Acta Odontologica Scandinavica, 2009; 67: 134–138

informa
healthcare

ORIGINAL ARTICLE

The human periodontal membrane – focusing on the spatial interrelation between the epithelial layer of Malassez, fibers, and innervation

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Abstract

Objective. The purpose of the present study was to map the spatial interrelation of fibers, peripheral nerves, and epithelial layer of Malassez in human periodontal membrane in areas close to the root surfaces. ***Material and Methods.*** Four healthy permanent teeth extracted from four patients during puberty due to orthodontic treatment planning were analyzed. The extracted teeth, fixed in 4% neutral buffered formaldehyde for 5 days, were decalcified in 0.5 M EDTA. Paraffin blocks were sagittally cut in 5 μm thick serial sections and mounted on Superfrost® Plus microscope slides. For survey, every fifth slide was stained with Alcian Blue/Van Gieson. Immunohistochemical reactions: Cytokeratin (wide spectrum screening) for epithelium, anti-vimentin for fibers, and anti-neuronal nuclei (NeuN) for innervation. ***Results.*** The study indicates that the epithelial layer of Malassez is a border between different fiber morphologies and innervation patterns. Innervation is identified predominantly in the periodontal layer with tightly packed fibers close to the root surface. ***Conclusion.*** It is suggested that the genetic composition of the epithelial layer of Malassez in the periodontal membrane may be the key to understanding the different functions of the periodontal membrane and also the individual differences of these functions.

Key Words: *Cells of Malassez, fibers, immunohistochemistry, innervation, periodontium*

Introduction

The periodontal membrane plays a decisive role in eruption, alveolar bone formation, tooth movement, and tooth resorption. These four processes are dependent on ongoing reorganization of tissue components in the periodontal membrane. As we do not know the etiology behind the physiological processes leading to eruption, alveolar bone formation, tooth movement, or tooth resorption, we do not understand the complete processes of the reorganization of the periodontal tissue components either. Studies of the human periodontal membrane are complicated because tooth extraction disrupts the periodontal membrane, and only remnants of the membrane attached to the root surface are available for investigation. This limitation has to be acknowledged in our attempt to gain insight into clinical questions on tooth eruption, alveolar bone formation, tooth movement, and tooth resorption in humans.

In previous studies on human tooth development, tissue types and the interaction between tissues have been in focus [1–3]. These tissues are: the dental lamina with ectodermal origin, the innervation from the neuroectoderm, and the ectomesenchyme from the neural crest. The tooth bud and its close surroundings have been investigated by molecular biologists using advanced molecular methods and in several different species [2,3]. From this early stage of human tooth development to later postnatal stages, the possibilities of research are dramatically reduced owing to accessibility of material and limited research methods. Thus, the postnatal stages are rarely investigated by basal biologists, though there are some interesting studies on the epithelial rests of Malassez and the relation to nerve endings [4]. In recent experimental studies, the focus has been on the epithelial cells lining the root [5,6].

In order to evaluate the continuous function of the tissues interacting in pre- and postnatal tooth development, it is important to study the tissue types, the epithelium, the nerve tissue, and the ectomesenchyme in the periodontal membrane, too. In the present study, the focus is therefore

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(Received 27 May 2008; accepted 29 December 2008)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350802714726

the epithelial components, peripheral nerves, and ectomesenchymal structures in the human periodontal membrane.

There is no exact knowledge of the etiology behind eruption. Different factors discussed have been: influence from innervation of the periodontal membrane [7–9]; influence from the epithelial layer of Malassez [10–12]; the periodontal fibers, cells, and the surrounding alveolar bone [13]. It is believed that these factors actually interact in the process of eruption [14].

During eruption, bone apposition occurs at the top of the alveolar bone. How these bony depositions are regulated has recently been discussed with a focus on innervation as a factor regulating alveolar bone apposition [15].

In orthodontic treatment with fixed and removable appliances, physical forces are applied to the teeth and thereby transferred to the periodontium, resulting in resorption of bone and rebuilding of the periodontal membrane.

How the above-mentioned tissue types, innervation, Malassez epithelium, and periodontal cells and fibers interact in these processes is not fully understood.

