Histology of Nonfluorosed and Fluorosed Dental Cementum - an Invitro Study

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Abstract

Background & Objectives: *The literature on effect of fluoride on dental caries is well discussed in contrast to periodontal tissues. However, a recent review has explored an epidemiological association between fluorosis and periodontal disease, but also the influence of fluorosis on periodontal structures along with the comparison of influence of periodontal treatment on fluorosed and non fluorosed teeth. During progression of periodontitis, there is a possibility of microhardness, mineral and histologic changes in cementum. Considering the higher incidence of periodontitis in endemic fluorosed area around Davangere, there is an opportunity to study the cemental changes due to fluorosis which would influence the initiation and progression of periodontal disease. Hence the aim was to study the histology of fluorosed and nonfluorosed cementum.*

Materials and Methods: *A total of 24 healthy nonfluorosed and fluorosed orthodontically extracted premolars were collected to assess and compare the histology of fluorosed versus non fluorosed cementum using light microscope.*

Results and Conclusion: *The results of the study showed that the thickness of acellular cementum in nonfluorosed teeth (23.88±11.77 microns) was found to be more than in fluorosed teeth (17.69 ±8.98 microns) but was statistically non-significant. Histologically, density of cells in cellular cementum of nonfluorosed teeth (4.36±1.27) was found to be statistically highly significant than in fluorosed teeth (1.60±1.01).*

Keywords: Dental fluorosis, periodontitis, dental caries, histology, dental cementum.

Introduction

Fluorine is a common element in the earth's crust and is an essential element for the calcification of bones and teeth. Fluoride ion has played a major role in dramatically reducing dental caries over past 40 years. Excessive systemic exposure to fluoride can lead to disturbances of bone homeostasis, enamel development [dental/ enamel fluorosis] and mineralization. The severity of fluorosis on periodontal hard and soft tissues is dose dependent and also depends on timing and duration of fluoride exposure during development [1].

The literature on fluoride and dental caries is well discussed in contrast to periodontal tissues. However, a recent review by Vandana K L has explored an epidemiological association between fluorosis and periodontal disease, and also the influence of fluorosis on periodontal structures along with the comparison of influence of periodontal treatment on fluorosed and non fluorosed teeth. There is a scarcity in literature dealing with effect of fluorosis on biological tissues like bone and cementum [2].

The tooth root cementum is a thin, mineralized tissue covering the root dentin that is present primarily as acellular cementum on the cervical root and cellular cementum covering the apical root. While cementum shares many properties in common with bone and dentin, it is a unique mineralized tissue and acellular cementum is critical for attachment of the tooth to the surrounding periodontal ligament (PDL). Cementum is likely the least understood of the mineralized tissues of the skeleton and dentition [3].

During progression of periodontitis, there is a possibility of mechanical, physical and chemical changes in cementum. Considering the higher incidence of periodontitis in endemic fluorosed area around Davangere, there is an opportunity to study the cemental changes due to fluorosis which would influence the initiation and progression of periodontal disease.

The possible histologic properties which may be different in fluorosed cementum would influence the pathogenesis of periodontal disease and /or outcome of periodontal treatment.

Hence, the comparison of fluorosed versus nonfluorosed cementum is a new area of interest in fluorosis research. Medline search using keywords fluorosed and nonfluorosed cementum does not reveal much data. So, present study aims to find out changes in histologic properties of non fluorosed versus fluorosed cementum.

Materials and Methods

A total of 24 healthy non fluorosed and fluorosed orthodontically extracted premolars were obtained from Department of Oral and Maxillofacial Surgery, College of dental sciences, Davangere. Subjects with age group of 18 to 25 years of both the sexes were included. Written consent was taken from all subjects and ethical clearance was obtained from the Institutional Review Board (IRB; Ref No. CODS/ 2184) of College of Dental Sciences, Davangere, Karnataka according to Rajiv Gandhi University of Health Sciences, Karnataka protocols.

The extracted teeth were required to meet the following inclusion criteria: fully erupted, extracted non-traumatically due to orthodontic reasons, no history of recent periodontal instrumentation or dental prophylaxis, for fluorosed teeth; the fluorotic enamel stains was confirmed by the clinical examination and history of the subjects hailing from natural high water fluoride areas in and around Davangere (fluoride concentration >1.5 ppm). The exclusion criteria were: teeth with proximal caries extending to the cementum, fillings extending beyond cementoenamel junction (CEJ), and intrinsic stains caused by other reasons such as porphyria, erythroblastosis fetalis, tetracycline therapy, etc. Sample size was 11.72 using $n= z^2 \sigma / (x1 - x2)^2$

