ISSN Print: 2394-7489
ISSN Online: 2394-7497
IJADS 2017; 3(4): 21-25

www.oraljournal.com
Received: 05-08-2017
Accepted: 06-09-2017

Dr. Suchetha A
Head of the department, Department of periodontology, DAPM RV dental college, Bengaluru, Karnataka, India

Dr. Spandana A
Post graduate student, Department of periodontology, DAPM RV dental college, Bengaluru, Karnataka, India

Dr. Sapna N
Reader, Department of periodontology, DAPM RV dental college, Bengaluru, Karnataka, India

Dr. Divya Bhat
Lecturer, Department of periodontology, DAPM RV dental college, Bengaluru, Karnataka, India

Dr. Apoorva SM
Reader, Department of periodontology, DAPM RV dental college, Bengaluru, Karnataka, India

Dr. Chitra Jayachandran
Post graduate student, Department of periodontology, DAPM RV dental college, Bengaluru, Karnataka, India

Correspondence
Dr. Spandana A
Post graduate student, Department of periodontology, DAPM RV dental college, Bengaluru, Karnataka, India

Hertwig's epithelial root sheath: A panaromic view

Dr. Suchetha A, Dr. Spandana A, Dr. Sapna N, Dr. Divya Bhat, Dr. Apoorva SM and Dr. Chitra Jayachandran

Abstract
The Hertwig's epithelial root sheath is a transient structure formed in the early period of root formation. It has an important role in regulation and maintenance of the periodontal ligament space, prevention of root resorption and ankylosis, maintenance of periodontal ligament homeostasis, Induction of acellular cementum formation, as a stem cell in periodontal regeneration and also as a stimulant in periodontal regeneration. Hence, this article aims to review HERS along with its functions in development as well as in periodontal regeneration.

Keywords: Hertwig's epithelial root sheath, Periodontal regeneration, Stem cell regeneration, Cementogenesis, Dentinogenesis

Introduction
The Hertwig's epithelial root sheath is a double-layered, tube-like sleeve of epithelial cells that play an important role in the regulation and maintenance of periodontal ligament space and function. It was first discovered by Oskar Hertwig in 1874 in an amphibian [1]. It is a transient structure assembled in the early period of root formation, elongation and subsequently, fenestration and reduction. Upon the deposition of the first layer of mantle dentin, the Hertwig's epithelial root sheath will fragment to form several discrete clusters/strands of epithelial cells known as epithelial cell rests of malassez [2]. These may act as a source of mesenchymal progenitor cells for cementoblasts and hence, this has gladdened interest in the role of Hertwig's Epithelial Root Sheath (HERS) in periodontal regeneration [3].
The objective of this review is to provide an overview of HERS development, origin, role in root formation, fate after root development, role in periodontal regeneration and stem cell regeneration.

Development and origin
The development of human teeth initiates from the interaction of the oral epithelial cells and the underlying mesenchymal cells. Tooth development is a continuous process which takes place in many stages. Each tooth develops through three successive early stages: i) Bud stage. ii) Cap stage iii) Bell stage. During these early stages the tooth germs grow, expand and the cells that are to form the hard tissues of the teeth (i.e enamel and dentin) differentiate. As the crowns are formed and mineralized the roots of the teeth begin to form. After the roots calcify the tooth supporting tissues (cementum, periodontal ligament, and alveolar bone) initiate to develop. Subsequently the completed tooth crown erupts. Root formation and cementogenesis continue until a functional tooth and its supporting structures are fully developed [4].
The development of the Hertwig's epithelial root sheath originates at the end of the crown stage in tooth development [2]. It is a complex process that includes a number of cascades and mechanisms with many necessary questions left unanswered [5]. Those include whether the Hertwig's epithelial root sheath is a cellular origin of cementoblasts, how the fragmentation of Hertwig's epithelial root sheath is initiated, how it withdraws from the root surface and the fate of the Hertwig's epithelial root sheath after tooth-root development [6].
The ectodermal derivatives, inner and outer enamel epithelium of the enamel organ (devoid of stratum intermedium and stellate reticulum) proliferate and give rise to a double-layered, tube-like sleeve of epithelial cells, known as the Hertwig's epithelial root sheath [1]. The development of the periodontium, including cementum, periodontal ligament and alveolar

