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Mini review

The epithelial cell rests of Malassez – a role in periodontal regeneration?

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This article reviews general aspects about the epithelial cell rests of Malassez (ERM). The historical and general morphological features of the ERM are briefly described. The embryological derivation of the ERM is presented as an important consideration in understanding the events associated with their origin and possible functional roles within the periodontal ligament. The ultrastructural description of the ERM is also included to complement the morphological characteristics which distinguish these cells as the unique epithelial element of the periodontal ligament. The unique ability of these cells to synthesize and secrete a number of proteins usually associated with cells of mesenchymal origin, rather than ectodermal origin, is discussed in light of their role in cementum repair and regeneration. Such considerations lead to our hypothesis that one of the functional roles of the ERM may lie not only their role in maintaining and contributing to the normal periodontal cellular elements and function but also contributing, in a significant manner, to periodontal regeneration.

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It is very difficult to find an extended review about the epithelial cell rests of Malassez (ERM) in the dental literature. The ERM are briefly mentioned in books and their existence is scarcely remembered by dental practitioners. These cells are part of the normal structures within the periodontal ligament and it is believed that they play an important functional role that needs further investigation.

The ERM from the periodontal ligament were first described in 1817 by Serres as 'restes de l'organe de l'email' (Rests from the enamel organ) (1). This investigator was also aware of Hertwig's root sheath, because he described its atrophy and near-disappearance with age. Subsequent studies by histologists, confirmed that epithelial cells underwent atrophy and were absent in adult periodontal structures. However, Malassez, in 1885 (2), presented the first description of the cells and their distribution. He examined cross sections and longitudinal sections of adult human teeth, made composite drawings and found that the epithelial rests formed a network around the root. Since then, credit has been given to Malassez for demonstrating, definitively, that epithelial cells persist in the adult periodontal ligament. Several other authors have subsequently described the morphology and localization of these particular cells (3–9).

The morphological characteristics of the ERM have been described (10) using conventional histologic and light microscopy methods. The ERM can be identified easily as small clumps of epithelial cells within the periodontal ligament, closely approximated to the radicular cementum surface. Each cell has a darkly stained nucleus and a small, barely distinguishable, peripheral rim of cytoplasm. The cells have a high nuclear : cytoplasm ratio. In oblique sections of the periodontal ligament, the cell rests can be seen, not as isolated groups of cells, but as a network, similar to a fishnet, surrounding the root (2,11–13) (Fig. 1A,B).

The ERM have been implicated in a number of dental pathologies. Their vicinity within the periodontal and peri-apical tissues surrounding the root leads to their proliferation subsequent to inflammatory developmental or neoplastic stimuli. Proliferation of the ERM has been implicated in developmental cyst formation, such as the gingival or lateral periodontal cyst. Inflammatory cysts, such as the paradental and peri-apical cysts, also arise

