities therefore also influence the morphology and function of the dental root and PDM.

The epithelial rests of Malassez (ERM) are development residues of HERS in the PDM. They are reported to appear in rabbits in cord- or net-like formations or as isolated islands near the cementum (Peters *et al.*, 1995).

The functions of the ERM are not fully understood; however, there are theories, including a possible role in regulating the homeostasis and width of the PDM, in PDM regeneration or in endocrine activity (Götz *et al.*, 2003).

Recently, immunoreactivity for nerve growth factor was observed in ERM cells in the cervical and furcation regions of molars in rats, but not in other non-neural cells such as osteoblasts, fibroblasts, odontoblasts, or cementoblasts (Yamashiro *et al.*, 2000). Denervation resulted in a marked decrease in the distribution area and size of ERM cells (Yamashiro *et al.*, 2000). A further study showed that denervation in rats resulted in dentoalveolar ankylosis associated with decreased numbers of ERM cells. It was concluded that ERM cells might protect the root against resorption (Fuiyama *et al.*, 2004).

Proliferation of ERM cells during tooth movements has also been described (Talic *et al.*, 2003).

Experimental studies investigating the distribution and function of ERM are usually animal based. Few have investigated these epithelial remnants in human PDM. Mizuno *et al.* (2005) studied ERM, which had been cultured from PDM removed from human teeth.

The aim of the present study was to describe the localization and extent of ERM in the PDM in normal erupting human third molars.

Subjects

All patients gave their informed consent to contribute to this research project.

The material comprised 24 normal human third molars surgically removed from patients with an age range from 15 to 27 years (six females and six males) due to lack of space or in connection with orthognathic surgery. The root lengths were from approximately half-length to complete apex closure (Table 1).

Method

Fixation, decalcification, and sectioning

The extracted teeth were fixed in 10 per cent neutral-buffered formalin for 5 days, and then decalcified in 0.5 M ethylene diamine tetra acetic acid (EDTA disodium salt, Titriplex II, Merck 8417, Darmstadt, Germany) for 6–10 weeks. After decalcification, the tissues were refixed in 10 per cent neutral-buffered formalin.

The tissue was dehydrated and embedded in paraffin using a double embedding method: in graded ethanols—methyl salicylate—1 per cent celloidine and finally embedded in paraffin.

Paraffin blocks were cut sagittally in 5 µm thick serial sections and mounted on Superfrost® Plus Microscope slides (Menzel, Braunschweig, Germany). The sections were dried overnight at 40°C.

Immunohistochemistry

Parraffin sections were deparaffinized and treated with Tris-EDTA (Merck), pH 9.0, for 2 hours at 60°C prior to immunohistochemical staining. This was undertaken using the EnVision+ dual link (K4065, Dako, Glostrup, Denmark) method as described below.

All incubations were performed at room temperature. Following 2 × 5 min rinses in [0.05 M Tris, 0.15 M NaCl, pH 7.6] (TBS), endogenenous peroxidase was blocked in blocking buffer (S2001, Dako). After washing in TBS, the sections were incubated with primary antibody, polyclonal rabbit anti-bovine cytokeratin (wide-spectrum screening, WSS; Z0622, Dako), and diluted 1:400 in antibody diluent (S2022, Dako). After rinsing in TBS (3 × 5 minutes), the sections were incubated with peroxidase-labelled polymer (K4065, Dako) for 30 minutes. Following washing (3 × 5 minutes) in TBS, the sections were incubated in Substrate/Chromogen DAB (Sol. 1, K4065, Dako), or Vector NovaRed (SK-4800, VWR International, Vector Laboratories, Burlingame, California, USA). The reaction was stopped in distilled water. The water and the sections were counter stained with haematoxylin Mayer (LAB00254, Bie & Berntsen, Rødovre, Denmark), dehydrated, and cover slipped using Pertex mounting media (Histolab, Göteborg, Sweden).