bone, occurs in a spatially and temporally ordered manner [7].
Initially, the Hertwig's epithelial root sheath divides two ectomesenchymal tissues: dental follicle and dental papilla [2]. On the tooth side, the inner epithelial cells of the Hertwig's epithelial root sheath stimulates dental papilla cells to differentiate into dentin-producing odontoblasts. Following dentin formation, this Hertwig's epithelial root sheath secretes a fine matrix of proteins, termed the hyaline layer of Hopewell Smith. Soon after the deposition of the first layer of mantle dentin matrix, Hertwig's epithelial root sheath begins to fragment that results in the formation of strands of epithelial cells known as the epithelial cell rests of Malassez. The disintegration of HERS allows the dental follicle cells to migrate and attach to the hyaline layer of Hopewell Smith where they differentiate to form cementum-producing cementoblasts. Dental follicle-derived periodontal ligament fibroblasts give rise to collagen fibers that are embedded in the cementum and termed Sharpey's fibers [8].
On the bone side, osteoblasts derived from dental follicle cells deposit alveolar bone lined up the tooth socket. Insertion of Sharpey's fibers into alveolar bone completes the development of periodontium [8]. Therefore, it appears that dental follicle cells have the capacity to differentiate into three cell types, namely cementoblasts, periodontal ligament fibroblasts and osteoblasts, which form cementum, Sharpey's fibers and alveolar bone, respectively [9].

Role in root formation (Dentinogenesis and Cementogenesis)

It is well accepted that HERS plays an important role in root development; however, the precise nature of this role remains unclear. Amongst the different functions attributed to these HERS cells are that of inducers and regulators of root formation, including the size, shape, and number of roots [10]. The external shape of each root is fully determined by the position of the surrounding Hertwig's epithelial root sheath [11].
The classic theory of root formation states that, as these cells divide, there is apical migration of HERS cells through the underlying dental ectomesenchymal tissues (dividing into dental papilla and dental follicle) [12]. Root formation starts when the enamel organ has reached its final size and the inner and outer cell layers of the enamel epithelium, which delineate the enamel organ, proliferate from the cervical loop to form Hertwig's epithelial root sheath. In an experimental culture it revealed that epidermal growth factor (EGF) initiated the expansion of stellate reticulum (SR) residing between the inner and outer epithelial layers in HERS and prevented the formation and growth of HERS and consequently root formation. In conflict, EGF- kinase, tyrphostin, resulted in the HERS development and the transition from crown morphogenesis to root formation [13]. Uninterrupted cell mitotic activity at the apical termination of Hertwig's root sheath leads to a coronoapical progression of this double cell layer. Its most apical portion, that is, the diaphragm, separates the dental papilla from the dental follicle. The inner and outer cell layer of Hertwig's root sheath is surrounded by a basement membrane. Similarly to the reciprocal epithelial mesenchymal interactions occurring during crown formation, cells originating from the peripheral dental papilla differentiate along the internal basement membrane of the diaphragm into odontoblasts [14].
Once the first matrix of radicular mantle dentin is formed by the maturing odontoblasts and before the mineralization of the dentin matrix reaches the inner epithelial cells, Hertwig's root sheath becomes discontinuous. Epithelial cell remnants of Hertwig's root sheath persist in the still developing and, later in time, in the aging periodontal ligament at an approximate distance of 30-60 pm remote from the root surface, where they are referred to as the epithelial rests of Malassez. The role of the Hertwig's epithelial root sheath in the initiation of dentinogenesis was first proposed as early as 1887 [15].
The cellular events occurring during root formation are of utmost importance for proper understanding of cementogenesis. Before the cementogenesis to begin, the newly formed surface of root dentin comes into contact with the undifferentiated cells of the dental sac (dental follicle) [1]. Then it stimulates the activation of cementoblasts to begin cementogenesis. It is believed that either HERS become interrupted or fragmented cells transform into cementoblasts [11]. Studies done by authors **(**Thomas & Kollar) may support, the hypothesis that the cementoblasts originate from epithelial cells of Hertwig's root sheath when they undergo an epithelial-mesenchymal transformation under the induction of $TGFB_1$ and develop cementum forming cells. HERS was shown to secrete cementum related proteins like Bone sialoprotein (BSP), Insulin-like growth factor 1, Osteopontin, Fibrillar collagen, Osteocalcin and high levels of Alkaline phosphatase(ALP). HERS cells also express ameloblastin (AMBN), due to which we can assume that AMBN is related to tooth root formation [16].
Hamamoto *et al*. (1996) reported the capacity of HERS cells to pro-duce enamel and express amelogenins in response to pulp in-flammation. However, this finding was achieved under pathologic Hamamoto *et al*. (1996) reported the capacity of HERS cells to pro-duce enamel and express amelogenins in response to pulp in-flammation. However, this finding was achieved under pa.