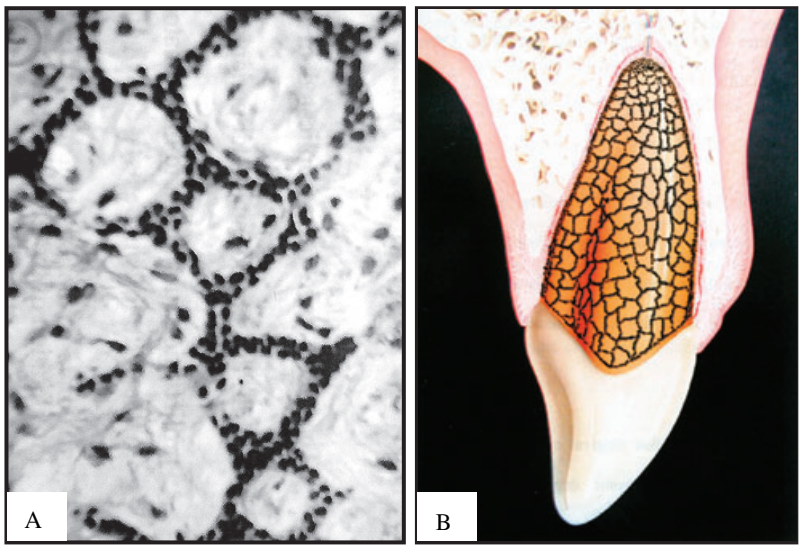

Fig. 1. (A) Histological appearance of a well-formed network of epithelial cell rests of Malassez. Areas of degenerative disruption to this network can be seen in the lower left-hand portion of the figure. Magnification: 250×. Reproduced with permission from Simpson HE. The degeneration of the rests of Malassez with age as observed by the Apoxestic Technique. *Journal of Periodontology* 1965; **36:** 28–31 (12). (B) Schematic representation of the mesh-like distribution of epithelial cell rests of Malassez around a root tooth. Adapted from Andreasen JO, Andreasen FM. Essentials of traumatic injuries to the teeth. Copenhagen: Munskgaard, 1990, 77 (95).

commonly from the ERM. A number of odontogenic tumors may also arise from the ERM. While the contribution of the ERM to pathological lesions is important, this review will largely focus on the role that the ERM play in normal periodontal ligament function and their potential role in periodontal regeneration.

Embryological origins of the ERM

The formation of the ERM occurs during the development of the root, which begins before eruption and largely coincides with this process. Amelogenesis has been completed by this time, so that the unerupted crown is covered by reduced enamel epithelium.

Root formation is initiated by mitotic activity occurring within the cervical loop of the enamel organ. This elongates in an apical direction, producing a double-layered, tube-like sleeve of epithelial cells, proliferating along the line of the future dentino–cemental junction of the root. This structure is called the root sheath of Hertwig and is directly responsible for inducing formation of the dentine. Its inner surface (inner dental epithelium of the dental papilla) induces odontoblast differentiation in the adjacent mesenchymal cells, and these cells begin to secrete dentine matrix thereafter (Fig. 2A).

As soon as the outer layer of dentine starts to calcify, the adjacent epithelial cells of the root sheath (outer dental epithelium) separate from its surface, and holes appear in the continuity of this previously continuous sleeve. The result is to produce strands of epithelial cells, which are referred to as ERM (Fig. 2B). Coincident with the fenestration of the epithelial root sheath, mesenchymal cells from the dental follicle move through the newly formed openings and attach to the outer surface of the newly forming dentine. The cells align themselves on this surface, differentiate into cementoblasts and commence the formation of cementum (14). Collagen fibre bundles are also laid down through the fenestrations and become incorporated into the developing cementum. Attachment of these fibres to the alveolar bone constitutes the functional attachment that provides support to the tooth. After tooth formation is completed, the remains of the enamel organ and the root sheath are represented by the reduced enamel epithelium and the ERM, respectively (15–17). Some authors have suggested that continuity exists between the ERM, the reduced enamel epithelium and subsequently the junctional epithelium of the gingival sulcus (18,19).

Ultrastructure of the ERM

Transmission electron microscopy (TEM) has been used to identify the ERM. Several studies have demonstrated the presence of tonofilaments and desmosomes. These ultrastructural characteristics differentiate the epithelial nature of these cells from fibroblasts and cementoblasts of the periodontium.

Early studies demonstrated that a basal lamina separated the ERM cell islands from the connective tissue (20,21). Hemidesmosomes and tight junctions are routinely observed between the cells. A large number of fine filaments (tonofilaments) are found within the cytoplasm (Fig. 3). These are bound together into bundles called tonofibrils. These do not appear to be in direct contact with the cementum. The average distance from the cementum to the epithelial cells was measured in three regions – apical 21 microns, middle radicular 33 microns, and cervical 41 microns – indicating a coronal migration away from the root surface. Further studies of human ERM (22) reported characteristic features of these epithelial cells, such as irregular nucleus with dense heterochromatin, cytoplasm containing tonofilaments, and abundant mitochondria, but a poorly developed rough endoplasmic reticulum.