Tooth resorption is a normal process in the primary dentition, but a pathological process in the permanent dentition. Differences between periodontal membranes surrounding primary root surfaces and permanent root surfaces in the same individual may be important in our understanding differences in resorption patterns. As the primary and permanent dentitions develop from the same mucosa in the same oral environments, there must be factors that are different in the periodontal membrane causing different patterns of resorption. In recent studies, it has been shown that patients with deviant root resorption in the primary dentition also have abnormal resorptions in the permanent dentition [16,17].

These different clinical aspects of the periodontal membrane demonstrate a lack of knowledge of the functional interrelation between the root surface, the surrounding bone, and the intermediate periodontal membrane with fibers and epithelial layers of Malassez and its possible association with innervation [4] during eruption, alveolar bone growth, and root resorption in humans.

The purpose of the present study was to map the spatial interrelation between the root surface and the periodontal membrane, including fibers, peripheral nerves, and epithelial layer of Malassez.

Material and methods

Four healthy permanent teeth extracted from four patients during puberty, owing to space problems or to orthodontic treatment planning, were analyzed.

Fixation, decalcification, and sectioning

Extracted teeth were fixed in 4% neutral buffered formaldehyde for 5 days and decalcified in 0.5 M EDTA (Ethylene Diamine Tetra Acetic Disodium Salt, Titriplex II; Merck 8417, Germany) for 6–10 weeks. After decalcification, the tissues were re-fixed in 4% neutral buffered formaldehyde.

The tissues were 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 sagittally cut into 5 μm thick serial sections and mounted on Superfrost® Plus microscope slides. Sections were dried overnight at 40°C.

Staining

Every fifth slide was stained with Alcian Blue/Van Gieson.

Immunohistochemistry

Three different antibodies were used in the study: Cytokeratin (wide spectrum screening), anti-vimentin, and anti-neuronal nuclei (NeuN).

Paraffin sections were deparaffinized and treated with Tris-EDTA (Merck, Germany), pH 9.0, for 2 h at 60°C prior to immunohistochemical testing. This was done using the EnVision+Dual Link (K4065; DAKO Denmark A/S, DK) 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), endogenous peroxidase was blocked in blocking buffer (S2001; DAKO Denmark A/S, DK). Following washing in TBS, 1×5 min sections were incubated with primary antibody, polyclonal rabbit anti-bovine cytokeratin (wide spectrum screening) (Z0622; DAKO) diluted 1:400 in antibody diluent (S2022; DAKO), monoclonal mouse anti-vimentin (M7020; DAKO) diluted 1:50 in antibody diluent, and mouse monoclonal antibody anti-neuronal nuclei (NeuN) (MAB377; Milipore, Chemicon, USA) diluted 1:50 in antibody diluent. All incubations were done at room temperature for 30 min.

After rinsing 3×5 min in TBS, the sections were incubated with peroxidase labeled polymer (K4065; DAKO) for 30 min. Following washing for 3×5 min in TBS, sections were incubated in substrate/chromogen DAB (Sol. 1, K4065; DAKO), or Vector NovaRed (SK-4800; VWR International, Denmark). The reaction was stopped in distilled H_2O for 5 min, and sections were counter-stained with haematoxylin Mayer (LAB00254; Bie & Berntsen,

Denmark), dehydrated, and cover slipped using Pertex mounting media (Histolab, Sweden).

The results were based on visual comparison of antibody reaction in the tissue sections using a light microscope (Leica, Germany).

Immunohistochemical controls

Two types of control reaction were carried out. In the first, the antibodies were tested on sections containing different tissues known to show positive reaction for WSS, anti-vimentin, and neuronal nuclei. Control sections showed a positive reaction in the epidermis, soft tissue, and nerves.

In the second control, the primary antibodies were deleted and sections were incubated only with antibody diluents. Control sections were processed in an identical fashion, as described above. This type of control section failed to display immunoreactivity.

Results

Survey staining

It was not possible in survey staining to distinguish either the structure or the location of fibers, the ectodermal nature of the epithelial layer of Malassez, or innervation (Figure 1A).

Immunohistochemistry

The epithelial layer of Malassez was a marked epithelial layer not completely continuous. There are few interruptions in the epithelial layer, which was organized parallel to the root surface (Figure 1B).

There was a distinct difference in the amount as well as in the orientation of the fibers in relation to the root surface and to the epithelial layer of Malassez (Figure 1C). Between the epithelial layer of Malassez and the root surface there was a zone rich in tightly packed fibers with no precise orientation. On the other side of this epithelial layer of Malassez (to the left in the figure), there were fewer fibers orientated parallel to the root surface (Figure 1C).

