MORPHOLOGICAL AND DEVELOPMENTAL CHARACTERISTICS OF THE HERTWIG'S EPITHELIAL ROOT SHEATH AND ITS INVOLVEMENT IN THE ROOT GROWTH AND DEVELOPMENT OF THE IMMATURE TEETH

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Abstract: *Onset of the root formation occurs after the dental crown reaches the final dimensions and the main role in this process plays an epithelial formation, Hertwig's sheath named after the author who described it in 1874.Hertwig's epithelial sheath appears as a proliferation of union between inner and outer adamantin epithelial layers of enamel organ in the underlying mesenchymal tissue making up as a thin epithelial sheath able to induce the appearance of dental hard tissue that will form the root.When the dental root reached its final length, Hertwig sheats disintegrates and the remaining epithelial elements are forming some isolated islands named Mallassez epithelial debris. This study aims to point out the most important morphological and developmental properties of Hertwig's sheath and how is directly involved in the root formation based on more or less recent data collected from literature.Together, all the studies indicate that during root formation, acts as a barrier that establishes the root shape and may mediate cementum formation, but does not secret cementum itself.Although, many studies have been made relating to the role played by this complex formation in the radicular maturation, there are still many questions related to its mechanism of action and involvement in apexogenesis and apexifications processes.*

Cuvinte cheie: *dezvoltare radiculară, teaca epitelială, resturi Mallassez*

Rezumat: *Debutul formării radiculare se produce după ce coroana dentară şi-a atins dimensiunile definitive iar rolul principal în acest proces îl joacă o formațiune epitelială numită teaca lui Hertwig după numele autorului care a descris-o în 1874.Teaca epitelială Hertwig apare ca o proliferare în țesutul mezenchimal subiacent a zonei dată de unirea epiteliilor adamantine interne şi externe din organul smalțului, constituindu-se sub forma unui manşon epitelial subțire ce are capacitatea de a induce apariția țesuturilor dentare dure ce vor forma rădăcina.Când rădăcina şi-a atins lungimea definitivă, teaca Hertwig se dezintegrează iar elementele epiteliale rămase formează nişte insule izolate, numite resturile epiteliale Mallassez. Scop.Această lucrare îşi propune să scoată în evidență cele mai importante proprietăți morfologice şi evolutive ale tecii Hertwig precum şi modul în care este implicată direct în formarea rădăcinii pe baza unor date mai mult sau mai puțin recente culese din literatura de specialitate. Concluzii. Împreună, toate studiile analizate sugerează că în timpul formării radiculare, teaca Hertwig se comportă ca o barieră care stabileşte forma radiculară şi mediază cementogeneza dar nu secretă cementul în sine.Deşi, s-au realizat numeroase studii referitoare la rolul jucat de această formațiune complexă în maturarea rădăcinii, încă există multiple semne de întrebare legate de mecanismul său de acțiune şi implicarea în procesele de apexogeneză şi apexificare.*

INTRODUCTION

After the total eruption and entry in the functional period, permanent tooth needs about three years to complete the formation and development of the root and apical closure.Start signal of developmental activity is given in the epithelial area resulting from union of inner with outer epithelial enamel layers which constitutes a thin-sleeve epithelial sheath named Hertwig's epithelial sheath (HERS).As the root grows, sheath ring becomes increasingly narrow and root gradually thins reaching that the mature tooth has only one apical hole through which vasculo-nervous package passes.Once the root has reached the final length, epithelial sheats disintegration starts and epithelial elements that persist are organized in the form of isolated island forming epithelial debris Mallassez (1).

1.Development and evolution of the Hertwig epithelial sheath

Hertwig's epithelial root sheath was not discovered in any mammalian species. Instead this epithelial structure was discovered by Oskar Hertwig in 1874 in an amphibian species.The reason behind Hertwig's early discovery in amphibians was likely related to the longevity of HERS in the amphibian dentition – mid-saggital sections of amphibian jaws reveal ample opportunities to view root sheaths at all stages of development. While in mammalian the HERS is rather a transient structure, in amphibians it is more or less a permanent one. Here the root epithelium does not fenestrate like in mammalians.

