ORIGINAL RESEARCH

Hyaline Layer of Hopewell-Smith: A Morphometric and Histochemical Analysis

Nimoshini G[1], Ramya Ramadoss[2], Swarnalakshmi R[3], Vasanthi V[4], A Ramesh Kumar[5], Bose Divya[6]

Abstract

Background: Hyaline layer of Hopewell-Smith is a homogenous layer between the most external layer of dentin and the internal layer of acellular cementum. The development and functions of intermediate cementum are always controversial.

Aim: To analyze the morphological and histochemical traits of hyaline layer of Hopewell-Smith.

Materials and methods: Analysis of morphological traits were carried out in ground sections of 30 adult teeth with an age range of 20–50 years by polarized light microscopy. Histochemical traits of hyaline layer of Hopewell-Smith was done by using Periodic Acid Schiff and Alcian blue staining.

Results: Morphological traits such as thickness in different regions of root were found to show significant regional variation. Histochemical staining revealed clear demarcation between the dentin and cementum, hyaline layer proved to be a part of the cemental layer than dentin.

Conclusion: Findings such as regional variation in thickness have a remarkable impact on periodontal regeneration. Further correlates with electron microscopic studies will help newer treatment modalities for periodontal pathologies.

Keywords: Acellular cementum, Cementum, Hopewell-Smith layer, Hyaline layer, Intermediate cementum.

CODS Journal of Dentistry (2021): 10.5005/jp-journals-10063-0085

Introduction

Cementum is a thin, mineralized, avascular tissue covering the root of teeth. Cementum is the least understood hard tissue. Intermediate cementum is a narrow zone at the junction between cementum and dentin in the root of human tooth. Intermediate cementum is 0.5–0.8 µm thick and initially unmineralized which mineralizes eventually. Hopewell-Smith described a thin homogeneous layer between the Tomes granular layer and the acellular extrinsic fibre cementum. The term intermediate cementum was used by Bencze to describe a narrow region of cellular elements present between dentin and cellular mixed stratified cementum.[1–5]

The origin of intermediate cementum is controversial in the literature. Inspite of being homologous and presenting histological similarities, the structure in relation to cellular mixed stratified cementum is referred to as intermediate cementum and the structure with respect to acellular extrinsic fiber cementum is referred to as hyaline layer of Hopewell-Smith. There are two schools of thought with respect to the origin of intermediate cementum. One group concluded that the intermediate cementum is a part of dentin and reported dentinal tubule continuity present between intermediate cementum and dentin. The other school of thought is that the intermediate cementum layer contains enamel matrix proteins and originates from Hertwig's Epithelial Root Sheath (HERS). Intermediate cementum or hyaline layer of Hopewell-Smith is gaining interest nowadays as it contributes to periodontal regeneration.[5] The present study intends to analyze the morphometric and histochemical traits of hyaline layer of Hopewell-Smith to emphasize on the origin by using special stains.

Materials and Methods

The study was approved by Institutional Review Board (SRMU/M&HS/SRMDC/2019/PG/015). 30 extracted teeth maxillary molars, mandibular molars, maxillary premolars, and mandibular premolars with type II cementoenamel junction (CEJ) of adults with an age range of 20–50 years were included in the study. Teeth with dental caries, fracture or any enamel defects were excluded from the study. The extracted teeth were formalin fixed and ground sections of 30 µm thickness were made manually. The ground sectioned teeth were mounted on glass slides. Analysis of morphological traits was carried out by examining the ground sections under polarized light microscopy. Photographs taken in the middle third of the tooth were selected for morphometric analysis using Image J software version 1.8. Morphometric analysis was done from type II cementoenamel junction to 100 µm on the cementum and from type II cementoenamel junction to 1000 µm on the cementum of the ground sections.

