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Morphological and structural analysis of cementum of teeth extracted due to pathological and non-pathological conditions: SEM and EDS Analysis

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ABSTRACT

Introduction: Cementum plays a vital role in periodontal attachment and tooth stability. Pathological conditions such as periodontitis can alter its structural and mineral composition, leading to compromised periodontal integrity. This study aimed to evaluate the morphological and compositional differences in cementum of teeth extracted due to pathological and non-pathological conditions.

Materials and Methods: A total of four extracted human teeth were included, comprising two pathological and two non-pathological samples. Specimens were cleaned, prepared, and analyzed using Field Emission Scanning Electron Microscopy (FE-SEM) for morphological assessment and Energy Dispersive X-ray Spectroscopy (EDS) for elemental analysis. Fourier Transform Infrared Spectroscopy (FT-IR) was used to assess functional group changes. Statistical analysis was performed using independent t-tests.

Results: FT-IR analysis revealed reduced phosphate peak intensity in pathological cementum, indicating decreased mineral content, along with increased amide peaks suggesting collagen exposure. SEM analysis showed regular globular morphology in normal cementum, whereas pathological cementum exhibited irregular surfaces with resorption areas. EDS analysis demonstrated significantly lower calcium and phosphate levels and higher carbon content in pathological cementum ($p < 0.001$).

Conclusion: Pathological conditions significantly alter the structural and mineral composition of cementum. These findings emphasize the importance of understanding cementum biology for improving periodontal regenerative therapies.

Key Words: *Cementum, SEM, EDS analysis, Periodontitis, Mineral composition, Tooth morphology, Periodontal regeneration, FT-IR analysis*

INTRODUCTION

Last few decades, there has been an increase in dental pathology resulting in tooth loss leading to poor oral health related quality of life1. Further, young people are losing teeth at an early age, making them edentulous in early life. Primary/Secondary periodontitis is a common oral disease responsible for loss of teeth. The disease process induces the changes in cementum over the period due to local factors such as inflammation, microorganisms etc. In periodontitis, there is inflammation of periodontal tissue causing demineralization as well as loss of cemental structure, often resulting in loss of teeth attachment with alveolus.

Regenerative periodontal therapy is one of the treatments for periodontitis in early stages. Regenerative periodontal therapy comprises techniques which are particularly designed to restore those parts of the tooth-supporting structures which have been lost due to periodontitis 1,2. The outcome of periodontal regenerative therapy depends upon several factors such as oral hygiene, disease progression, and operator skill. Bone grafting is a commonly accepted treatment for bone loss due to periodontitis.

Recently several biocompatible bone grafting materials were developed.[3] A new calcium sulfate-based bioactive bone cement (BioCaS) has been found to induce bone formation from periodontal stem cells. Tricalcium phosphate (TCP) is another bone substitute that has been growing in recent years [4]. It induces the cell differentiation into osteoblast. However, these have limited success. There could be several reasons including the surrounding in vivo environment to bone graft materials. Most materials have focused on bone regeneration to fill the defect and tooth attachment to alveolar bone. However, cementum role in attachment has been neglected for unknown reasons.

Cementum is an important part of periodontal structure and responsible for attachment of teeth. Periodontitis or inflammation can induce the demineralization of cementum leading to loss of periodontal fibers attachments. Zahraa M.N. et al observed the difference in cementum structure as well as composition in gingivitis and periodontitis. This clearly suggests disease processes can change composition and can have an impact on periodontal treatment outcome. Further, limited success of bone grafting material success could be due to inadequate understanding of cementum structural loss as well as mineral structure.

The better understanding of change in cementum composition and structure characteristics features at physiology and pathology could help in developing better periodontal reattachment therapy. Considering this need, it was intended to conduct a study to evaluate the morphological changes in cementum of tooth loss due to pathology and compared it with normal cementum. Morphological investigations clearly revealed the change of hydroxyapatite mineral orientation in pathological conditions. In addition, we carried out element analysis to find changes in mineral composition in cementum in these teeth. Functional group assessment by FT-IR explicates the deficiency of mineral components in the pathological tooth.

Aims of the present study

To assess mineral composition and structural changes in cementum of teeth extracted due to pathological and non-pathological conditions.

P: Extracted teeth

I: SEM and EDS analysis

C: Pathological and non-pathology

O: Mineral composition

MATERIALS AND METHOD

Teeth Collection

A true experimental study, carried out among extracted human teeth, which were collected from patients, attended Dental Hospital during the time period June 2022. The total number of teeth was 4; they were indicated for extraction according to the treatment plan made by one of the consultants in the Periodontology Department. Extraction was done by one of the dentists in the Department of Oral and Maxillofacial Surgery. Two freshly lost/extracted/removed teeth due to either mobility or pathology were collected. Similarly, 2 teeth extracted electively for orthodontic treatment were collected. All teeth were cleaned with hydrogen peroxide to remove debris. Those patients having systemic diseases that might Have affected the thickness of cementum, parafunctional habits, localized periapical pathology, pulpitis, hypercementosis or root resorption, trauma from occlusion were excluded.

