A Possible Continuity between Epithelial Rests and Epithelial Attachment in Miniature Swine

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There may be a continuity between the epithelial rests of Malassez and the attachment epithelium. Observations in miniature swine indicate that the network of epithelial rests may form a continuum with the reduced enamel epithelium before eruption and with the attachment epithelium after eruption. This possible continuum was seen in disease free and in periodontally diseased teeth.

While the epithelial structures in the periodontal ligament are of subject intensive study, no reported connection of epithelial rests with the attachment epithelium was found in the available literature. As a result, the root epithelium (epithelial rests) is regarded as a series of disconnected remnants of Hertwig's root sheath, persisting in the periodontal ligament. The primary purpose of this paper is to describe a possible continuum between the network of epithelial cells around the root and the attachment epithelium as seen in microscopic specimens of miniature swine.

Review of the Literature

Structural Arrangement

Black (1898) and Noyes (1912) searched for a possible connection between the rests and the epithelial attachment or gingival epithelium, but could find none. Gaunt and Miles (1967) observed that the "continuity between Hertwig's sheath and the epithelial dental cap is broken at an early stage of root formation." Although Malassez (1885) thought that the rests were disconnected remnants of the epithelial root sheath, there is considerable evidence to indicate that they form a network that enmeshes the root. This arrangement is apparent in sections that are cut tangential to the root. Black (1887) wrote that "the endolympathics formed a network over the whole root," while Ohrlin (1911) credits Fischer with the discovery. Simpson (1965), using aposexual techniques, has confirmed the mesh-like arrangement of the root epithelium. In a further study (1967) he found that the rests were present as perforated sheath in newly erupted teeth. With aging, the sheath appeared to break down into a network with small meshes and thick strands. This structural arrangement was then succeeded by a wide meshed net with thin strands and finally by isolated epithelial cords and rosettes. Brust, however observed a network arrangement around the tooth of a 58-year-old man.22

Prevalence and Distribution

The dominant opinion that the root epithelium is a vestigial tissue, persisting as islands or meshes near cementum, spurred a number of studies on the prevalence and distribution of the "rests."

Black described the rests as being most numerous near the gingiva, lying close to cementum but not touching it "except in some isolated cases in the pig." Wentz, Weimann and Schour studied the distribution of rests in rat molars and found them most numerous in the supra-alveolar area and in decreasing frequency in the midroot, bifurcation and apical areas. The rests occurred more frequently around young teeth.15 Valderhaug found that the cells were farthest from cementum in the supra-alveolar area and nearest to cementum at the apex, in young human teeth.

Morphology and Incidence

Resting, proliferating and degenerating forms of rests have been reported.14 Black (1898) and Reeve and Wentz (1962) found that the rests were reduced in numbers in older individuals. Occurrence of rests varies with species. The rests are numerous in pigs and sheep, sparse in the rat and mouse. Rests, said to be difficult to identify in hamsters, became prominent in animals treated with a carcinogen.15

Renewal

The persistence of the rests throughout life implies that a cell turnover takes place. Wentz, Weimann and Schour found a mitotic figure within a rest, while Reeve and Wentz described epithelial rests with a proliferative morphology, but observed no mitosis. Ramfjord observed an uptake of tritiated thymidine in rests following gingivectomy. Cell turnover, however, may be of a lower order since Diab and Stahl found no mitotic activity in rests in rat periodontium using tritiated thymidine. Trowbridge and Shibata using the same tracer, however, did observe mitotic activity. Grupe saw mitosis in tissue culture of these cells.

Function

Aside from pathological significance, any function assigned to the root epithelium is speculative. Serres (1817), who may have first described the rests, called them "glandulae tartarica" and postulated that they secreted dental calculus. Legros and Magnot (1850)21

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concurrent with Kolliker that the cells were epithelial and were of pathological significance in cyst formation. Malassez (1885), who gave the root epithelium his name, confirmed their relationship to cyst formation in several works. In calling the rests epithelial debris, he implied a lack of function. Black (1887) first believed that the structures were lymphatics and later interpreted them to be glands, an opinion shared by some other investigators. Noyes felt that some function was probable since they persisted through life. Orban and Weinmann thought that the rests might be involved in pocket formation in periodontosis.

Speculating that the cells might act as organizers, Orban felt that they might influence cementogenesis while Diab and Stallard doubt that the cells have any influence on the deposition of cementum. Nevertheless, the root epithelium is extensive in animals with heavy cemental deposition, such as the pig and sheep. Waerhaug and Loë felt that the cells might act to protect the root surface from resorption. Reitan noted root resorption during orthodontic tooth movement to question this hypothesis.

Ten Cate found histochemical evidence of low metabolic activity in the rests and concluded that the possibility of a functional role for the epithelial rests in the adult could be discounted. Waerhaug and Nylen examining the ultrastructure of the cells, concluded that “rest” cells was an appropriate appellation.