Tooth root formation starts after the crown morphogenesis is complete. After the bell stage in tooth development, the transition between crown and root is seen as the appearance of HERS whose development begins with the formation of a bilayered extension of the inner and outer dental epithelium from the cervical loop of the enamel organ.The epithelial double layer continues to grow in apical direction

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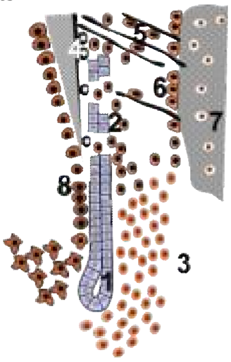
outlining the shape of the future root of the tooth (2).

Figure no. 1. Schematic representation of HERS: 1) the HERS; 2) epithelial debris Mallassez; 3) dental follicle; 4) cementoblasts; 5) periodontal ligament; 6) alveolar cells; 7) bone; 8) odontoblasts



Befor the Hertwig's appearance, between the two epithelial layers is present stellete reticulum (SR) whose disappearance may be the key events to control the timing of onset of the Hertwig's sheath formation.In this procces an important role is played by an epidermal growth factor (EGF).In the experimental culture it showed that EFG stimulated the expansion of SR residing between the inner and outer epithelial layers in HERS and inhibited the formation and growth of HERS formation and consequently root formation.In contrast, EFG- kinase, tyrphostin, resulted in the HERS formation and the transition from crown morphogenesis to root formation (3).Pattern of proliferation of HERS is genetically determined and thus it is formed according to that short or long, straight or curved roots and an apex which is close later or faster (1). Evolutionary stages in HERS evolution are : *cap, sheath and net.*Together, many studies reveal a gradual progression of HERS morphology and function from fish to human.This studies allow the distinction of three stages of HERS in vertebrates:*1*.A narrow epithelial cap laterally and apically confined by the cervical loop *2*.Continuous and elongated epithelial sheaths covering the coronal portion of the root shaft throughout the life of the tooth *3*.HERS has emerged as a transient structure evolving from a brief shaft associated with the initiation of root formation to a filigree network at later stages of root development and a subsequent collapse of the network into epithelial rests of Malassez.At this stage, there is an intimate association between the penetration of HERS' epithelial cell barrier by dental follicle derived connective tissue cells and the subsequent establishment of a periodontal ligament replacing HERS as the principle tissue occupying the root surface (2).

Hertwig's epithelial root sheath degenerates immediately after root dentin is formed.However,odontogenic tumors or cysts may originate from residual cells, although little is known about how HERS proliferates and disappears.These results may indicate that HERS cells migrate into the periodontal ligament or die immediately after root dentin is formed and that various types of cell death such as apoptosis and cytoplasmic type occur in the tissues surrounding the root during tooth development.