Fate of hers after root development

During initial tooth-root formation, the Hertwig's epithelial root sheath cells undergo an extensive period of proliferation but, the proliferation rate of the Hertwig's epithelial root sheath at the later stages of tooth-root development is not in synchrony with the surrounding root-forming connective tissue cells, as space between the Hertwig's epithelial root sheath cells increases, where the number of the Hertwig's epithelial root sheath cells decreases on the maturing root surface. Different mechanisms have been put forward to explain the observed decrease in the number of Hertwig's epithelial root sheath cells [1].
One proposed mechanism is that some of the Hertwig's epithelial root sheath cells migrate to the periodontal ligament away from the root surface and form the epithelial cell rests of Malassez. It is also suggested that the Hertwig's epithelial root sheath cells become incorporated into the advancing cementum front or differentiate into cementoblasts and some undergo apoptosis [17]. Analysis of expression of Dlx-2 and ameloblastin indicate that some HERS express both the markers and some express only one, thus indicates that there are more than one type of cells present in the tissues.
However, a study using the terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) assay showed positive labelling of only some of the nuclei in Hertwig's epithelial root sheath cells. This indicates that while some of the Hertwig's epithelial root sheath cells may undergo apoptosis, many of them remain viable and become part of the adult periodontal ligament through other pathways [18].

Role of hers in periodontal regeneration

Periodontal regeneration is indicated to restore the original

architecture and function of the periodontium. Review of embryology shows the importance of Hertwig's epithelial root sheath, involved in cementogenesis and root formation. The remnants of these cells are found in normal periodontal ligament as the epithelial cell rests of Malassez (ERM). The cells of ERM are known to retain the functions of HERS by expression of various proteins and growth factors. These cells have stem cell characters and known to express stem cell related genes and thus lead to the hypothesis that they can contribute to true periodontal regeneration [19].

Currently the periodontal therapies have been directed towards obtaining predictable regeneration of the periodontium [20]. Successful tooth regeneration was achieved using the combination of tooth germ derived epithelial and mesenchymal single cells. The epithelial or mesenchymal cells alone generated keratinized epithelium-like structures or bone, respectively [21]. Although the use of periodontal ligament stem cells can result in significant periodontal regeneration, the combination of the Hertwig's epithelial root sheath/epithelial cell rests of Malassez and dental mesenchymal stromal/ stem-cell populations gave rise to better outcomes in periodontal regeneration [22].

In combination with dental pulp cells, porcine epithelial cell rests of Malassez can differentiate into ameloblast-like cells and generate enamel-like tissues *in vivo*, as shown by positive amelogenin staining [23]. Co-culture of dental follicle cells and the Hertwig's epithelial root sheath cells significantly increases bone/cementum related gene expression as well as *in-vitro* mineral nodule formation [22]. When transplanted into rat omenta, dental follicle cells pre-exposed to the Hertwig's epithelial root sheath give rise to cementum like and periodontal ligament-like structures, while control cells only produce fibrous tissues [22].

Collectively, the improved regenerative outcomes using the combination of the Hertwig's epithelial root sheath/ epithelial cell rests of Malassez and dental mesenchymal stromal/stem cells have highlighted the essential roles of the Hertwig's epithelial root sheath/epithelial cell rests of Malassez in the regenerative procedures [1].

