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ORIGINAL ARTICLE

The inter-relation between epithelial cells of Malassez and vessels studied immunohistochemically in the periodontal membrane of human primary and permanent teeth

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Abstract

Background. Only few immunohistochemical studies have focused on the periodontal membrane in human primary teeth. Recently, studies on epithelial cells of Malassez and innervation have been published. Studies on the inter-relation between vessels and the epithelial cells of Malassez are seemingly lacking. ***Aim.*** The aim of this immunohistochemical study is to describe the histological inter-relation between epithelial cells of Malassez and vessels in the periodontal membrane close to the root surface of human primary and permanent teeth. ***Methods.*** Twenty-nine human primary teeth and 15 permanent teeth were extracted in connection with dental treatment. The teeth were fixated, embedded in paraffin, cut in serial sections and examined immunohistochemically for epithelial cells of Malassez using wide spectrum screening and vessels using Von Willebrand Factor VIII. ***Results.*** The study showed that vessels and epithelial cells of Malassez are seen parallel to the root surface. The vessels are seen on that side of the epithelial cells of Malassez, which are not facing the root surface. ***Conclusion.*** The vascularization appeared similar in primary and permanent teeth.

Key Words: *blood vessels, epithelial cells of Malassez, periodontal ligament*

Introduction

Histological description of the human periodontal membrane is fundamental in order to comprehend detailed experimental studies on the pathogenesis of resorption, ankylosis and periodontal diseases. Only a few immunohistochemical studies have focused on mapping the inter-relation between epithelial cells of Malassez and other structures in the human periodontal membrane such as peripheral nerves, vessels and fibers [1,2]. Even fewer studies have focused on differences between the periodontal membrane in human primary and permanent teeth [2,3].

Several experimental studies support the view that epithelial cells of Malassez are important for maintaining the homeostasis of the periodontal membrane [4–6]. It has been indicated that the epithelial cells of Malassez are involved in the pathogenesis of root resorption, ankylosis and periodontal diseases [5–12].

Recently, the periodontal membrane attached to surgically removed human third molars has been analyzed and the following layers close to the root surface have been described [2]:

- an inner layer close to the root surface displaying expression of neuronal nuclei (innervation) covered by a thick layer of vimentin expression (cells producing periodontal fibers),
- a middle layer with epithelial cells of Malassez collected in more continuous net-like strands and
- an outer layer with less expression of neuronal nuclei (innervation) and vimentin.

Few immunohistochemical studies have focused on the periodontal membrane in human primary teeth. In human primary teeth the epithelial cells of Malassez are seen in clusters along the root surface without resorption, whereas epithelial cells are either dissolved or not present in relation to root surfaces with resorption [3].

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The distribution of epithelial cells of Malassez is different in human primary teeth [3] and in human third molars [2]. Studies on the inter-relationship between vessels and epithelial cells of Malassez are seemingly still lacking in primary and permanent teeth.

We hypothesize that there is an overall coordination between epithelial cells of Malassez and vessels in the periodontal membrane under normal conditions and that changes in these structures might occur during pathological conditions.

The aim of this immunohistochemical study is to describe the histological inter-relation between epithelial cells of Malassez and vessels in the periodontal membrane close to the root surface of human primary and permanent teeth.

Materials and methods

The materials comprised 29 human primary teeth (19 primary molars and 10 primary canines) from 24 children aged 8–16 years and 15 human permanent pre-molars from eight adolescents/adults aged 13–27 years.

All primary teeth were extracted due to ectopia of the permanent successor and all permanent pre-molars were extracted in connection with and before orthodontic treatment. The teeth were from patients seen at the Department of Orthodontics or forwarded from municipal clinics in Zealand communities, Denmark, after written request. Teeth with caries or apical infections were not included.

Permission for this study was granted from the biomedical research ethics committee of Copenhagen (H-C-2008-133).

Fixation, decalcification, sectioning and pre-treatment

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

After decalcification the teeth were re-fixed in 4% neutral buffered formaldehyde for 1 day. The teeth were then dehydrated and embedded in paraffin using a double embedding method: 80% ethanol, 90% ethanol, 96% ethanol, 99% ethanol, 99% ethanol/methyl salicylate, methyl salicylate, 1% celloidin in methyl salicylate, methyl salicylate/paraffin, several changes of paraffin and, finally, embedded in paraffin. Paraffin blocks were serially cut in 3 μm thick sections and dried overnight at 37°C.

Sections were dewaxed in xylene, rehydrated in graded alcohols and pre-treated with Tris-EDTA pH 9 at 60°C for 1–2 h. Sections were washed in tris-buffered saline (TBS 0.05 M Tris, 0.15 M NaCl, pH 7.6) (Bie & Berntsen, Herlev, Denmark) for 5 min and encircled with a Dako pen S2002 (Dako, Glostrup, Denmark) and then washed twice in TBS for 5 min.

Vessels were visualized using two different staining systems (Dako EnVision™+ HRP (AEC) Detection System K4008 (Dako) and Dako REAL™EnVision™ Detection System K5007) and protocols. This was done in order to visualize vessels in relation to epithelial cells. Epithelial cells were only visualized using a Dako REAL™EnVision™ Detection System K5007.