The results were based on the visual comparison of WSS immunoreactivity in the tissue sections, using a light microscope (Lecia, Wetzlar, Germany).

Table 1 The third molars included in the study.

Subject	Age (years)	Teeth	Root development
1	15	38, 48	<½
2	19	38, 48	>½
3	20	38, 48	>½
4	16	38, 48	>½
5	18	48	<½
6	19	18, 38, 48	>½
7	16	38, 48	<½
8	20	18, 28, 38, 48	Apex closed
9	24	48	Apex closed
10	27	18, 28	Apex closed
11	21	38, 48	>½
12	16	38	>½

Fédération Dentaire Internationale notation used for identification of teeth.

Immunohistochemical controls

Two types of control reactions were performed. In the first, the antibody was tested on sections containing different tissue known to show positive reaction for WSS. Control sections showed a positive reaction in the epidermis and bile ducts in liver and in the second, the primary antibody was deleted and the sections were incubated only with antibody diluent. Control sections were processed in an identical fashion as described above. This type of control section failed to display WSS immunoreactivity.

Other antibodies tested were monoclonal mouse anti-vimentin (Klon Vim 3B4), Dako M 7020, and NGF Receptor ($p75^{NGFR}$)/Neurotrophin Receptor Ab-1 (Clone NGFR5), Lab Vision Corporation, Neomarkers, Fremont, California, USA.

Both tested antibodies did not show reaction in the ERM.

Results

The ERM cells were distributed in the PDM in a network-shaped manner along the root surface and in the furcation region (Figure 1a–d).

The distribution of ERM was more prominent in teeth with incomplete root formation (Figure 1a,c).

Discussion

In the present investigation, the remnants of HERS were not randomly distributed cell clusters in the PDM, but appeared as an almost continuous chain of epithelial cells with regular breaks. This appearance is consistent with a network as described in animal studies (Peters *et al.*, 1995).

During root maturation, disintegration of HERS takes place leaving ERM in the PDM. It was therefore also expected that the ERM would be more prominent in teeth with incomplete root formation. This is in agreement with the findings of Wesselink and Beertsen (1993), who