Specimen preparation

The teeth were cleaned with 10 % hydrogen peroxide by sonication using an ultra bath sonicator for 2 minutes. Further, these teeth were rinsed with double distilled water and dried with the help of a hot air oven for further examination. For FE-SEM (Field Emission Scanning Electron Microscopy [JEOL -JSM – IT 800]) analysis teeth were coated with platinum for 30 sec to induce the conductivity on the sample surface before transferring it to the FE-SEM chamber of assessment. FE-SEM scans were taken from all the prepared samples. It is essential to mention that FE-SEM images were also taken from a similar pair (incisor and canine). The Energy Dispersing X-ray (EDX) analysis (Inca Energy software, Oxford Instruments Ltd.) was employed to determine whether there are mineral component compositional changes between cementum from pathologically and non-pathological affected teeth. EDX analysis was performed on the same sections employed for FE-SEM imaging. X-ray spectra were collected from the rectangular areas of the cementum. The composition of cementum between pathologically affected teeth and normal teeth were compared using student independent t-tests to determine the difference in cementum composition. Diseased teeth are denoted at DT and normal tooth is denoted at NT.

The study was approved by the Research ethics committee of Saveetha Dental College and Hospitals, Chennai

RESULTS

FT-IR Spectra

Phosphate vibrations indicate the presence of hydroxyapatite; here it was intended to analyze the alterations in the bondings of PO_4^{3-} in natural as well as in pathological tooth cementum. Sharp stretching was observed in the range around 550 cm^{-1}-600 cm^{-1} in both the FT-IR spectra. Distinct peaks were noted in the normal tooth cementum rather than the pathological tooth cementum that resembles the deficient mineral component in the pathological tooth cementum. The peak centered at around 1000 cm-1 indicates the phosphate vibration and intensity of this peak was drastically reduced in pathological tooth cementum, which indicates the decrease in mineral content in the pathological tooth cementum. Amide groups were observed in the regions of 1722 cm-1, 2350 cm-1, 2950 cm-1 and these vibrations are more prominent in pathological tooth cementum than the normal teeth cementum that explicates exposure of collagen mesh rather than the hard mineral component on the tooth surface. Overall, these results shows that the mineral hydroxyapatite component is drastically reduced in the pathological tooth cementum may be due to the microbial exposure.

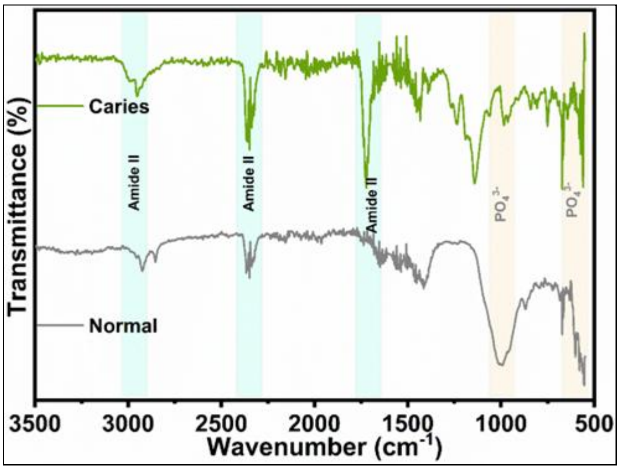


Fig. 2. FT-IR spectra of natural tooth and the caries tooth.

FE-SEM and EDX analysis of Pathological and Normal tooth cementum surfaces

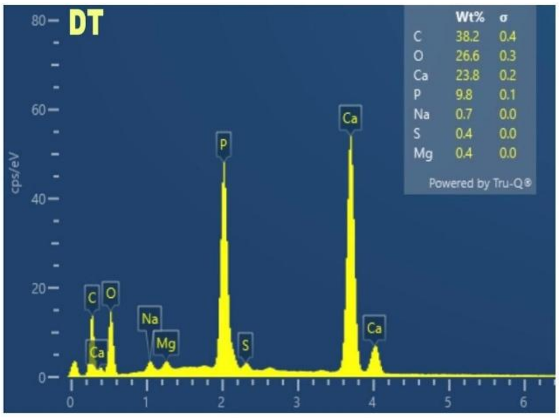
The FE-SEM analysis of the cemental surface of normal teeth showed a regular, globular pattern without any evidence of fracture or loss of tooth structure. The cementum surface observed was indicative of resting stage or slow rate of activity. The sharpes fiber in the mineralized stage were seen as mounds or surface projects in regular patterns which were indicative of normal remodeling activity. In pathological teeth cementum, the surface showed irregular surface along with uneven surface mound/ projection indicative of remodeling activity in the form of resorption as well as loss of cemental surface (resorption bay) at few places. This is supported by the elemental analysis which shows higher amounts of calcium and phosphate in normal teeth cementum as compared to pathological tooth cementum ($p<0.001$) (Table 1). Further, carbon content was more in pathological teeth cementum compared to normal teeth cementum indicative of more organic content such as collagen fibers ($p<0.001$) (Table 1).