**METHODS AND MATERIALS**

Six miniature swine (Pitman-Moore Strain) 26 weeks (5) and 52 weeks (1) old were used in this study. The specimens were fixed in a 10% formalin solution, decalcified in nitric acid-formalin and prepared for nitrocellulose embedding in the routine manner. The embedded blocks of tissue were sectioned at 7 μ to 40 μ. Alternate cut sections were stained with hematoxylin and eosin, Mallory’s connective tissue stain, P.A.S., Alcian Blue and with Verhoeff’s iron hematoxylin stain.

**OBSERVATIONS**

The cementoenamel junction of an unerupted tooth can be seen in a thick section (40 μ) stained with Verhoeff’s iron hematoxylin (Fig. 1). Enamel formation has been completed and the reduced enamel epithelium borders the enamel space and terminates at cementum. Just coronal to the terminus of the reduced enamel epithelium, a thin cord of epithelium juts irregularly into the developing periodontal ligament. The thickness of the section provides a three-dimensional view of the jagged epithelial cord. One can visualize a mesh-like arrangement. Medial to the cord, scattered and disconnected masses of epithelial cells extend to the cementum. These epithelial masses are probably parts of an interconnected network that is continuous with the reduced enamel epithelium. In thinner sections they would appear as epithelial islands, the rests of Malassez.

Ameloblasts are still visible, bordering the enamel space of a partly erupted tooth (Fig. 2). Just coronal to the cementoenamel junction, a cord of epithelium projects latero-apically from the attachment epithelium into the gingival lamina propria. This cord seems to arc toward some interconnected masses of epithelial cells that have curved outward from cementum. The epithelial masses extend in an almost uninterrupted line alongside the cementum. Laterally, nerve fibers can be seen projecting from the periodontal ligament into the gingival lamina propria. The alveolar crest is visible.

The epithelial rests of Malassez are seen to extend coronally in file-like juxtaposition alongside the ce-

![Figure 1. A network of epithelial strands projects apically from the reduced enamel epithelium of an unerupted tooth.](Image)
EPI THELIAL ATTACHMENT IN MINIATURE SWINE

Near the cementoenamel junction, the rests are laterally and coronally away from cementum, where they become lost among the dense fiber bundles of the dentoparotidal and alveolar crest fiber groups. A small projection can be seen extending from the attachment epithelium toward the epithelial rests in a fashion similar to that seen in the previous specimen.

The area of the cementoenamel junction of a disease-free, functional tooth may be seen (Fig. 4). Just coronal to the apical end of the pigmented attachment epithelium, a cord of epithelium projects apically toward two islands of epithelial rests. Further coronally, a second epithelial cord projects into the gingival lamina propria toward a series of pigmented and disconnected epithelial islands and cords that extend apically.

Epithelial rests were found along the entire root surface. In thick longitudinal sections, they often appeared as a long interconnected line (Fig. 3). In tangential sections, the rests appeared as an interconnected network of rosettes (Fig. 5). In the apical area the network appeared to run into and out of cementum.

Cylinder-like strands are the most frequent form of rest cell groups seen in longitudinal sections (Fig. 6). The cylinder-like epithelial masses are pointed toward cementum and appear as dark bodies in thick sections (25 X) stained with Verhoeff’s iron hematoxylin. The rests may lie close to the cemental surface or even abut the root.

The shape of the epithelial cell groups is influenced by the direction of an tension within the fiber bundles. Oblique periodontal ligament fiber bundles can be seen extending from cementum toward the alveolar process (Fig. 7). Cementoblasts line the cemental surface. Close to cementum, elongated strands of epithelial rests lie between tensely stretched fiber bundles. The epithelial rests extend coronally, have close in cementum. Near the cementoenamel junction they are laterally and coronally away from cementum and are lost among the dense fiber bundles of the dentoparotidal and alveolar crest groups. A slight projection from the attachment epithelium extends apically in an analogous fashion and position to that seen in Figure 2. (Verhoeff’s iron hematoxylin. Original magnification 150X).

**Figure 2.** Just coronal to the cementoenamel junction of a fully erupted, functioning tooth. Epithelial rests extend coronally, have close in cementum. Near the cementoenamel junction they are laterally and coronally away from cementum and are lost among the dense fiber bundles of the dentoparotidal and alveolar crest groups. A slight projection from the attachment epithelium extends apically in an analogous fashion and position to that seen in Figure 2. (Verhoeff’s iron hematoxylin. Original magnification 150X).