Studies related to hertwig's epithelial root sheath

S.no	Year	Author	Type of study	Result
1	2004	Yamamoto H *et al*	Animal study	Conducted a study to examine root formation of the first molar in mice. The results showed migration of HERS cells did not occur during root formation and both cell adhesion and cell proliferation are essential for root development [24].
2	2009	Huang X *et al*.	Animal Study	Conducted a study to investigate the morphological fate and analyze the dynamic movement of HERS cells *in vivo* in tooth root development. Most of the HERS cells are attached to the surface of the cementum, and others separate to become the epithelial rest of Malassez. During root development, the HERS is not interrupted, and instead the HERS cells continue to communicate with each other through the network structure [17].
3	2004	Bosshardt DD *et al*	Ex-vivo study	They have assumed that HERS' cells occasionally have a lingering ameloblastic activity at the beginning of root formation, but the results did not support the hypothesis of a causal relationship between EMPs and cementogenesis and thus lend to support the concept of an epithelial origin of cementoblasts [9].
4	2003	Margarita Zeichner-David *et al*[1]	*In-vitro* study	The results of this study suggest that the acellular and cellular cementum are synthesized by two different types of cells, the first one by HERS-derived cementoblasts and the later by neural crest-derived cementoblasts [25].
5	2013	Hirose N *et al*	Animal study	Conducted a study to identify the role of Ameloblastin in the differentiation HERS derived cells. The results suggest that the expression of AMBN in HERS functions as a trigger for normal root formation [16].
6	2014	Chen J *et al*	Animal study	Earlier studies have shown that transforming growth factor beta 1 (TGF-β1) and fibroblast growth factor 2 (FGF2) are involved in inducing EMT. The TGF-β1 regulated the differentiation of HERS cells into periodontal ligament fibroblast-like cells, and FGF2 directed the differentiation of HERS cells into cementoblast-like cells [26].
7	2011	Hyun Nam *et al*	*In vitro* study	In this study, they investigated whether HERS/ERM cells have primitive stem cell characteristics: those of embryonic stem cells as well as of epithelial stem cells. To overcome these problems, immortalized primary HERS/ERM cells isolated from human periodontium [3].
8	2012	Jimin Xiong *et al*	Animal study	The results of the study demonstrate that ERM cells share similar phenotypic and functional attributes with mesenchymal stem cells by their capacity to differentiate into diverse lineages indicative of mesodermal and ectodermal origin [27].
9	2005	Rincon JC *et al*	*In-vitro* study	The results of the study provide evidence that ERM express mRNA for at least one bone/cementum-related protein like osteopontin or bone sialoprotein [28].
10	2014	Rajendran *et al*	Review	Described the functional role of ERM not only in maintaining the periodontal ligament homeostasis but also in contributing to periodontal regeneration [19].
11	2002	Shimizu-Ishiura M *et al*	*In vitro* study	The results of the study suggest that enamel matrix derivative (EMD; Emdogain) is an effective biological matrix for enhancing new trabecular bone induction after pure bioinert titanium (Ti) implantation in the rat femur [29].
12	2016	HD Miniggio *et al*	Clinical review	Epithelial cell rests of Malassez have the ability to maintain their structure within the periodontal ligament through the constant release of a polypeptide known as epidermal growth factor (EGF). The receptor for this growth factor (EGFr) is made up of transmembrane proteins that activate tyrosine kinase intracellularly and trigger cellular events that lead to cell division that explains their role in regeneration of periodontal tissue [30].

Clinical significance of hers

1. The regulation and maintenance of the periodontal ligament space.
2. The prevention of root resorption and ankylosis.
3. The maintenance of periodontal ligament homeostasis.
4. Induction (i.e., not secretion) of acellular cementum formation.
5. HERS as a stem cell in periodontal regeneration.

It has been recently reported that the epithelial cell rests of malassez contain unique stem-cell populations that are capable of undergoing epithelial mesenchymal transition (EMT) [27]. However, HERS/ERM cells have been difficult to sufficiently expand for *in vitro* characterization and *in vivo* transplantation. To overcome these limitations, immortalization of HERS/ERM cells can be done. The use of these immortalized HERS/ERM as a regenerative medicine in the periodontium can be expected [3].

6. Emdogain (Enamel-related matrix protein secreted by HERS) as a stimulant in periodontal regeneration.

Emdogain is a commercially available mixture of enamel matrix derivatives (EMDs), has been shown to enhance the osteogenic potential of bone marrow by enhancing the proliferation of osteoblasts, promoting cell differentiation, and stimulating migration and viability of osteoblasts, which can lead to improved bone and periodontal regeneration [31].