The innervation was predominantly located in the periodontal layer with tightly packed fibers close to the root surface (Figure 1D).

Discussion

The present study indicates that the epithelial layer of Malassez, recently demonstrated with cytokeratin in human periodontal tissue [12], is a border between different fiber morphologies and between different innervation patterns. Nerve fibers have been identified in the dental pulp and in the periodontal ligament in rats [18]. The present study showed that the epithelial layer of Malassez seemingly divides areas rich in innervation from areas with only sparse innervation. Kvinnsland et al. [19] found neuro-endocrine cells in the Malassez epithelium in the rat. In the present study, it was difficult to locate nerve fibers specifically in the epithelial layer of Malassez. The reaction for peripheral fibers was intense in the region between the epithelial layer of Malassez and the root surface. It is hypothesized that the epithelial layer of Malassez is decisive in the different functions of the periodontal membrane and may therefore protect the root surface from resorption processes. Experimental denervation plays a role in healing after replantation [20,21]. Fujiyama et al. [9] found that denervation resulted in dento-alveolar ankylosis, which was associated with a decrease in Malassez epithelium. Thus, it can be presumed that the epithelial cell layer in its location in the periodontal membrane retains the periodontal space. The epithelial cell layer is also considered important in the reorganization of the membrane during eruption and tooth movement [11].

Insulin-like growth factor-II and its binding protein-6 were recently localized in human epithelial cells of Malassez, which may be a key to understanding the function of these cells [22]. Hasegawa et al. [23] found immunoreactivity for bone morphogenic protein-II in the epithelial layer of Malassez, which indicates that the epithelial layer may be functionally related to cementum repair. Localization of blood vessels was not studied in the present work, but Kat et al. [10] found that in areas with physiological resorption the Malassez epithelial network adjacent and surrounding vessels are stimulated to proliferate and may provoke root resorption. Also, gene expression and bone-related proteins in the epithelial cells in the periodontal membrane have been studied [24]. Thus, the epithelial layer of Malassez may be important in the different functions of the periodontal membrane.

The role of the cementum covering the root was not considered in the present study. Recent studies have focused on the molecular and cell biology of the cementum. Bosshardt & Selvig [25], Diekwisch [26], and Saygin et al. [27] have all suggested that cementum is a dynamic tissue covering the root surface. In future studies on root resorption, the specific qualities of cementum and the expression of RANK and RANKL [28,29] should be related to findings in the epithelial layer of Malassez. In 1982, Lindskog & Hammarström found that the matrix of the intermediate cementum was formed by the epithelial root sheath of Hertwig [30].

The study draws special attention to the periodontal layer between the epithelial rests of Malassez and the root surface. This layer ought to be further investigated. In a recent study, Liu et al. [31] reported that the inhibitory effects of clodronate on tooth movement and osteoclasts may in part be due