**Figure 3.** The cementoenamel junction area of a fully erupted, functioning tooth. Epithelial rests extend coronally, have close in cementum. Near the cementoenamel junction they are laterally and coronally away from cementum and are lost among the dense fiber bundles of the dentoparotidal and alveolar crest groups. A slight projection from the attachment epithelium extends apically in an analogous fashion and position to that seen in Figure 2. (Verhoeff’s iron hematoxylin. Original magnification 150X).
lial bodies are not only elongated, but lie parallel to the
direction of the fiber bundles.

Three elongated epithelial rests, shaped like tadpoles,
lie between stretched fiber bundles (Fig. 8). A portion
of the central group extends into the cementum and
seems about to be entrapped in cementoid. The flanking
epithelial cell groups about the tooth surface. Epithelial
rest cells were frequently observed being entrapped in
cementum. These epithelial rests take a rounded form
when they lie in the loose connective tissue between
fiber bundles (Fig. 9). A P.A.S. positive membrane cir-
cumscribes the cell groups. When cells are about to be
entrapped in cementum however, the membrane is not
demonstrable.

The epithelial rests show an Alcian Blue positive
membrane, indicating the presence of an acid muco-
polysaccharide (Fig. 10).

Follicular or duct-like forms of epithelial rests can
best be seen in thin sections (7 μ) (Fig. 11). The rests
about cementum lying in loose connective tissue. Ce-
mentocytes within lacunae, with interconnecting can-
aliculi, are present. The canaliculi communicate with the
periodontal ligament and with dentinal tubules.

Calified deposits and bacterial plaques are joined
to the cemental tooth surface in a severe periodontitis
(Fig. 12). Lateral to the pocket, the bordering epithe-
lum has proliferated extensively into the lamina pro-
pra. The sub-epithelial connective tissue is densely
infiltrated by inflammatory cells. The infiltrate is con-
centrated around the pocket, while more deeply, fiber
bundles are intact, with a few nests of inflammatory
cells gathered along vascular channels. A thin strand
of epithelium is extended apically from the base of the
attachment epithelium. It lies in the connective tissue
close to cementum and seems to be projected toward
some islands of epithelial rests.

The proliferating attachment epithelium of a tooth
with periodontitis is surrounded by inflammatory cells

![Figure 4: The cementocrenel junction area of a fully functional, periodontally healthy tooth may be seen. Just coronal to the apical end of the pigmented attachment epithelium a long cord projects toward two detached epithelial islands. Further coronally, a thin cord of epithelium extends toward a line of pigmented epithelial rests. (Verhoeff's iron hematoxylin. Original magnification 250X)](image1)

![Figure 5: The net-like character of the root epithelium is apparent in sections cut tangential to the root. Near the root apex, the rests seemed to run into and out of cementum. (P.A.S. hematoxylin. Original magnification 25A.)](image2)
Although the epithelial rests are the subject of intensive study, a search of the available literature failed to uncover evidence that the rests might be connected to the attachment epithelium or to the gingival epithelium. Black (1898) wrote that “the glands lay in close proximity to the gingival aperture,” but that he could find “no duct to it.” Noyes (1912) found “no contact (of the rests) with the gingival space.”

Since most studies of the “rests” have been in animals where they are less numerous than in the miniature pig, a possible continuity of the rests with the attachment epithelium may not have been apparent. In addition, most studies have used thinner sections (5 μ to 15 μ) in order to better visualize cellular detail. In such sections, the epithelium around the root appears as detached islands when viewed in cross-sections or in sections cut longitudinal to the root. Groser details of structural arrangement are not apparent and are difficult to reconstruct in these sections. However, when thick microscopic sections (25 μ to 40 μ) are used, a three dimensional perspective is provided that indicates that there may be a continuum between the reduced enamel epithelium and the root epithelial network (rests of Malassez). This continuity is best demonstrated in unerupted teeth and in newly erupted teeth. In older, functional
teeth, free of disease, a continuum is more difficult to demonstrate. The epithelial network may become masked by the dense fiber bundles in the gingival lamina propria, or there may have been a loss of continuity because of cell degeneration. Observations to suggest a persisting continuity are found only after diligent search. It is possible that a continuity, if it does exist, may be transitory in nature; that is, it may disappear and then be reestablished under certain conditions, such as in disease or in the shedding of teeth. This interpretation would conform with the findings of Simpson that the network arrangement of rests may be lost in aging.

In thick sections, the rests formed a continuous line over long areas of the root. In tangential sections, the rests formed a lattice network. The existence of an interconnected network arrangement in cells once thought to be isolated epithelial remnants may also mean that a connection of this network with the attachment epithelium could exist. Since Hertwig's epithelial root sheath, when first formed, is continuous with the enamel organ, it is entirely possible that the successors to the root sheath and the enamel organ, the epithelial root network and the attachment epithelium, may be continuous as well. The observations in this paper enhance the possibility that such a continuum may be present or at least transiently present.