Several studies have described the same structures in cultured porcine ERM cells (23–26). The ultrastructure of ERM from rat periodontal ligament sections resembles that of humans and other animals (27–29). Other studies have localized and studied the ERM around mouse molars (30,31). Again, the epithelial nature of these cells was

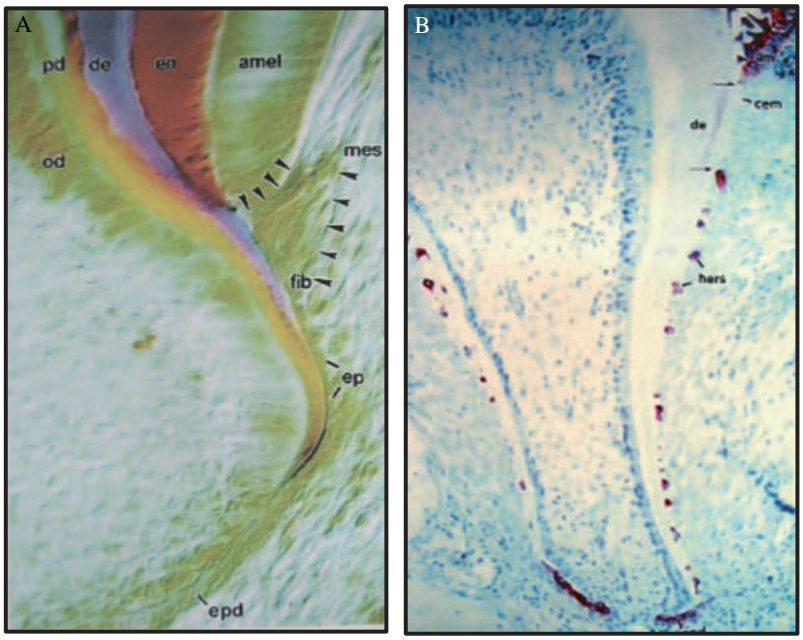

Fig. 2. (A) Histological appearance of Hertwig's epithelial root sheath (HERS) and subsequent formation of epithelial cell rests of Malassez in a 10-d postnatal mouse molar stained with AZAN. A bilayer of HERS is distinguishable at the apex but has lost its continuity at the level of the ameloblast layer. Mesenchymal cells and fibrous structures occupy the developing root surface, which is no longer covered by HERS. Isolated epithelial cells were localized within the mesenchymal tissues. Magnification 600×. (B) Labelling of epithelial cells during initial cementogenesis using an anti-keratin immunoglobulin in a 20-d postnatal mouse molar root. Magnification 720×. amel, ameloblasts; cem, cementum; de, dentine; en, enamel; ep, epithelial cells; epd, cells of epithelial diaphragm; fib, fibrous elements; hers, Hertwig's epithelial cell sheath; mes, mesenchymal cells; od, odontoblasts; pd, predentine. Reproduced with permission from Diekwisch TG. The developmental biology of cementum. Int J Dev Biol. 2001; **45:**695–706

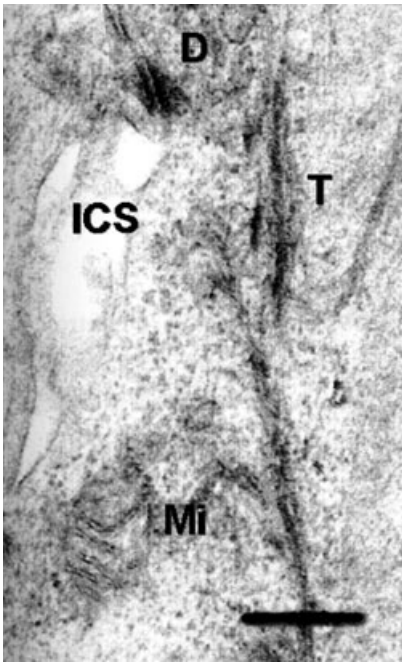

Fig. 3. Transmission electron microscopy of cultured porcine epithelial cell rests of Malassez. A desmosome (D) joins the two epithelial cells across the intercellular space (ICS). Mitochondrion (Mi) bulge into the intercellular space. Tonofilaments (T) can also be seen. Bar, 500 nm.