Following morphometry, histochemical traits were analyzed using Periodic Acid Schiff (PAS) and Alcian Blue staining. For PAS staining, 15 ground sections were stained with 0.1% toluidine blue for 3 minutes, rinsed in distilled water for 5 minutes and dehydrated in series of alcohol. Sections were then cleaved in xylene, dried and mounted in DPX mount. The remaining 15 ground sections were placed in 1% Alcian blue in 3% acetic acid solution for 30 minutes. The sections were

[1,3,4-6]Department of Oral Pathology and Microbiology, SRM Dental College, Chennai, Tamil Nadu, India

[2]Department of Oral Pathology and Microbiology, Saveetha Dental College, Chennai, Tamil Nadu, India

Corresponding Author: Nimoshini G, Department of Oral Pathology and Microbiology, SRM Dental College, Chennai, Tamil Nadu, India Phone: +91 9677954910, e-mail: nimoshini94gowrinathan@gmail.com

How to cite this article: Nimoshini G, Ramadoss R, Swarnalakshmi R, *et al.* Hyaline Layer of Hopewell-Smith: A Morphometric and Histochemical Analysis. CODS J Dent 2021;13(1):3–5.

Source of support: Nil

Conflict of interest: None

gently rinsed in tap water for 10 minutes. They were counter stained in 0.1% nuclear fast red for 5 minutes, rinsed in tap water for 15 dips, cleared in xylene, air dried and mounted in DPX mount.

The distance from CEJ to 100 µm and from CEJ to 1000 µm in molars and premolars was measured using Image J software version 1.8 (Fig. 1). Stained sections (PAS, Alcian blue) were examined under light microscope and polarized microscope for difference in staining intensity between dentin and cementum.

The data obtained were statistically analyzed using SPSS software (Version 22, Chicago, IL, USA). The morphometric comparison was made using Student's *t*-test. The difference in the mean scores of the molar and premolar between the distance from CEJ to 100 µm and the distance from the CEJ to 1000 µm distance was done using inferential statistics and paired *t*-test accordingly. *P* value ≤ 0.05 was considered statistically significant.

Results

The mean of distance from CEJ to 100 µm in molars and premolars were 27.54 ± 232.11 and 30.1 ± 13.01, respectively. The difference was not statistically significant ($p = 0.64$). The mean distance from CEJ to 1000 µm in molars and premolars were 30.16 ± 13.18 and 33.04±20.07, respectively. The difference was not statistically significant ($p = 0.62$). Though not significant, morphological traits such as thickness in different regions of root were found to show significant regional variation (Table 1).

Periodic acid Schiff stain showed intense pink staining of intermediate cementum. Dentin and the lacunae in the cellular cementum showed pale pink staining (Fig. 2). Alcian blue stain showed pink staining of intermediate cementum and cementum. There was adequate contrast between the cementum and dentin in all the two stains. There was significant demarcation in the staining intensity between cementum and dentin. Histochemical staining revealed clear demarcation between the dentin and cementum, hyaline layer proved to be a part of the cemental layer than dentin. The intermediate cementum was better observed in Periodic Acid Schiff than the Alcian blue staining.

Discussion

Hertwig's epithelial root sheath (HERS), the double layered epithelial tube is the core component in the formation of root. During odontogenesis, once the crown formation is complete, HERS induces the differentiation of odontoblasts from the dental papilla. Once root dentin is formed, the enamel protein matrix is secreted by HERS, termed the hyaline layer of Hopewell-Smith. Following the deposition of a layer of mantle dentin, HERS undergoes disintegration to allow the cells of the dental follicle to migrate and get attached to the hyaline layer of Hopewell-Smith and gets differentiated into cementoblasts and fibroblasts. Recent studies report that HERS can be considered in regenerative medicine as a stem cell in periodontal regeneration. Emdogain, enamel matrix protein secreted by HERS also plays a role in periodontal regeneration.[6] The remnants of HERS in periodontal ligament, namely the epithelial cell rests of Malassez is being extensively studied in terms of periodontal regeneration as it possesses stem cell properties.[7]

Hyaline layer of Hopewell-Smith or intermediate cementum is less studied, least understood hard tissue of the tooth. There are very few studies that have studied the cemental ground sections. The present study was to attempt with light and polarizing microscopic features of intermediate cementum using special stains and correlate with origin and composition.