Developmental anomalies/pathologies associated with hers

1) Enamel pearls:

Some cells of HERS remain adherent to the dentin surface and differentiate into fully functioning ameloblasts, producing enamel. Such droplets of enamel, called ENAMEL PEARLS, are sometimes found in the area of furcation of roots of permanent molars [4].

2) Periapical cyst:

The remnants of HERS in the periapical region have the potential for reactive proliferation in response to an adjacent focus of infection resulting in formation of Periapical cyst [4].

3) Accessory root canals:

If the continuity of HERS is broken prior to dentin formation, a defect in the dentin wall of the pulp consequences. Such defects are found in the furcation or on any point of the root itself resulting in development of accessory root canals opening on periodontal surface of the root [4].

4) Periodontal pocket:

The periodontal pocket is a focus of infection which occurs as a localized area of chronic inflammation in the connective tissues adjacent to the epithelial rests [32].

Conclusion

HERS is the definitive governor of the periodontal ligament. The role of the remnants of Hertwig`s root sheath cannot be overlooked because of their presence in the periodontal ligament as the cell rests of Malassez and their efficiency for cellular activity. This highlights that ERM cells, rather than being cell rests as indicated by their name in the literature, are an important stem cell source that might play pivotal role in periodontal regeneration. Application of enamel matrix proteins in the form of Emdogain has set a modern standard for periodontal regeneration therapy. Surgical periodontal treatment of deep intrabony defects with EMD promotes periodontal regeneration. Further studies are needed to explain the basic role of these HERS cells within the regenerating periodontal ligament.

References

1. Luan X, Ito Y, Thomas GH, Diekwisch. Evolution and development of hertwig's epithelial root sheath. Developmental dynamics, 2006; 235:1167-1180.
2. Stan JX, Gronthos S, Bartold PM. Role of the epithelial cell rests of malassez in the development, maintenance and regeneration of periodontal ligament tissues. Periodontology. 2000-2013; 63:217-233.
3. Hyun Nam, Jaewon Kim, Jaewan Park, Joo-Cheol Park, Jung-Wook Kim, Byoung-Moo Seo *et al*. Expression Profile of the Stem Cell Markers in Human Hertwig's Epithelial Root Sheath/Epithelial Rests of Malassez Cells. Mol. Cells. 2011; 31(355-360):30.
4. Bhaskar SN, Kumar GS. Editors. Orban's oral histology and embryology, 14th edition. Elsevier publications. 2015.
5. Ten Cate AR. The development of the periodontium–a largely ectomesenchymally derived unit. Periodontol. 2000; 1997:13:9-19.
6. Tomazela-Herndl SA, Arana-Chavez VE. Ultrastructure of early mineral deposition during hyaline layer formation in rat molars. Arch Oral Biol. 2001; 46:305-311.
7. Lin NH, Gronthos S, Bartold PM. Stem cells and periodontal regeneration. Aust Dent J. 2008; 53:108-121.
8. Xiong J, Gronthos S, Bartold PM. Role of the epithelial cell rests of Malassez in the development, maintenance and regeneration of periodontal ligament tissues. Periodontol. 2000-2013; 63(1):217-33.
9. Bosshardt DD, Nanci A. The Hertwig's epithelial root sheath, enamel matrix proteins, and initiation of cementogenesis in porcine teeth. J Clin Periodontol. 2004; 31:184-192.
10. Ten Cate AR. The role of epithelium in the development, structure and function of the tissues of tooth support. Oral Dis. 1996; 2:55-62.
11. Von BA. Über die Ausdehnung des Schmelzorganes and seine Bedeutung fur die Zahnbildung. Arch mikrosk Anat entwmech. 1887; 29:367-395.
12. Ten Cate's Oral Histology, Nanci, Elsevier, 2013, 207.
13. Wentz FM, Weinmann JP, Schour I. The prevalence, distribution, and morphological changes of the epithelial remnants in the molar regions of the rat. J Dent Res. 1950; 29:637-646.
14. Daniela eşian 1 al. Monea. Morphological and developmental characteristics of the hertwig's epithelial root sheath and its involvement in the root growth and development of the immature teeth. Acta medica transilvanica march. 2011; 2(1)257-260.
15. Fijiwara N, Kagiya T *et al*. EFG prevents formation of HERS during developing mouse molar tooth *in vitro*, European Cells and Materials, 2007; 14(2):54.
16. Wallace JA, Vergona K. Epithelial rests' function in replantation: is splinting necessary in replantation? Oral Surg Oral Med Oral Pathol. 1990; 70:644-649.
17. Hirose N1, Shimazu A, Watanabe M, Tanimoto K, Koyota S, Sugiyama T *et al*. Ameloblastin in Hertwig's epithelial root sheath regulates tooth root formation and development. PLoS One. 2013; 8(1):54449.
18. Huang X, Bringas PJr, Slavkin HC, Chai Y. Fate of HERS during tooth root development. Dev Biol. 2009; 334:22-30.