Immunohistochemical detection of vessels using Dako EnVision™+ HRP (AEC) detection system K4008

Sections were incubated with Peroxidase blocking solution K4008 (Dako) for 10 min and then washed in TBS for 5 min.

For detection of endothelial cells, sections were incubated for 60 min in the rabbit polyclonal primary antibody Von Willebrand Factor VIII A0082 (Dako) diluted 1:750 in Antibody diluent S2022 (Dako).

Sections were then washed for 2 × 5 min and incubated in secondary antibody labeled polymer-HRP K4008 (Dako). Sections were washed for 3 × 5 min and then incubated in substrate/chromagen K4008 (Dako) for 10 min and then washed in distilled water.

Sections were counter stained in Carazzi's hematoxylin (Bie & Berntsen), washed and coverslipped with Kaiser's glycerol gelatine 1.09242.0100 (Bie & Berntsen (Merck)).

Immunohistochemical detection of epithelial cells of Malassez and vessels using Dako REAL™EnVision™ Detection System K5007

Sections were incubated with Peroxidase blocking solution S2023 (Dako) for 10 min and then washed in TBS for 5 min.

Sections were incubated in primary antibodies for 60 min: One section was incubated in rabbit polyclonal Cytokeratin Wide Spectrum Screening (WSS) Z0622 (Dako) diluted at 1:5000 for detection of epithelial cells of Malassez and another section was incubated in rabbit polyclonal Von Willebrand Factor VIII A0082 (Dako) diluted at 1:750. All primary antibodies were diluted in Antibody diluent S2022 (Dako).

Sections were then washed for 2 × 5 min and incubated in secondary antibody coupled with peroxidase K5007 (Dako). Sections were washed for 3 × 5 min and then incubated in Substrate buffer/DAB+ chromagen K5007 (Dako) for 10 min and then washed in distilled water.

In staining machine Shandon Varistain Gemini A78010402 (Thermo Electron Corporation, Copenhagen, Denmark) sections were counter stained in Carazzi's hematoxylin (Bie & Berntsen), washed and dehydrated in graded alcohols. Sections were coverslipped with Pertex 00811 (Histolab, Göteborg, Sweden).

Control of immunohistochemistry

In the controls the primary antibody was deleted and the sections were incubated in Antibody diluent S2022 (Dako) only. Sections were otherwise processed according to protocols.

Results

Localization of epithelial cells of Malassez

In human primary and permanent teeth, immunoreactivity for epithelial cells of Malassez were seen close to the root surfaces. The cells were seen in small clusters parallel to the root surface (Figures 1A and 2A), predominately in the cervical area of the periodontal membrane.

Localization of vessels in relation to epithelial cells of Malassez

Vessels were predominately seen in the coronal part of the periodontal membrane. In both primary and permanent teeth, vessels was seen parallel to the root surfaces and predominately in the outer layer of that part of the periodontal membrane, which is localized close to the root surface. Vessels were occasionally seen in close proximity to the epithelial cells of Malassez (Figure 1). It was difficult to locate the epithelial cells in the counter stain, when they were not immunohistochemically stained (Figure 1B). In the present material the pattern of vascularization appeared similarly in primary (Figure 1B) and permanent teeth (Figure 2B).

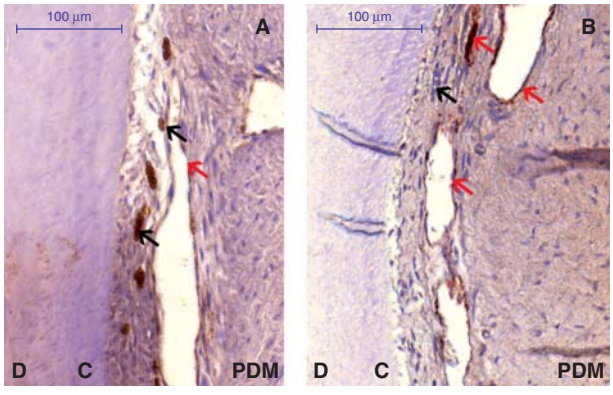

Figure 1. The figure shows two sections of the same primary tooth. The periodontal membrane (PDM) is seen to the right, the cement (C) in the middle and the dentin (D) to the left in (A) and (B). (A) This section is stained for epithelial cells of Malassez using Wide Spectrum Screening, visualized by DAB (K5007, Dako). The epithelial cells are seen close and parallel to the root surface (brown, exemplified by black arrows). A vessel (red arrow) is seen in close relation to the epithelial cells of Malassez. (B) This section is stained specifically for vessels using Von Willebrand Factor VIII, visualized by AEC (K4008, Dako). The vessels (red-brown, exemplified by red arrows) are predominately seen parallel to the root surface. The black arrow points at a cluster of cells, which is presumed to be epithelial cells of Malassez.