Histological stains bind to tissues and the tissues interacts with the staining reagent. From these binding and interactions, the staining of tissues differs based on affinity. Slavkin et al.[8] Lindskog et al.[9,10] and Nalbandian et al.[11] proposed that even before development of root, deposition of enamel protein matrix occurs. Craig et al.[12] proposed that matrix is deposited ahead of cementogenesis. Lindskog et al reported that epithelial root sheath cells undergoes morphological changes which proves that HERS contributes to the intermediate cementum formation.[9,10,13,14]

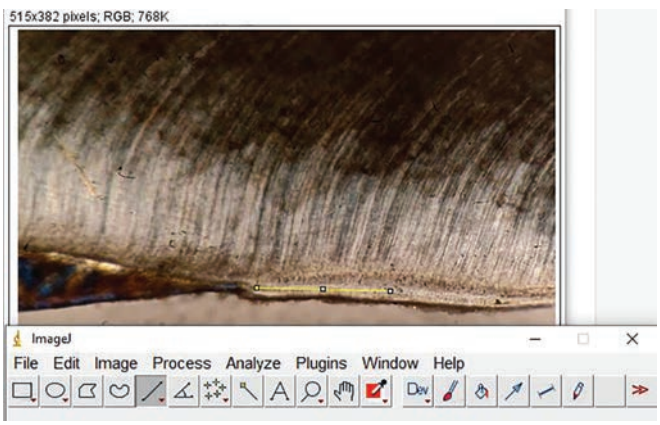

Fig. 1: Morphometric analysis from CEJ using ImageJ

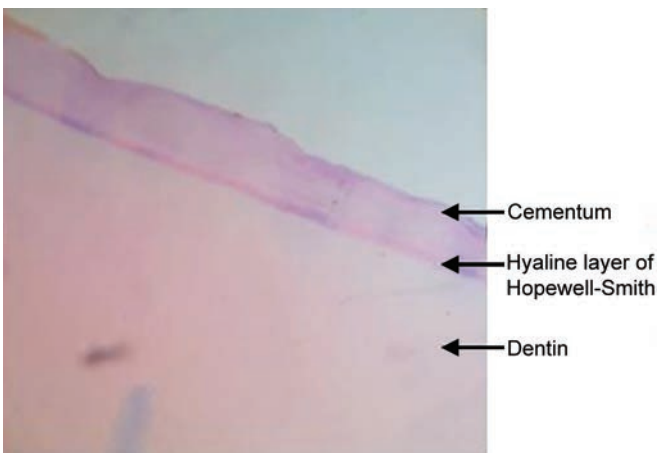

Fig. 2: PAS staining of hyaline layer of Hopewell-Smith

Table 1: Comparison of distance from CEJ in molars and premolars

	CEJ to 100 µm			*CEJ to 1000 µm*		
	Mean	*SD*	*p value*	*Mean*	*SD*	*p value*
Molar	27.54	232.11	0.64	30.16	13.18	0.62
Premolar	30.1	13.01		33.04	20.07	

*p value <0 .05 is considered significant; CEJ: cementoenamel junction

As the reparative role of dentin is more or less dependent on pulp, similarly the reparative action of cementum is also dependent on the periodontal ligament. Reparative cementum is formed on mechanically denuded surfaces of root during procedures such as scaling or curettage.[15,15] Cherian also documented that the tissue between root dentin and cementum is hypermineralised similar to enamel and can be separated as a sock.[17] Emdogain®, a commercially available enamel matrix derivative protein is being reported to stimulate cementum reproduction in periodontal disease which may be attributed to the fact that enamel matrix-like proteins are present within the hyaline layer.[18]

Conclusion

This fact represents that these findings such as regional variation in thickness has a remarkable impact on periodontal regeneration. Further correlates with electron microscopic studies will throw more light on its clinical relevance in terms of periodontal pathology and regeneration.