The role and mechanism of action of Hertwig epithelial sheath Hertwig's sheath together with Nasmyth's membrane and the odontoblasts are important parts of an epithelial mantle which everywhere protects the enamel and dentine of the teeth from the vascular tissue of the tooth follicle and pulp.In such conditions as the resorption of deciduous teeth and idiopathic resorption of the teeth ("pink spot"), this protective barrier is broken through, leading to destruction of dentine and/or enamel (4).It was Isaac Schour, who in his classic textbook wrote: "As soon as the dentin of the root begins to form, while the developing tooth is still within its bony crypt, connective tissue cells of the dental sac break through Hertwig's epithelial sheath and arrange themselves along the dentinal surface" (5).The role of HERS cells in root formation is widely accepted; however, the precise function of these cells remains controversial.Functions suggested have ranged from structural (subdivide the dental ectomesenchymal tissues into dental papilla and dental follicle), regulators of timing of root development, inducers of mesenchymal cell differentiation into odontoblasts and cementoblasts, to cementoblast cell precursors.These studies 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 (6).Acellular or fibrillary cementum cover radicular dentin and the section shows numerous perpendicular grooves to the external surface, corresponding insertion of periodontal fibers.In the absence of cells, the development of metabolic processes is ensured by a fine system of ducts with a radial arrangement.Cellular or secondary cementum is located on the outskirts of acellular cementum and is located mainly in the apical or furcation area (7). While the distance between individual HERS cells increased progressively, mesenchymal cells populated the spaces between the epithelial cells.The epithelial diaphragm as the most apical portion of HERS remained intact throughout all stages investigated.The results showed that the cell number of inner epithelial layer was greater than that of outer epithelial layer in HERS during root formation.Previous studies have reported that inner cells are square, while outer cells are rectangular (8).The difference in the numbers of cells between inner and outer may be due to cell shape.

One of the crucial questions regarding HERS is whether the cells migrate.They remained intact during root formation in our *in vitro* organ culture for 3 days.This suggests that no special growth point exists in the HERS.This study showed that both inner and outer epithelial cells do not migrate during root formation (2).

The classic theory on epithelial root sheats function relates HERS with the establishment of root shape during root formation (8).This hypothesis has found support in an extensive study on autotransplantation of premolars in which variations in root growth were linked with damage to HERS (9).In addition, there have been a number of isolated clinical studies on the later-life remnants of HERS.Using fluorescent dyes and transmission electron microscopy, we have provided experimental evidence visualizing the massive migration of dental follicle cells (10) and their perforation of HERS in support of Schour's original concept.In sites of initial cementogenesis we have also documented that dental follicle cells accessed the root surface subsequent to penetration of the HERS barrier while HERS cells remained confined through a basal lamina, indicating that dental follicle cells and not HERS cells secrete initial cementum (11).In another study the authors are adding evidence for the continuous fenestration of HERS and its collapse as rests of Malassez providing access for dental follicle/ periodontal ligament cells to attach to the root surface (2). Although Hertwig's epithelial root sheath performs an important function in the formation of the tooth root, the developmental mechanisms that control HERS growth and differentiation remain to be thoroughly elucidated. Bone morphogenetic protein 4 (BMP4), which is secreted by mesenchymal cells, acts on the dental epithelium as a regulator of cell differentiation during crown formation (5).The HERS cells's secretion are the enamel matrix proteins (12).Based on this finding, investigators have used enamel matrix proteins to regenerate the periodontium and this therapy successfully produced periodontal tissues (13). Before and during disintegration of HERS, its cells displayed the cytologic features of protein synthesis and secretion. While some cells assumed an ameloblast-like phenotype, others extended their territory away from the root surface. A collagenous matrix filled the widening intercellular spaces and tonofilaments and desmosomes were still present in cells featuring the morphologic characteristics of cementoblasts. Labeling was associate with organic matrix deposits that were sporadically and randomly distributed both along the root surface and away from it among the dissipated epithelial cells (14). It is generally accepted that cementoblasts originate in the process of differentiation of mesenchymal cells of the dental follicle.