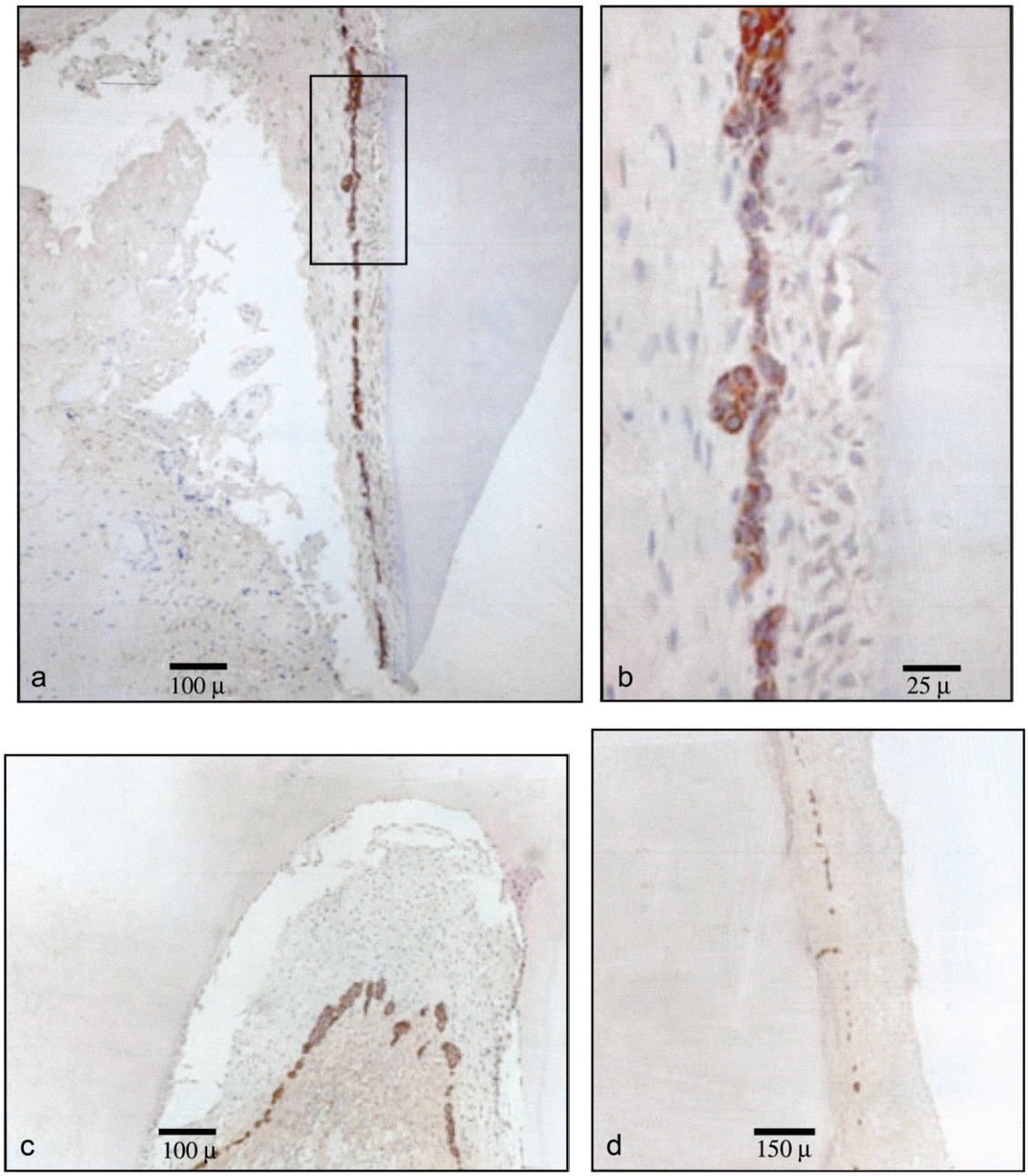

Figure 1 Epithelial rests of Malassez from (a) subject no. 4, (c) subject no. 3, and (d) subject no. 2. Immunohistochemical reaction showing wide spectrum screening (brown colour) distributed in cells of ectodermal origin. The cells appear in a network along the root surface. The marked area in (a) is shown in (b). Magnification: a and c, ×63; d, ×40; and b, ×400.

reported that the number of ERM in mice diminished with age. However, it has been demonstrated that they persist in the PDM (Ten Cate, 1996), suggesting possible physiologic functions such as maintenance of the PDM space and prevention of ankylosis.

The functional significance of the ERM is still unknown; however, some studies have shown that ERM might play a role for maintenance and remodelling of the PDM. It has been shown that ERM synthesize and secrete latent collagenase (Ragnarsson *et al.*, 1985), and that they proliferate and increase in size in connection with experimental tooth movement (Talic *et al.*, 2003). Furthermore, it has been stated that the ERM play a significant role in maintaining periodontal ligament space thereby preventing ankylosis (Shimono *et al.*, 2003; Fuiyama *et al.*, 2004).

That HERS and ERM might play an important role in preventing calcification and ankylosis of the PDM has been illustrated in a study comparing vertebrate ankylosis-type attachment and mammalian 'true' periodontium (McIntosh *et al.*, 2002). That study demonstrated how the root of a

gecko was free of HERS and ERM and how it was connected to bone via ankylosis. Whereas in the crocodilian and mouse, root size and shape were developed from HERS, and in connection with disintegration of the HERS into ERM the periodontal ligament was established.

In an investigation of root resorption following orthodontic treatment (Kjær, 1995), it was noted that abnormal morphology of the teeth was closely related to root resorption. Deviations such as taurodontism of the molar roots, slender pipette-shaped roots, short roots, invagination in the incisors, small, narrow crowns, especially of the lateral incisors, and screwdriver-shaped central incisors were described. Since the root is developed from HERS, it is likely that deviations in root morphology can be linked to deviations in HERS, and thereby deviations in the ERM also would be present, and the homeostasis of the PDM could be influenced.