confirmed by the presence of tonofilaments, desmosomes and a basal lamina, with hemidesmosomes surrounding the cell rests. The ultrastructure of bovine ERM (32) is similar with regard to the presence of desmosomes and tonofilaments. One electron microscopy study has also demonstrated close apposition between Ruffini-like, free nerve endings, and the basal lamina of the epithelial cell rests (33). Epithelial cell clusters from periodontal ligament ERM have been demonstrated by TEM in areas of orthodontic repair (34). Recently, immunogold labelling and TEM has been used to co-localize the ERM cytokeratins within the tonofilaments (35).

Protein expression of the ERM

Several studies have investigated the expression of different types of proteins by the ERM. These proteins can be broadly classified into a number of groups, such as cytokeratins and neuropeptides, as well as extracellular matrix and cell-surface proteins including a variety of growth factors, cytokines and extracellular matrix-degrading proteinases.

Cytokeratins

Cytokeratins have been described in the ERM from different sources, such as human peri-apical lesions (36), human sinus tracts (37), rabbit periodontal ligament (35), monkey and human periodontal ligaments (38), rat periodontal ligament (39) and bovine periodontal ligament (32) (Table 1).

Immunohistochemistry has been used to demonstrate the expression of various epithelial proteins by the ERM. The expression of keratins 5 and 19 has been shown in normal human periodontal ligament and peri-apical lesions (36). The expression of a wide range of keratins (cytokeratins 5, 7, 8, 14, 15, 17, 18 and 19), some desmosomal cadherins (Dsg2 and DSC 2), and a cytoplasmatic desmosome-associated protein (plakophilin I) has also been demonstrated using immunohistochemical techniques on sections containing ERM from in rabbit and human sources (35). More recently, cytokeratin expression by ERM in human and monkey periodontal ligaments has also been described (40). In this study, cytokeratins 1, 5, 10 and 14 (34BE12 antibody), cytokeratins 1, 2, 5, 6, 7, 8, 10, 11, 16 and 19 (KL1 antibody), cytokeratin 16 (LL025 antibody), cytokeratin 18 (DC 10 antibody) and cytokeratin 19 (A 53-B/A2 antibody), were specifically identified. Bovine ERM have also been shown to express a variety of cytokeratins, including cytokeratins 1, 5, 6, 8, 13 17 and probably 19 (32). No cytokeratins 1, 4, 10, 11 and 18 were demonstrated in this study. Acidic

Table 1. Expression of cytokeratins by epithelial cell rests of Malassez (ERM) with different antibodies

Cytokeratin specificity	Antibody	Species and publication	Test	ERM reaction
5	RCK 102	Human	Immunohistochemistry	+ + +
19	LP2K	(36)		+ + +
1, 5, 10, 14	34,E12			+ + +
1,2,5,6,7,8,10,11,16,19	KL1	Monkey		+ + +
16	LL025	and	Immunohistochemistry	+ +
18	DC 10	human		+ +
19	A53-B/A2	(40)		+ +
5, 6, 8, 17, 19	MNF-16			+ + +
1, 5, 6, 8	PCK-26	Bovine	Immunohistochemistry	+ + +
13	KS-1AB	(32)		+ + +
Acidic cytokeratins	AE-1	Porcine (41)	Immunofluorescence	+ + +
5	Lu-5			+ + +
5	AE 14			+ + +
7	RCK-105			+ + +
7	K_s 7.18			+ + +
8	K_s 8.42	Rabbit		+ + +
8	K_s 8–17.2	and		+ + +
8	M20	human	Immunofluorescence	+ + +
14	CKB 1	(35)		+ + +
15	Krit 15			+ + +
17	E3			+ + +
18	KS 18.27			+ +
18	K_s 18.174			+ +
18	C 04			+ +
18	GP9			+ +
19	BA 16			+ + +
19	K_s 19.2			+ + +

+ + +, strong immunoreaction; + +, weak immunoreaction.

cytokeratins expressed by the ERM can be identified using the AE-1 antibody (41). Other studies have used the AE1/AE3 pan cytokeratin antibody for immunohistochemical identification of ERM cytokeratin expression in a variety of species (Fig. 4) (42,43). These observations and findings confirm the epithelial phenotype of those cells.