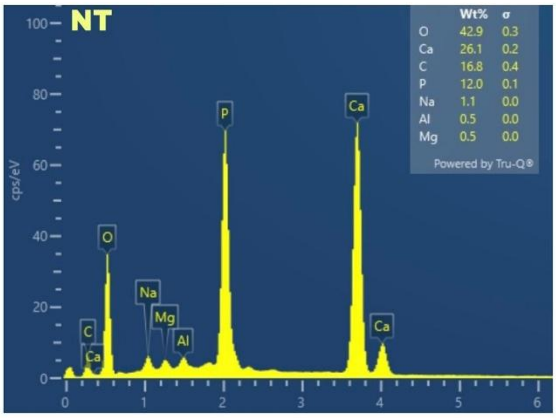
Table 1: Comparison of Cementum composition between pathological teeth and normal teeth

	Groups	No. of site	Mean	SD
Phosphate	Normal Teeth	5	12.0	0.0707
	Pathological Teeth	5	9.78	0.0837
Carbon	Normal Teeth	5	16.8	0.0548
	Pathological Teeth	5	38.12	0.0837
Calcium	Normal Teeth	5	26.1	0.0548
	Pathological Teeth	5	23.82	0.0837

Figure 1: Percentage of elements present in pathological teeth (A) and normal (B) cementum analyzed with help of energy dispersive X-ray spectrometry. There was a difference in calcium, phosphate, and carbon content in pathological teeth cementum as compared to normal teeth cementum.

Analysis of the energy dispersive X-ray spectrometry photomicrographs showed a difference in the content of calcium and phosphorus. The diseased cementum had relatively less calcium (M = 9.78, SD = 0.08) and phosphorus (M = 23.82, SD = 0.08) whereas higher carbon (M = 38.12, SD = 0.08) as compared to normal cementum.





DISCUSSION

Cementum is an important structure for tooth support. periodontitis and periapical disease cause the loss of cementum resulting in tooth mobility. Loss of periodontal ligament attachment is one of the reasons for mobility. Periodontal regenerative therapies intended to achieve reattachment of periodontal fibers. However, most of these therapies focused on attachment of fibers on alveolar bone side neglecting the cementum which is an important component of periodontium. Furthermore, for better periodontal fiber attachment with cementum, it is necessary to understand the structural changes in cementum in pathological conditions.

The present study analyzed the morphology of cementum of normal teeth and pathological teeth using SEM. Normally, cementum undergoes remolling which is visualized in the form of uneven but regular cemental surface. In addition, normal cementum surface shows denser and conspicuous collagen fibers with their interfibrillar matrix [5] (Bilgin E et al.). Similarly, Jones S J et al reported the presence of extrinsic fibers almost 100 % of the surface in acellular cement [6]. However, pathology affected cementum showed irregular surface with loss of cementual structure as compared to normal cementum which is evident on SEM Backscattered Electron (BSE) micrographs. These irregular surfaces are due to irregular remodeling activity leading to uneven and excessive loss of surface structure and minerals similar to observations present in our study. Also, there is a decrease in cementum thickness. These changes may be the result of pathological processes such as inflammation and local factors such as morphology, topography, and involvement of bacterial metabolites. Further, these factors can induce the structural changes in the collagen matrix in the resorption front in diseased/pathological cementum. The impact of these morphological changes in remineralization need to be explored to improve the outcome of regenerative techniques.

The cementum recovery depends upon several factors such as removal of etiology, inflammation, and bacteria. However, the most important factor is formation of cementum by cementoblast as well as mineralization of cementoid. Usually, periodontal treatment can remove etiology, reduce inflammation. Further, increase the formation of periodontal fibers which get embedded in cementum [7]. Although current traditional as well as new regenerative applications are able to generate the periodontal ligaments, they are not able to mimic the original morphology. One of the reasons could be improper mineralization of new cementoid material. This could be due to mechanisms involved in demineralization of cementum as well as mineral content and their structural changes. During the pathological process, exposure, interaction of inflammatory markers, and bacterial metabolites may impact on cementum structure as well as remineralization. However, there is no sufficient evidence on this aspect. Upon the comparison of mineral composition between normal tooth cementum and pathology associated tooth cementum, we found the decrease levels of phosphate and calcium in pathology associated tooth cementum compared to normal tooth cementum. Similar findings were reported by Soliman Amro et al who found a significant decrease in the calcium and phosphate contents in diseased tooth cementum in comparison to the normal tooth cementum. In contrast, Attila et al found elevated Ca and P content in diseased cementum surfaces [8]. This could be justified by the small size of the present study as well as racial difference. Scanning electron microscopy and EDS can help in understanding the microscopic changes in diseased cementum.

CONCLUSION

This study provides a detailed FE-SEM analysis of the microscopic anatomy and elemental analysis of normal and diseased tooth cementum. The study clearly indicated the changes in mineral composition of cementum is affected due to disease process. Considering the difference in mineral composition, clinicians need to adapt new methodology to achieve the reattachment of periodontal ligament. Further, future studies should focus on understanding structural change in cementum due to change in Ca and P content and how it may impact the reattachment of periodontal fibers to cementum

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