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ORIGINAL ARTICLE

Apoptosis in the human periodontal membrane evaluated in primary and permanent teeth

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Abstract

Objective. Recent studies revealed a highly innervated layer in close proximity to the root surface in the periodontal membrane of human teeth. Persistence of the epithelial cells of Malassez along root surfaces without resorption has also been demonstrated. It is hypothesized that resorption is connected to apoptosis of the epithelial cells of Malassez. The purpose of this study is to localize cells undergoing apoptosis in the periodontal membrane of human primary and permanent teeth. ***Materials and methods.*** Human primary and permanent teeth were examined immunohistochemically for apoptosis and epithelial cells of Malassez in the periodontal membrane. All teeth examined were extracted in connection with treatment. ***Results.*** Apoptosis was seen in close proximity to the root surface and within the epithelial cells of Malassez. This pattern of apoptotis is similar in the periodontal membrane in primary and permanent teeth. ***Conclusions.*** The inter-relationship between apoptotis and root resorption cannot be concluded from the present study. Apoptosis seen in close proximity to the root surface presumably corresponds to the highly innervated layer of the periodontal membrane. The function of this layer still needs to be elucidated.

Key Words: *root resorption, epithelial cells of Malassez, apoptosis, periodontal regeneration*

Introduction

Identification of patients susceptible to resorption of permanent teeth represents a great challenge in orthodontics. Patients with ectodermal morphological characteristics (invaginations, taurodontia, short roots, slender roots) are known to be predisposed for resorption [1–3]. The epithelial root sheath of Hertwig, which later develops into the epithelial cells of Malassez, is important for shaping the root. The epithelial root sheath of Hertwig and the epithelial cells of Malassez are of ectodermal origin. The epithelial cells of Malassez in human primary teeth are seen only as clusters along the root surfaces without resorption [4], which is different from the more continuous strands surrounding the entire root surface of permanent third molars [5].

In patients with hyper-IgE syndrome the primary teeth do not resorb. Histological studies of primary teeth from children with hyper-IgE syndrome have revealed a persistent epithelial layer along the root surface [6,7]. Studies on human primary teeth [4] and permanent teeth [8] showed that the epithelial cells of Malassez are never seen directly above a root surface with resorption [4]. It has therefore been suggested that the epithelial cells of Malassez protect the root surface against resorption.

It has been shown immunohistochemically on rats that the epithelial cells of Malassez occasionally undergo apoptosis [9,10] and a recent study has hypothesized that, in connection with the eruption of the permanent successor, the epithelial cells of Malassez in human primary teeth undergo apoptosis and that the root surface consequently resorb [4].

It has been shown in human permanent teeth [5] and in human primary teeth [4] that the part of the periodontal membrane in close proximity to the root surface is strongly innervated. The importance and function of such a layer still need to be elucidated, but it is known from case reports that resorption and

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arrest in root formation can occur following viral infections in nerves [11–14]. Seemingly, no studies exist specifically on the human periodontal membrane that can demonstrate where apoptosis occurs in the periodontal membrane.

The purpose of this study is to demonstrate where apoptosis occurs in the human periodontal membrane along the root surface of primary and permanent teeth.

Materials and methods

The material consisted of human primary and permanent teeth.

Primary teeth

The primary teeth were forwarded from municipal clinics in Zealand after written request. All teeth were extracted due to ectopic eruption of a permanent successor (30 primary teeth from 23 patients aged 8–16 years).

Permanent teeth

The permanent teeth were from patients at the Department of Orthodontics or forwarded from municipal clinics in Zealand after written request. The permanent teeth were extracted before orthodontic treatment (13 permanent premolars from eight adolescents/young adults).

Permission was given by the biomedical research ethics committee of Copenhagen (H-C-2008-133).

Fixation, paraffin embedding and sectioning

The primary teeth were fixed in 4% neutral buffered formaldehyde for 2–8 days and afterwards decalcified in 4M formic acid for 8–12 weeks. After decalcification the primary teeth were fixed in 4% neutral buffered formaldehyde overnight.