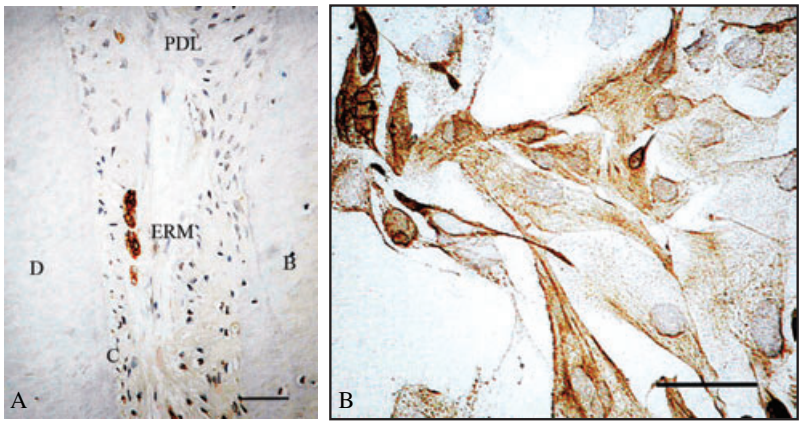

Fig. 4. (A) Epithelial cell rests of Malassez (ERM) immunostained with AE1/AE3 antibody in a section of human periodontal ligament (PDL). The ERM are closely located to the cementum (C). The alveolar bone (B) can also be seeen. Bar, 100 μm. (B) Immunostaining for cytokeratins of cultured porcine epithelial cell rests of Malassez using AE1/AE3 antibody. Cytoplasmic limits are very well defined despite the absence of stained cell membranes. The patterns of tonofilaments stain brown in the cytoplasm and around the nuclei. Bar, 100 μm.

Neuropeptides

A number of neuropeptides, including calcitonin gene-related peptide (CGRP) (44,45), substance P (SP), vasoactive intestinal peptide (VIP) (45,46), tyrosine receptor kinase A (TrkA) – a high-affinity receptor of nerve growth factor (47,48) – and parathyroid hormone-related protein (PTHrP) (49) may be expressed by the ERM.

Extracellular matrix and cell-surface proteins

A number of extracellular matrix proteins, growth factors and cytokines are also expressed by the ERM. While it has been recognized for some time that Hertwig's epithelial root sheath expresses both amelogenin and enamelin (50), more recent *in situ* hybridization studies have demonstrated that the ERM express both of these proteins with some regional differences (39). Other matrix macromolecules expressed by the ERM include glycosaminoglycans, hyaluronic acid, dermatan sulphate, chondroitin sulphate and type IV collagen fibronectin, laminin and laminin-5 (28,51–53). Interestingly, these cells also synthesize several proteins more commonly associated with mesenchymal tissues rather than epithelial tissues, such as osteopontin, bone sialoprotein and osteoprotegerin (42,54–57).

Other proteins expressed by the ERM include cell-surface molecules, such as calbindin D28 (which are vitamin D-dependent calcium-binding proteins) and epidermal growth factor receptor, growth factors such as epidermal growth factor and bone morphogenetic proteins 2 and 4, various cytokines, including interleukin-1α, interleukin-6, interleukin-8 and granulocyte–macrophage colony-stimulating factor (GM-CSF), β defensin (BD-1) and prostaglandins E and F (58–64).

The ERM also have a demonstrated ability to degrade collagen, both intracellularly and extracellularly, by secretion of collagenase, gelatinases and human tissue inhibitor

metalloproteinases (TIMP) (24,65,66). The same research group has demonstrated that some pathogen-virulence factors enhance epithelial tissue (including ERM) secretion of collagenases through the induction of matrix metalloproteinases (MMPs) (67) (Table 2). This group has also shown that ERM production of MMPs is inhibited by tetracyclines (68). Chymotrypsin-like enzymes comprise a large family of serine proteases that degrade peptide bonds. This enzyme has also been also purified from cultured ERM (69).