Orcid

Ramya R http://orcid.org/0000-0001-9408-3158

References

1. Gonclaves PF, Sallum EA, Sallum AW, et al. Dental cementum reviewed: development, structure, composition, regeneration and potential functions. Braz J Oral Sci 2005;4:651–658. DOI: 10.20396/bjos.v4i12.8641790
2. Consolaro A, Renata B, Consolaro LA, et al. Methods for studying tooth root cementum by light microscopy. Int J Oral Sci 2012;4:119–128. DOI: 10.1038/ijos.2012.57
3. Bosshardt DD, Selvig KA. Dental cementum: the dynamic tissue covering of the root. Periodontol 2000 1997;13:41–75. DOI: 10.1111/j.1600-0757.1997.tb00095.x
4. Shukla D, Vinuth DP, Soumya S, et al Cemetum made more visual. J Forensic Odontostomatol 2012;30:29–37.
5. Yamamoto T, Hasegawa T, Yamamoto T, et al. Histology of human cementum: its structure, function, and development. Jpn Dent Sci Rev 2016;52:63–74. DOI: 10.1016/j.jdsr.2016.04.002
6. 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;63:217–233. DOI: 10.1111/prd.12023
7. Sucheta A, Spandana A, Sapna N, et al. Hertwig's epithelial root sheath: a panaromic view. Int J Appl Dent Sci 2017;3:21–25
8. Slavkin HC, Boyde A. Cementum: an epithelial secretory product? J Dent Res 1974;53:157.
9. Lindskog S. Formation of intermediate cementum. I: early mineralization of aprismatic enamel and intermediate cementum in monkey. J Craniofac Genet Dev Biol 1982;2:147–160.
10. Lindskog S. Formation of intermediate cementum. II: a scanning electron microscopic study of the epithelial root sheath of Hertwig in monkey. J Craniofac Genet Dev Biol 1982;2:161–169.
11. Nalbandian J, Frank RM. Electron microscopic study of the regeneration of cementum and periodontal connective tissue attachment in the cat. J Periodontal Res 1980;15:71–89. DOI: 10.1111/j.1600-0765.1980.tb00261.x
12. Craig KR, Harrison JW. Wound healing following demineralization of resected root ends in periradicular surgery. J Endod 1993;19:339–347. DOI: 10.1016/S0099-2399(06)81360-9
13. Boyde A, Jones SJ. Scanning electron microscopy of cementum and Sharpey fibre bone. Z Zellforsch Mikrosk Anat 1968;92:536–548. DOI: 10.1007/BF00336664
14. Morris ML. The reattachment of human periodontal tissues following surgical detachment: a clinical and histological study. J Periodontol 1953;24:220–228. DOI: 10.1902/jop.1953.24.4.220
15. Lindskog S, Blomlof L, Hammarstrom L. Repair of periodontal tissues in vivo and in vitro. J Clin Periodontol 1983;10:188–205. DOI: 10.1111/j.1600-051x.1983.tb02207.x
16. Listgarten MA, Rosenberg MM. Histological comparison of periodontal wound healing in the beagle dog with and without citric acid conditioning. J Periodontal Res 1979;50:330–344.
17. Cherian G. Harvesting cementum from root surface: a new paradigm in the study of cementum and the cemento-dentinal junction. J Adv Oral Res 2011;2(2):17–20. DOI: 10.1177/2229411220110203
18. Esposito M, Grusovin MG, Papanikolaou N, et al. Enamel matrix derivative (Emdogain®) for periodontal tissue regeneration in intrabony defects. Aust Dent J 2010;55(1):101–104. DOI: 10.1111/j.1834-7819.2009.01186.x