The teeth were dehydrated in graded alcohols: 80% ethanol; 90% ethanol; 96% ethanol; 99% ethanol. The teeth were embedded in paraffin: 99% ethanol/methyl salicylate; methyl salicylate; 1% celloidin/methyl salicylate; 2:1 methyl salicylate:paraffin; 1:1 methyl salicylate:paraffin; 1:2 methyl salicylate paraffin and finally several changes of paraffin.

Teeth embedded in paraffin blocks were cut into serial sections of 3 μm thickness and dried at 37°C for 2 days.

Immunohistochemical staining with cleaved caspase-3 and wide spectrum screening

Sections were dewaxed in graded alcohols. Sections were pre-treated with Tris-EDTA pH 9 at 60°C for 1 h and washed afterwards in tris buffered saline (TBS) for 5 min. Sections were encircled with a Dako pen (S2002, Dako, Glostrup, Denmark) and washed afterwards in TBS for 5 min.

Sections were blocked for endogenous peroxidise using Dako Real™ Peroxidase-Blocking Solution (S2023, Dako, Glostrup, Denmark) for 10 min.

Sections were washed afterwards in TBS for 2 × 5 min and then incubated for 1 h in primary antibodies. Detection of epithelial cells of Malassez was performed according to Nolting and Kjær [5] using Wide Spectrum Screening (N1512, Dako) diluted 1:5000 in antibody diluent (S2022, Dako). For detection of apoptosis, Cleaved Caspase 3 antibody (Asp175, MEDINOVA Scientific, Glostrup, Denmark) diluted 1:250 in antibody diluent (S2022, Dako) was used.

Sections were washed afterwards in TBS for 2 × 5 min and then incubated for 30 min in secondary antibody using peroxide labelled polymer (K5007, Dako). Sections were then washed in TBS for 3 × 5 min, then incubated for 10 min in DAB$^+$ Chromogen/Substrate Buffer (K5007, Dako) and afterwards washed in distilled water for 5 min. In a staining machine (Shandon Varistain Gemini A7801402, Thermo Electron Corporation, Copenhagen, Denmark) sections were counter stained in Carazzi's haematoxylin (Bie & Berntsen, Herlev, Denmark), washed in tap water and then dehydrated in graded alcohols. Sections were cover slipped using Pertex (Pertex 00811, Histolab, Göteborg, Sweden).

Negative controls were performed by deleting the primary antibody and incubation in antibody diluent (S2022, Dako) only. Sections were otherwise processed according to protocol.

Results

Primary teeth

Apoptosis was predominately seen in close proximity to the root surface (Figure 1), presumably corresponding to the highly innervated layer in the periodontal membrane.

Apoptosis was occasionally seen within the epithelial cells of Malassez (Figure 1) and also in relation to vessels in the periodontal membrane. The localization of epithelial cells of Malassez was confirmed immunohistochemically.

Permanent teeth

Apoptosis was predominately seen in close proximity to the root surface (Figure 2), but also within clusters of epithelial cells of Malassez (Figure 2) and vessels. The localization of epithelial cells of Malassez was confirmed immunohistochemically. Epithelial cells were predominately seen in clusters along the root surface.

The pattern of apoptosis seems to be similar in the periodontal membrane in primary and permanent teeth.