ERM and periodontal regeneration

A major goal of periodontal therapy is restoration of the damaged tissues to their original form and function and requires regeneration of the destroyed periodontal connective tissues through the formation of new cementum, new bone and attachment of new connective tissue fibers (70–74). In general, regeneration of the periodontium involves collaboration and interaction of several cell types, including gingival connective tissue fibroblasts (GF), periodontal ligament fibroblasts (PDLF), cementoblasts and osteoblasts, endothelial cells and macrophages (75). An often-overlooked cellular element within the periodontal ligament is the ERM. To date, there is no certainty about the functional role of the ERM, despite the fact that they surround the dental root and are located in close proximity to radicular cementum and blood vessels. Within this framework it is noteworthy that the ERM can express a number of bone/cementum-related proteins which could implicate them in a regenerative role (Fig. 5).

Data from *in situ* hybridization analysis indicate that the adhesion molecules osteopontin (OPN) and bone sialoprotein (BSP) are expressed by cementoblasts along the root surface during the early stages of tooth root development also on mature fully erupted tooth root surfaces. In contrast, cells expressing collagen, proteoglycans and a number of other extracellular matrix macromolecules are noted throughout the surrounding

Table 2. Mammalian matrix metalloproteinases (MMP) expressed by epithelial cell rests of Malassez (ERM)

Protease	MMP number	ERM
Collagenases		
Collagenase-1	MMP-1	–
Collagenase-2	MMP-8	–
Collagenase-3	MMP-13	+ (66)
Collagenase-4	MMP-18	–
Gelatinases		
Gelatinase A	MMP-2	+ (67)
Gelatinase B	MMP-9	+ (67)
Membrane-anchored MMPs		
MT1-MPP	MMP-14	–
MT2-MPP	MMP-15	–
MT3-MPP	MMP-16	–
MT4-MPP	MMP-17	–
MT5-MPP	MMP-24	–
MT6-MPP	MMP-25	–
Stromelysins		
Stromelysin-1	MMP-3	+ (67)
Stromelysin-2	MMP-10	–
Stromelysin-3	MMP-11	–
Matrilysins		
Matrilysin	MMP-7	–
Matrilysin-2/Endometase	MMP-26	–
Other MMPs		
Metalloelastase	MMP-12	–
RASI, Stromelysin-4	MMP-19	–
Enamelysin	MMP-20	–
XMMP	MMP-21	–
(chicken)	MMP-22	–
(human homologue)	MMP-27	–
CA-MMP	MMP-23 A,B	–
Epilysin	MMP-28	–

+, Reported expression of MMP.

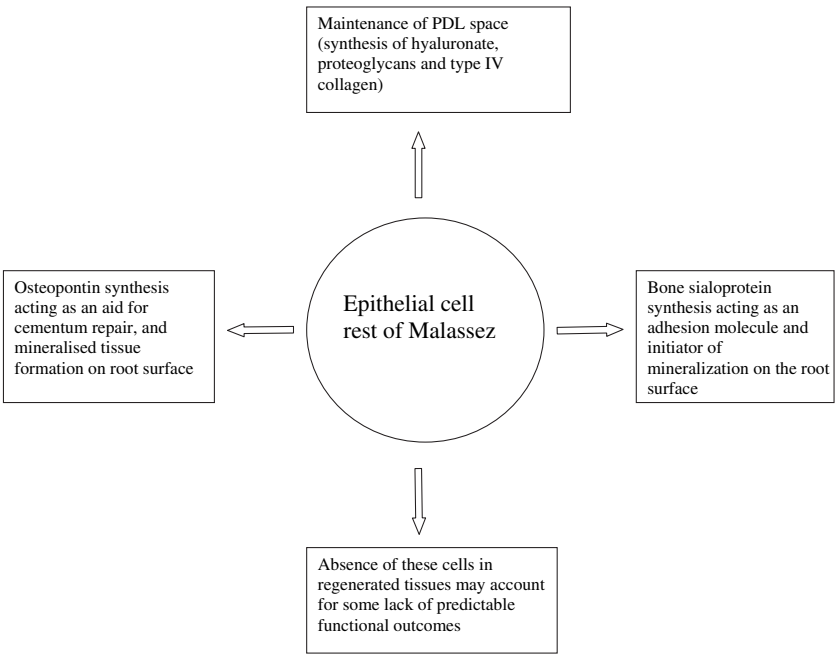

Fig. 5. Putative functions of the epithelial cell rests of Malassez in periodontal regeneration.