The concentration of study on individual cell form, origin and function has yielded pertinent information, but it is not likely to illuminate grosser structural relations. Examination of individual cells showed them to vary in form from squamous to columnar. They were generally aggregated in the form of islands surrounded by a basement membrane. The form that the islands took depended upon the density and tension of the fiber bundles as well as upon the angles at which the sections were cut. When the rests lay between tensely stretched fiber bundles, they were seen as elongated strands. In looser connective tissue, they appeared as ducts or follicles.

Black described the rests as lying close to cementum but not touching it "except in some isolated cases in the pig." In the current investigation, the root epithelium was found close to cementum and frequently abutting cementum, except near the cementoenamel junction. There it would arc outward, sometimes to be met by projecting cords from the attachment epithelium.

Rests were numerous along the entire root surface, ex-

\[\text{FIGURE 8. Four epithelial rests may be seen. The uppermost rest is rounded in form. The more apical rests are elongated. A cell from the central group extends into cementum about to be entrapped. (Verhoeff's iron hematoxylin. Original magnification 250X.)}\]

\[\text{FIGURE 9. Lying in lower connective tissue, rests are rounded in form. Each cell group is surrounded by a P.A.S. positive membrane, indicating the presence of a glycoprotein. When cells were about to be entrapped, a membrane was not demonstrable. (P.A.S. hematoxylin. Original magnification 500X.)}\]
except in root resorption bays during the shedding of primary teeth. They were rarely present near the resorption lacunae. The occasional epithelial aggregate that could be identified was usually seen further away from the root surface, near bone and even in marrow spaces. This may indicate that the rests were "left behind" as progressive root resorption occurred, indicating that the rests do not protect the root from resorption. Some epithelial cells were also seen being entrapped in cementum. Prior to entrapment in the calcifying tissue, the cell aggregates appeared to lose the membrane that encircled them.

The entrapment of rest cells in cementum could sometimes be followed by observing the fate of the melanin pigment found in the rest cells. Pig oral mucosa is pigmented, as is the dental lamina and the root epithelium. Pigment was seen in the rest cells, in cemental lacunae, in the periodontal ligament and cementoid. It may be assumed that the pigment found in cementum and in the periodontal ligament may have come from a breakup of rest aggregates. In tangential sections near the root apex, the root epithelium appeared to run into and out of cementum.

While the responses of the attachment epithelium in periodontal inflammation have been extensively described, only speculative attention has been paid to the role of the rest epithelium. Oral and Weinmann postulated that the rests might play a part in the sudden appearance of deep pockets in periodontosis. They speculated that the rests might proliferate and become confluent with the attachment epithelium, which could then separate from the tooth to form a pocket.

The observations of periodontal disease made in this
study cannot support or refute this thesis. They may, however, suggest a mode of participation of the rests in periodontal disease. The proliferating cords from the attachment epithelium that seemed almost confluent with the root epithelium could be a response to inflammation. This may be evidence for the participation of attachment and root epithelium in the events of periodontitis. On the other hand, it could also be evidence for a continuum that has either persisted, or has been renewed, or is newly established. A continuum might become visible because the destruction of fiber bundles in the area of inflammation might make it more apparent. It might also mean that a newly established confluence has taken place because of proliferation of attachment and root epithelium. Such a continuum (persistent, renewed or newly established) might play a part in pocket formation and in the eventual expulsion of a tooth.

**SUMMARY**

1. There may be a continuity of the root epithelial network with the reduced enamel epithelium before eruption and the attachment epithelium after eruption.

This continuum may be persistent or it may be transient. The possible presence of a continuity is supported by the following observations:

(a) Cords of epithelial cells were seen arising from the reduced enamel epithelium of an unerupted tooth and projecting apically into the periodontal ligament. These cords seemed to be continuous with the epithelial rests in the upper third of the root of this tooth.

(b) In the erupted tooth, the epithelial rests were found close to cementum, except near the cemento-enamel junction, where they swung outward and coronally, toward an epithelial cord that projected from the attachment epithelium.

(c) The root epithelium formed a lattice network encircling the tooth when viewed in sections cut tangential to the root. At the apex, the network appeared to run into and out of cementum. A network-like structure could also be discerned in the cords that projected from the reduced enamel epithelium. In thick longitudinal sections, the root epithelium was continuous (parallel to cementum) over long lengths.
of the root. Near the cementoenamel junction the
roots would be outward away from the tooth toward
the attachment epithelium.

2. The shape of the rest groups was influenced by
the direction and tension of the periodontal fiber bun-
dles. Rests were seen lying close to cementum, without
wounding it. Other rests abutted cementum and some
rest cells were observed becoming entrapped in ce-
mentum.

3. The rest groups were encircled by a membrane
that was P.A.S. and Alcian Blue positive. When rest
cells were about to be entrapped in cementum, the
membrane was not demonstrable.

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