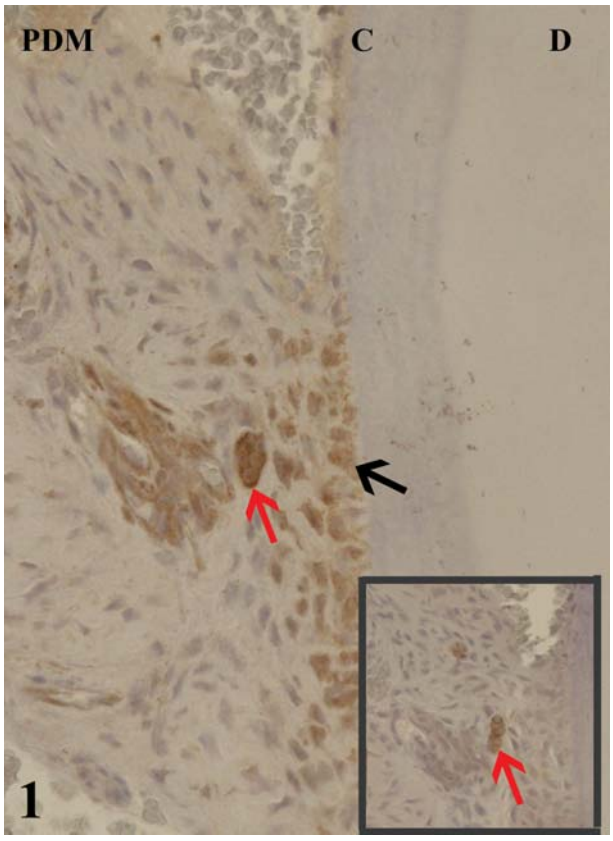

Figure 1. The figure shows a section of a primary tooth. The section demonstrates a part of the tooth root without resorption. The periodontal membrane (PDM) is seen to the left, the cement (C) in the middle and the dentin (D) to the right. This section is stained with Cleaved Caspase 3 (brown color), which is an apoptosis marker. Apoptosis is seen in epithelial cells of Malassez (red arrow) and in close proximity to the root surface (black arrow). The little square in the bottom right corner is a neighbor section of the same tooth. The section in the little square is stained with WSS (brown color) to confirm the presence of epithelial cells of Malassez (red arrow).

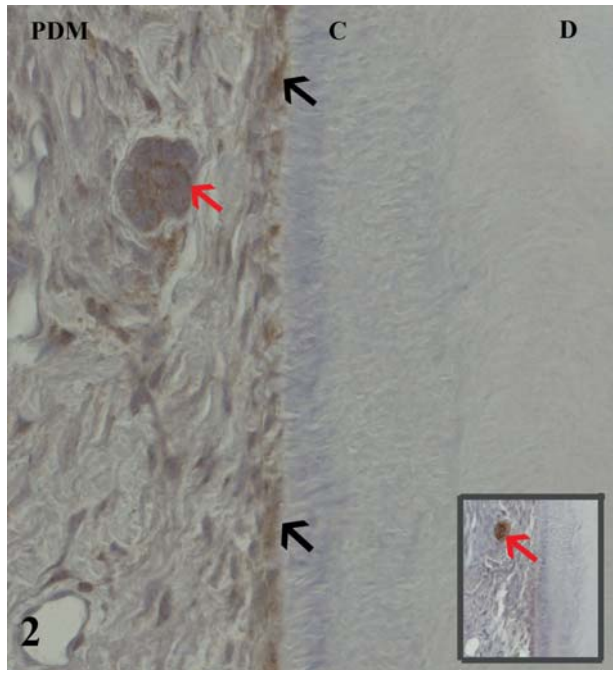

Figure 2. The figure shows a section of a permanent tooth. The periodontal membrane (PDM) is seen to the left, the cement (C) in the middle and the dentin (D) to the right. This section is stained with Cleaved Caspase 3 (brown color), which is an apoptosis marker. Apoptosis is seen in epithelial cells of Malassez (red arrows) and in close proximity to the root surface (black arrows). The little square in the bottom right corner is a neighbor section of the same tooth. The section in the little square is stained with WSS (brown color) to confirm the presence of epithelial cells of Malassez (red arrow).

Discussion

The present study shows apoptosis in the periodontal membrane of human primary teeth. Apoptosis was seen in epithelial cells of Malassez. This study supports the view that epithelial cells of Malassez undergo apoptosis prior to resorption, as observed in studies on rats [9,10]. An inter-relationship between root resorption and apoptosis in the human periodontal membrane cannot be concluded from the present study. Apoptosis was also seen in close proximity to the root surface. A highly innervated layer in close proximity to the root surface has previously been immunohistochemically documented in human primary teeth [4]. It is possible that it is this highly innervated layer that undergoes apoptosis.