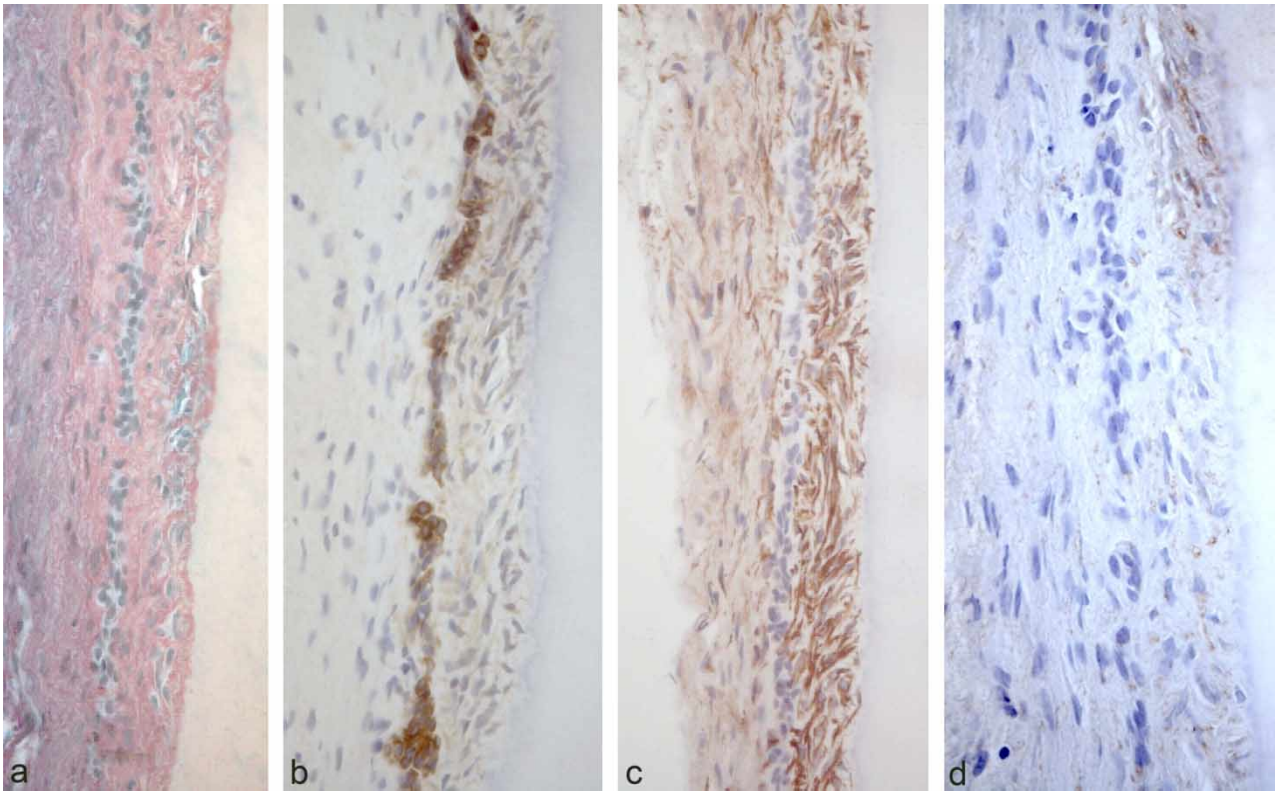

Figure 1. Four sections of a human root surface with attached periodontal tissue. In each section, the root surface is to the right and the periodontal tissue to the left; ×400. A. Alcian Blue/Van Gieson survey staining. It is not possible to distinguish either the structure or the location of the fibers, peripheral nerves, or the nature of the epithelial layer of Malassez, demonstrated as a grayish line of cells parallel to the root surface and located in the center of the figure. B. Immunohistochemical staining with antibody cytokeratin (wide spectrum screening) indicating the epithelial layer of Malassez as the brown colored, not completely continuous, layer parallel to the root surface. There are a few holes in the layer located in the center of the figure. C. Immunohistochemical staining with antibody anti-vimentin showing a distinct difference in the amount as well as in the orientation of the fibers in relation to the root surface and to the epithelial layer of Malassez. Between the epithelial layer of Malassez and the root surface there is a zone rich in brown fibers that are tightly packed with no precise orientation. On the other side of this epithelial layer of Malassez (to the left in the figure), there are fewer brown fibers orientated parallel to the root surface. D. Immunohistochemical staining with anti-neuronal nuclei (NeuN) showing that the innervation (brown color) is located in the region with tightly packed fibers between the epithelial layer of Malassez and the root surface (C). Nerve paths are only sporadically identified in the layer with few fibers (left).

to the inhibition of COX-2-dependent PGE_2 production and RANKL expression in periodontal cells. It would be interesting in the future to study in which periodontal cells/layers these changes occur.

It has recently been hypothesized in the periodontal literature that the rest of the epithelial cells of Malassez are decisive in periodontal gingivitis [32]. In this review article, it is also documented how the epithelial layer of Malassez forms a network around the root [32].

It is well known that teeth with deviant morphology due to ectodermal deviations, such as invaginations or taurodontia, are especially exposed to root resorption in the permanent dentition [33]. The connection between this clinical observation of the interrelationship between tooth morphology and tooth resorption supports studies on gene expression in the human periodontal ligament [23]. The genetic composition of the epithelial layer of Malassez in the periodontal membrane may be the key to understanding the different functions of the periodontal membrane and also the individual differences of these functions. With the present study, the morphological relationship between cells and fibers in the area close to the root surface has been elucidated in normal individuals. The study is intended as a reference for future immunohistochemical studies of the periodontal membrane in patients with eruption deviations and root resorption.

Acknowledgments

The IMK Foundation is acknowledged for funding and Maria Kvetny for linguistic support and manuscript preparation.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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