soft and hard connective tissues (76–79).

The concept that the ERM may be associated with cementoblast development is also emerging. Observations that a large number of epithelial cells, showing well-developed rough endoplasmic reticulum and Golgi apparatus, but lacking intracellular labelling for enamel matrix proteins, supports the idea that the presence of secretory granules in Hertwig's epithelial root sheath may not solely be for producing enamel matrix proteins (80). Hertwig's epithelial root sheath cells may have the ability to produce other proteins, such as cementum matrix constituents (81,82). Whether the ERM descendents of these cells retain similar functions remains to be fully established.

Both BSP and OPN are expressed by cells linked to the formation of mineralized tissues, such as bone and cementum, while OPN also is expressed by cells within the newly forming periodontal ligament (83). BSP is localized in cementum and bone, meanwhile OPN is strongly distributed in the periodontal ligament as well as in cementum and bone (84). OPN may also be involved in cementum repair (55). Recent studies have demonstrated a strong expression of OPN by the ERM, suggesting a potential function for these cells in mineralized tissue formation along tooth root surfaces during both development and, possibly, regeneration (42, 54–57).

OPN has been linked to the regulation of ectopic crystal formation, where evidence supports a role for OPN in controlling the extent of hydroxyapatite crystal nucleation and/or growth (85,86). Moreover, OPN has been reported to inhibit apoptotic events, such as those associated with inflammation, and this ability may have some significance in the regulation of cells at sites during development of cementum and also during wound healing (87,88). Suggested roles for BSP include acting as an adhesion molecule to maintain applicable cells at the root surface, and as an initiator of mineral formation along the root surface (89). The temporal and spatial expression of BSP during cementogenesis and bone formation is consistent with a role for this molecule in promoting mineral formation. Thus, while both BSP and OPN may have a role in recruiting and maintaining selective cells at the root surface, an equally important role may be related to the control of mineralization along the root surface (90).

One function of the ERM may be in the maintenance of the periodontal ligament space (91). This was proposed after experimental replantation of teeth and the observation that the ERM were always found in vital periodontal ligament areas of replanted teeth. Additional evidence, that maintenance of ligament space by the ERM may play a role in periodontal regeneration, has come from observations that an absence of these cells in regenerated periodontal ligament is associated with a narrowing of the periodontal ligament (92,93). However, two recent studies have reported an absence of ERM in the periodontal ligament of monkeys and humans after periodontal regenerative procedures (38,40). It was suggested that the ERM may not be able to regrow after periodontal regeneration. Given that these cells can be successfully isolated and cultured, such a conclusion may be unwarranted and it may be that the local environment of the repairing periodontium may not be conducive to ERM migration and proliferation. Furthermore, because of the important strategic position of these cells in healthy periodontal ligament, together with their ability to secrete matrix molecules conducive to cementum formation, it is our hypothesis that these cells will be crucial for successful and predictable periodontal regeneration. Indeed, the concept of periodontal regeneration is based on the goal of regeneration of the supporting tissues lost as a consequence of inflammatory periodontal disease (94). Therefore, ERM should be present after periodontal regenerative procedures as part of the reconstructed structural and functional elements of the periodontal ligament.

Conclusion

In conclusion, because the ERM play an important role within the periodontal apparatus of the normal periodontium, their presence after, and possibly during, periodontal regenerative therapies would be expected to be essential. Further studies are needed from a biomolecular basis and cell biological approach to explain the basic role of these cells within the regenerating periodontal ligament.

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