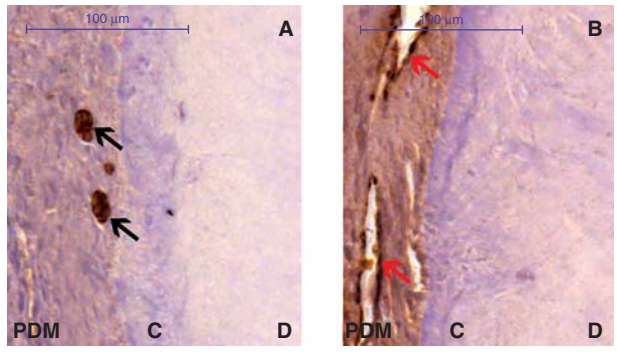

Figure 2. The figure shows two sections of the same permanent tooth. The periodontal membrane (PDM) is seen to the left, the cement (C) in the middle and the dentin (D) to the right in (A) and (B). (A) This section is stained for epithelial cells of Malassez using Wide Spectrum Screening, visualized by DAB (K5007, Dako). The epithelial cells are seen parallel to the root surface (brown, exemplified by black arrows). (B) This section is stained specifically for vessels using Von Willebrand Factor VIII, visualized by DAB (K5007, Dako). The vessels (brown, indicated by red arrows) are predominately seen parallel to the root surface.

Vessels were visualized using two different staining systems: Dako EnVision™+ HRP (AEC) Detection System K4008 and Dako REAL™EnVision™ Detection System K5007 in order to visualize vessels in relation to epithelial cells. However, a little more background staining was seen using Dako REAL™EnVision™ Detection System K5007 compared to Dako EnVision™+ HRP (AEC) Detection System K4008.

The controls did not show any chromagen staining (Figure 3).

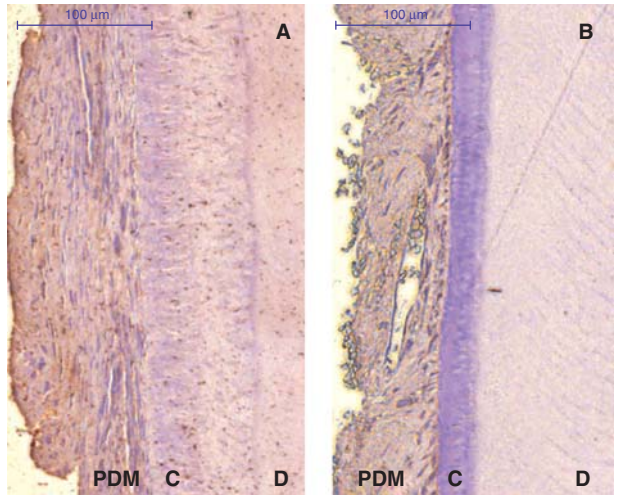

Figure 3. The figure shows two sections of two different teeth The periodontal membrane (PDM) is seen to the left, the cement (C) in the middle and the dentin (D) to the right in (A) and (B). (A) This figure shows sections of a primary tooth, where the primary antibody has been omitted. The section has otherwise been processed according to the protocol (K5007, Dako). (B) This figure shows sections of a permanent tooth, where the primary antibody has been omitted. The section has otherwise been processed according to the protocol (K4008, Dako).

Discussion

In the present study, primary teeth extracted due to ectopia of the permanent successor and permanent teeth from patients with a need for orthodontic treatment were used to describe the inter-relation between epithelial cells of Malassez and vessels in the periodontal membrane close to the root surface. Studies of the periodontal membrane of human teeth imply several technical and ethical challenges. Only parts of the periodontal membrane, predominately the cervical part of the periodontal membrane, are attached to the root following tooth extraction. This complicates the evaluation along the entire root surface. Studies performed on animals eliminate some of these challenges, but the information gained from such studies cannot uncritically be transferred to humans.

The present study shows an occasionally close interrelation between epithelial cells of Malassez and vessels in the periodontal membrane close to the root surface of both primary human teeth and permanent human teeth. In some teeth, this inter-relation was confirmed by comparing the localization of epithelial cells of Malassez and vessels in neighboring sections. This close interrelationship in human teeth may support the hypothesis of a functional coordination between epithelial cells of Malassez and vessels. In the present study the epithelial cells of Malassez, seen as clusters along the root surface, appeared similar in human primary teeth and premolars. The distribution of epithelial cells of Malassez has previously been described in human third molars as a nearly continuous layer with few interruptions [2]. This more continuous distribution of epithelial cells of Malassez was not seen in pre-molars in the present study. The explanation for this might be related to different stages of eruption and root development.

Conditions such as asthma and arthritis have been connected with root resorption [13–15]. In this context it is interesting that neuroendocrine peptides (CGRP, SP, VIP), which are important in vasoregulation and vascular permeability, have been demonstrated within the epithelial cells of Malassez in cats [5,16,17]. Similar immunohistochemical studies of human teeth that include the localization of neuroendocrine peptides might in the future provide substantial information.

We suggest that the regulation of the periodontal membrane during functional processes, such as eruption and/or resorption, is dependent on vascularization. The process might be dependent on an interaction between epithelial cells of Malassez and vessels. Disturbances in this interaction may result in arrested eruption and/or root resorption.

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