Another phenomena in the dentition, which until now has not been understood, is the eruptional process and in particular what goes wrong when ankylosis occurs during normal eruption. It is well-known that the stage of eruption before penetrating the oral mucosa is dependent on the crown follicle (Marks and Schroeder, 1996). It is likely that continued eruption after mucosal penetration is dependent on remodelling activity in the PDM; however, the exact factors responsible for this phase of tooth eruption are unknown. In order to contribute to this phase of tooth eruption, the PDM must be provided with two types of regulatory mechanisms: one is a calcification mechanism for differentiation of osteogenic cells for bone production and the other is a non-calcification mechanism for maintaining a fixed space. It can be hypothesized that the ERM participate in this phase of tooth eruption.

Yamashiro *et al.* (2000) found, in rat periodontal tissue, that innervation was closely associated with ERM and that denervation lead to ankylosis and infraposition of erupting molars.

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References

Fujiyama K, Yamashiro T, Fukunaga T, Balam T A, Zheng L, Takano-Yamamoto T 2004 Denervation resulting in dento-alveolar ankylosis associated with decreased Malassez epithelium. Journal of Dental Research 83: 625–629

Götz W, Lossdörfer S, Krüger U, Braumann B, Jäger A 2003 Immunohistochemical localization of insulin-like growth factor-II and its binding protein-6 in human epithelial cells of Malassez. European Journal of Oral Sciences 111: 26–33

Kjær I 1995 Morphological characteristics of dentitions developing excessive root resorption during orthodontic treatment. European Journal of Orthodontics 16: 25–34

Marks Jr S C, Schroeder H E 1996 Tooth eruption: theories and facts. The Anatomical Record 245: 374–393

McIntosh J E, Anderton X, Flores-De-Jacoby L, Carlson D S, Shuler C F, Diekwisch T G H 2002 Caiman periodontium as an intermediate between basal vertebrate ankylosis-type attachment and mammalian 'true' periodontium. Microscopy Research and Technique 59: 449–459

Mizuno N *et al.* 2005 Characterization of epithelial cells derived from periodontal ligament by gene expression patterns of bone-related and enamel proteins. Cell Biology International 29: 111–117

Peters B H, Peters J M, Kuhn C, Zoller J, Franke W W 1995 Maintenance of cell-type-specific cytoskeletal character in epithelial cells out of epithelial context: cytokeratins and other cytoskeletal proteins in the rests of Malassez of the periodontal ligament. Differentiation 59: 113–126

Pispa J, Thesleff I 2003 Mechanisms of ectodermal organogenesis. Developmental Biology 262: 195–205

Ragnarsson B, Carr G, Daniel J 1985 Isolation and growth of human periodontal ligament cells *in vitro*. Journal of Dental Research 64: 1026–1030

Shimono M *et al*. 2003 Regulatory mechanism of periodontal regeneration. Microscopy Research and Technique 60: 491–502

Talic N F, Evans C A, Daniel J C, Zaki A E 2003 Proliferation of epithelial rests of Malassez during experimental tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 123: 527–533

Ten Cate A 1996 The role of epithelium in the development structure and function of the tissues of tooth support. Oral Disease 2: 55–62

Ten Cate A 2003 Oral histology. Development, structure and function. Mosby, Inc, St Louis

Wesselink P R, Beertsen W 1993 The prevalence and distribution of rests of Malassez in the mouse molar and their possible role in maintenance of the periodontal ligament. Archives of Oral Biology 38: 399–403

Yamashiro T, Fujiyama K, Fukunaga T, Wang Y, Takano-Yamamoto T 2000 Epithelial rests of Malassez express immunoreactivity of TrkA and its distribution is regulated by sensory nerve innervation. Journal of Histochemistry and Cytochemistry 48: 979–984