The present study also shows apoptosis in the periodontal membrane of human premolars. It is impossible to tell whether the apoptosis and distribution of epithelial cells seen in human premolars occur in connection with a reorganization of the periodontal membrane or whether it occurs in connection with a degeneration of the periodontal membrane. Also, the growth of the alveolar bone during childhood and puberty may influence the apoptosis pattern. The reason for this is that the tooth eruption and the alveolar bone growth are two inter-dependent processes. The exact tissue changes in the periodontal membrane during tooth eruption are not known. It can be hypothesized that the erupting tooth requires constant reorganization of that part of the periodontal membrane, which is close to the root surface, and that the innervation controls this process. Apoptosis could be a part of this reorganization process.

When evaluating the results it has to be taken into consideration that immunohistochemical studies of extracted human primary and permanent teeth include several challenges. In the present study some autolysis of the tissue in the time between extraction and fixation in formalin may have occurred.

Apoptosis was observed in epithelial cells of Malassez. How these findings interfere with root resorption in human primary teeth cannot be concluded from the present study. Meanwhile, we presume that apoptosis seen in close proximity to the root surface corresponds to the highly innervated layer previously shown in human teeth [4,5]. We hypothesize that this highly innervated layer is important for

the root surface integrity and reorganization during tooth eruption.

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References

[1] Kjær I. Morphological characteristics of dentitions developing excessive root resorption during orthodontic treatment. Eur J Orthod 1995;17:25–34.

[2] Thongudomporn U, Freer TJ. Anomalous dental morphology and root resorption during orthodontic treatment: a pilot study. Aust Orthod J 1998;15:162–7.

[3] Bille ML, Kvetny MJ, Kjær I. A possible association between early apical resorption of primary teeth and ectodermal characteristics of the permanent dentition. Eur J Orthod 2008;30:346–51.

[4] Bille ML, Nolting D, Kjær I. Immunohistochemical studies of the periodontal membrane in primary teeth. Acta Odontol Scand 2009;21:1–6.

[5] Kjær I, Nolting D. The human periodontal membrane: focusing on the spatial interrelation between the epithelial layer of Malassez, fibers and innervation. Acta Odontol Scand 2009; 67:134–8.

[6] O'Connell AC, Puck JM, Grimbacher B, Facchetti F, Majorana A, Gallin JI, et al. Delayed eruption of permanent teeth in hyperimmunoglobulinemia E recurrent infection syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;89:177–85.

[7] Becktor KB, Kjær I, Koch C. Tooth eruption, epithelial root sheath and craniofacial profile in hyper-IgE syndrome: report of two cases. Eur J Paediatr Dent 2001;2:185–90.

[8] Brice GL, Sampson WJ, Sims MR. An ultrastructural evaluation of the relationship between epithelial rests of Malassez and orthodontic root resorption and repair in man. Aust Orthod J 1991;12:90–4.

[9] Cerri PS, Katchburian E. Apoptosis in the epithelial cells of the rests of Malassez of the periodontium of rat molars. J Periodont Res 2005;40:365–72.

[10] Gonçalves JS, Sasso-Cerri E, Cerri PS. Cell death and quantitative reduction of rests of Malassez according to age. J Periodont Res 2008;43:478–81.

[11] Solomon CS, Coffiner MO, Chalfin HE. Herpes zoster revisited: implicated in root resorption. J Endodontics 1986;12: 210–13.

[12] Bang E, Kjær I, Christhensen LR. Etiologic aspects and orthodontic treatment of unilateral localized arrested tooth-development combined with hearing loss. Am J Orthod Dentofacial Orthop 1995;108:154–61.

[13] Nielsen SH, Becktor KB, Kjær I. Primary retention of first permanent mandibular molars in 29 subjects. Eur J Orthod 2006;28:529–34.

[14] Ramchandani PL, Mellor TK. Herpes zoster associated with tooth resorption and periapical lesions. Br J Oral Maxillofac Surg 2007;45:71–3.