Recently, a different hypothesis for the origin of cementoblasts has been proposed. Hertwig's epithelial root sheath cells undergo the epithelial-mesenchymal transformation to differentiate into cementoblasts.This study concludes that the epithelial-mesenchymal transformation does not occur in Hertwig's epithelial root sheath in rat acellular or cellular cementogenesis and that the dental follicle is the origin of cementoblasts, as has been proposed in the original hypothesis (15) Several hypothesis have been put forward to explain this role, ranging from just a barrier to separate dental papillae mesenchyme from periodontal ligament to being responsible of root formation and acting as inducers for cell migration, proliferation and differentiation.These results suggest that HERS cells, through their secreted products, might have and effect on cell attachment. In conclusion , the suppression of proliferation might suggest that perhaps the proteins secreted by HERS cells might induce differentiation of dental follicle cells into odontoblast cells and/or periodontal ligament cells (16). Together, our studies indicate that during root formation, HERS acts as a barrier that establishes root shape and may mediate cementum formation, but does not secrete cementum itself.The clinical data presented above confirm Heretier’s hypothesis that the absence rather than the presence of HERS epithelial cells is an essential requirement for the onset of cementogenesis (17) yet, we are not excluding the possibility of an inductive role of HERS toward the initiation of acellular cementogenesis (2). Regarding the role of Hertwig sheath in apexification, a number o authors argue that Hertwig sheath may resume activity after endodontic treatment and provide apical growth.Other authors, however, through their research undertaken showed that after the occurrence of an infectious process in periapical region there was no longer odontogenetics activity.In this case, the apical process of building is the result of a secondary calcified connective tissue proliferation and not the result of epithelial sheath activity(1)

The morphological disintegration of HERS

During the development of HERS, both cell proliferation and cell death in the HERS are important.Once the root has reached its final length HERS disintegration begins.Epithelial elements dissociates and gradually disappear leaving only some isolated epithelial islands called epithelial debris Mallassez. The morphological disintegration of HERS begins with the dissociation of the outer basement membrane of HERS.At this stage, HERS cells lose their cuboidal form and become flattened, and the outer epithelial layer breaks up before the inner one (18).Four possible mechanisms for the reduction of the number of HERS cells are generally discussed, either that HERS cells undergo apoptosis (19), that they are incorporated into the advancing cementum front (20), that they undergo epithelial-mesenchymal transformation (21) and/or that they migrate away from the root surface (18).

This studies provide strong support for Lester’s original 1969 discovery that epithelial cells of the root surface may be incorporated by cementum.Throughout the period of root elongation, HERS continues to dissociate while windows of mesenchymal cells in between cords of the ever thinning HERS network increase in size until HERS’ final collapse into epithelial rests of Malassez.

Together, these clinically-oriented studies suggest that Malassez’ epithelial rests are not only an accidental left-over of early embryonic development but rather play significant roles in:

- the regulation and maintenance of the periodontal ligament space
- the prevention of root resorption and ankylosis
- the maintenance of periodontal ligament homeostasis
- induction (not secretion) of acellular cementum formation

CONCLUSIONS

1. Together, these studies indicate that while some HERS cells may undergo apoptosis at a rate representative for developing tissues, many HERS cells remain vital and become part of the adult periodontal ligament as rests of Malassez.
2. Incorporation of HERS by the thickening cementum layer does occur,but only in the mature root; not all HERS cells or rests of Malassez were incorporated by the advancing cementum front - the majority of rests of Malassez remained in close proximity to the root surface
3. As concerne the migratory capacity of HERS cells, some authors proposed that some of them migrate away from the root surface to form the rests of Malassez.Yet, it is not clear whether HERS cells simply move away from the root surface or whether other mechanisms contribute to their reduction and displacement over time.
4. Moreover, it has been demonstrated that the number of HERS cells decreases throughout human development (22).It is therefore conceivable that only a portion of the cells that set out to form HERS remained viable and stayed within the root sheath (2)
5. HERS as the ultimate governor of the periodontal ligament, the regulator of its width and homeostasis and the shield against resorption and ankylosis. During development, HERS fenestration allows mesenchymal cells from the dental follicle to penetrate the epithelial barrier and deposit cementum a part of this function being related to the induction of acellular cementogenesis (2).It seems that although ,many studies have been done so far, epithelial root sheats still remains unclear mystery and a perennial source of scientific discoveries concerning the complex role played in the formation and development of dental root.