19. Thomas HF. Root formation. Int J Dev Biol. 1995; 39:231-237.
20. The rest cells in periodontal regeneration - a review maheaswari rajendran int j cur res rev, 2014; 06(05).
21. Roxanne a. Lowenguth & timothy m. Bliede. Periodontal regeneration: root surface demineralization. Periodontology 2000, 1993:1.
22. Liu Y, Zheng Y, Ding G, Fang D, Zhang C, Bartold PM, *et al.* Periodontal ligament stem cellmediated treatment for periodontitis in miniature swine. Stem Cells 2008: 26: 1065-1073.
23. Bai Y, Matsuzaka K, Hashimoto S, Fukuyama T, Wu L, Miwa T *et al.* Cementum- and periodontal ligament-like tissue formation by dental follicle cell sheets co-cultured with the Hertwig's epithelial root sheath cells. Bone 2011; 48:1417-1426.
24. Shinmura Y, suchiya S, Hata K, Honda MJ. Quiescent the epithelial cell rests of Malassez can differentiate into ameloblast-like cells. J Cell Physiol, 2008: 217: 728-738.
25. Yamamoto, Tsuneyuki, Takahashi, Shigeru. Hertwig's epithelial root sheath cells do not transform into cementoblasts in rat molar cementogenesis. Annals of Anatomy = Anatomischer Anzeiger, 191(6):547-555.
26. Margarita Zeichner-David1, Keiji Oishi1, Zhengyan Su1, Vassili Zakartchenko1, Li-Sha Chen1, Higinio Arzate *et al.* Pablo Bringas Jr.1 Role of Hertwig's epithelial root sheath cells in tooth root development. 2003; 228(4):651-663.
27. Chen J1, Chen G, Yan Z, Guo Y, Yu M, Feng L *et al.* TGF-β1 and FGF2 stimulate the epithelial-mesenchymal transition of HERS cells through a MEK-dependent mechanism. J Cell Physiol. 2014; 229(11):1647-59.
28. Xiong J, Mrozik KM, Gronthos S, Bartold PM. Epithelial cell rests of Malassez contain unique stem cell populations capable of undergoing epithelial-mesenchymal transition. Stem Cells Dev, 2012, 21.
29. Rincon JC1, Xiao Y, Young WG, Bartold PM. Production of osteopontin by cultured porcine epithelial cell rests of Malassez. J Periodontal Res. 2005; 40(5):417-426.
30. Shimizu-Ishiura M(1), Tanaka S, Lee WS, Debari K, Sasaki T. Effects of enamel matrix derivative to titanium implantation in rat femurs. J Biomed Mater Res. 2002; 60(2):269-76.
31. HD miniggio; EJ Raubenheimer. Epithelial cell rests of Malassez: From quiescent remnants to front-runners in periodontal homeostasis and regeneration. A brief review. S. Afr. Dent. J. 71 n.2 Johannesburg Mar. 2016.
32. Vandana J Rathva. Enamel matrix protein derivatives: role in periodontal regeneration. Clinical, Cosmetic and Investigational Dentistry 2011; 3:79-92.
33. Spouge JD, A New Look at the Rests of Malassez. A Review of Their Embryological Origin, Anatomy, and Possible Role in Periodontal Health and Disease. J. Periodontol. August, 1980.