REFERENCES

1. Bratu Elisabeta , Glavan Florica – Practica pedodontica , Editia a III-a ,Timisoara 2005
2. Xianghong Luan, Yoshihiro Ito, and Thomas G.H. Diekwisch- Evolution and Development of Hertwig’s Epithelial Root Sheath, Dev. Dyn , 235 (5), may 2006, 1167-1180
3. Fijiwara N. , Kagiya T et al.- EFG prevents formation of HERS during developing mouse molar tooth in vitro, European Cells and Materials ,vol.14 ,suppl.2,2007,p54
4. Scott J.- Nasmyth’s membrane , Irish Journal of Medical Science, vol.26(7), 334-337, 1951
5. Schour I. Noyes- Histology and Embryology. 5. Philadelphia, Pennsylvania: Lea & Febiger; 1938. p. 157. .

6. Zeichner-David M, Oishi K, Su Z, Zakartchenko V, Chen LS, Arzate H, Bringas P Jr - Role of Hertwig's epithelial root sheath cells in tooth root development.Dev. Dyn. 2003 Dec;228(4):651-63.
7. Monea Pop Monica, Monea Al. – Caria simpla . Ed. University Press Târgu-Mures , 2006
8. Owens PD.- Ultrastructure of Hertwig's epithelial root sheath during early root development in premolar teeth in dogs. Arch Oral Biol. 1978;23:91–104.
9. Andreasen JO, Paulsen HU, Yu Z, Bayer T. A long-term study of 370 autotransplanted premolars. Part IV. Root development subsequent to transplantation. Eur J Orthod. 1990;12:38–50.
10. Diekwisch TG. Pathways and fate of migratory cells during late tooth organogenesis. Connect Tissue Res. 2002;43:245–256.
11. Diekwisch TG. The developmental biology of cementum. Int J Dev Biol. 2001;45:695–706
12. .Hammarstrom L.- The role of enamel matrix proteins in the development of cementum and periodontal tissues. Ciba Found Symp. 1997;205:246–255. discussion 255–260
13. H. Yamamoto1, S.-W. Cho1, E.-J. Kim1, J.-Y. Kim1et al.- Developmental Properties of the Hertwig's Epithelial Root Sheath in Mice , Journal of Dental Research ,September 2004 vol. 83 no. 9 688-692
14. Dieter D. Bosshardt, Antonio Nanci - Hertwig's epithelial root sheath, enamel matrix proteins, and initiation of cementogenesis in porcine teeth, Journal of Clinical Periodontology, Volume 31, Issue 3, pages 184–192, March 2004
15. Yamamoto, Tsuneyuki,Takahashi, Shigeru- Hertwig's epithelial root sheath cells do not transform into cementoblasts in rat molar cementogenesis , Annals of Anatomy 20 November 2009, Pages 547-555, 191(6)
16. Gee J. , Zakhartenko V., Bringas P., and Zeichner-David M. - Role of Hertwig's Epithelial Root Sheath (HERS) on Attachment and Proliferation of Dental Papilla Mesenchyme, Periodontal Ligament and Fibroblast Cells In Vitro , March 2002 San Diego Convention Center Exhibit
17. Heretier M.- Experimental induction of cementogenesis on the enamel of transplanted mouse tooth germs. Arch Oral Biol. 1982;27:87–97
18. Andujar MB, Magloire H, Hartmann DJ, Ville G, Grimaud JA.- Early mouse molar root development: cellular changes and distribution of fibronectin, laminin and type-IV collagen. Differentiation. 1985;30:111–122.
19. Cerri PS, Katchburian E.- Apoptosis in the epithelial cells of the rests of Malassez of the periodontium of rat molars. J Periodontal Res. 2005;40:365–372.
20. Lester KS.-The incorporation of epithelial cells by cementum.J Ultrastruct Res.1969;27:63-87.
21. Thomas HF.- Root formation. Int J Dev Biol. 1995;39:231–237
22. Yamamoto T, Domon T, Takahashi S .- Immunolocation of proteoglycans and bone - relatenoncollagenous glycoproteins in developing acellular cementum of rat molars. Cell Tissue Res. 2004;317:2