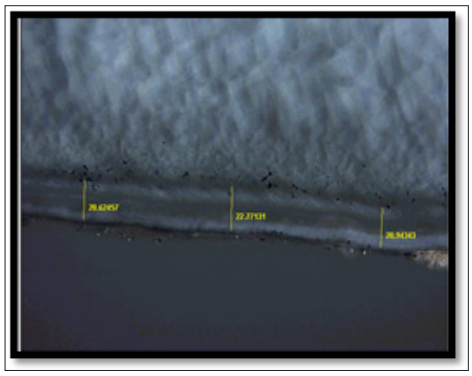

Figure 1a : Acellular cementum thickness of nonfluorosed cementum

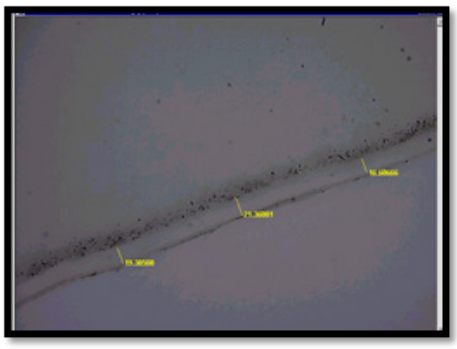

Figure 1b : Acellular cementum thickness of fluorosed cementum

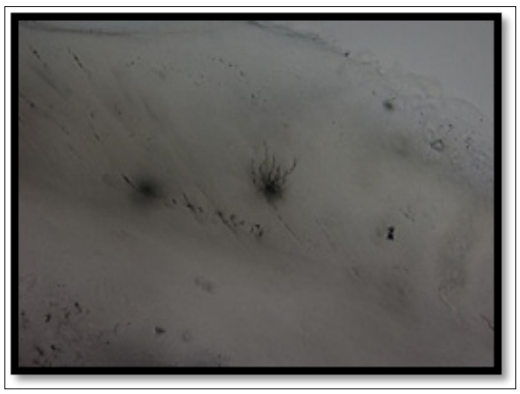

Figure 2a : Cellular Density of fluorosed Cementum

Figure 2b : Cellular density of nonfluorosed cementum

Procedural steps

Collection of teeth specimens

Healthy nonfluorosed and fluorosed teeth were collected and were immediately washed in running tap water and stored in bottles containing 0.9% saline [4].

Embedding and sectioning of teeth

Each tooth was embedded in the acrylic blocks and processed for hard tissue microtome [5]. Mesio-distal sections about 100 µm thick were cut parallel to the longitudinal axis of each tooth [6]. The sections were allowed to air dry at room temperature. When thoroughly dried, sections were mounted on the glass slide, a cover glass was applied using DPX as mounting media and examined unstained under the light microscope with 20X and 40X magnification [7].

Parameters assessed were as follows

- Thickness of acellular cementum – was measured at middle third of cementum at 3 points and taking average using image pro analyser under 20X magnification. (fig.1a, 1b)
- Number of cells in cellular cementum –cells were measured under 40X magnification taking the average of cells present in 5 fields. (2a,2b)
- Presence or absence of cellular cementum –was observed under 40X magnification.

Statistical analysis

The data obtained from the histological assessment was subjected for statistical analysis. Data was compiled on MS-excel sheet. Mean and standard deviation (SD) of fluorosed and nonfluorosed teeth (cementum) was analyzed using SPSS 17.0.P value < 0.05 was considered to be statically significant. NS (p>0.05) = not significant; HS(p<0.01) = Highly significant

Results

Thickness of acellular cementum	Mean ± S.D	P value*(μ)
Nonfluorosed	23.88 ± 11.77	p = 0.162 (NS)
Fluorosed	17.69 ± 8.98	t = - 1.44

* p value calculated using unpaired t test (NS) = non-significant

Table 1: Thickness of acellular cementum in nonfluorosed and fluorosed teeth

Density of cells in cellular cementum	Mean ± S.D	P value*
Nonfluorosed	4.36 ± 1.27	p = 0.000 (HS)
Fluorosed	1.6 ± 1.01	t = - 5.86

* p value calculated using unpaired t test (HS) = Highly significant

Table 2: Density of cells in cellular cementum in nonfluorosed and fluorosed teeth

A total of 24 healthy nonfluorosed and fluorosed orthodontically extracted premolars were collected to assess and compare the histology of fluorosed versus non fluorosed cementum using light microscope. The results of the study are interpreted in table 1 and 2

Thickness of acellular cementum in nonfluorosed and fluorosed teeth

In the nonfluorosed premolars of 18 to 25 years, acellular cementum thickness was higher

(23.88±11.77 microns) as compared to fluorosed teeth (17.69 ±8.98 microns).

Density of cells in cellular cementum in nonfluorosed and fluorosed teeth

In the nonfluorosed teeth, density of cells in cellular cementum was significantly higher (4.36±1.27, p= 0.000) as compared to fluorosed teeth (1.60±1.01).

Discussion

The tooth and its supporting tissues are unique by virtue of being home to four distinct mineralized tissues, the enamel, dentin, tooth root cementum and alveolar bone, and their associated hard–hard and hard–soft tissue interfaces. The tooth root cementum is a thin, mineralized tissue covering the root dentin surface. Cementum is primarily present in two varieties, the acellular extrinsic fiber cementum (AEFC, acellular or primary cementum) and cellular intrinsic fiber cementum (CIFC, cellular or secondary cementum), though mixed stratified cementum exhibits layers of both types in some species [8-10]. Acellular cementum covering the cervical portion of the root is critical for tooth attachment to the adjacent periodontal ligament (PDL), while cellular cementum covering the apical root is hypothesized to play a role in post-eruptive tooth movement and adaptation to occlusion [10].

In our study 24 teeth were collected according to sample size determination. However, sample size varied from [11-17]. The following paragraphs relates to the different methodologies used by different authors in conducting their study which is compared with our study.

Various sectioning techniques such as longitudinal sections [18,19], cross sections [12,20] with corresponding thickness ranging from 200 microns [14] to 2mm [12] have been described by various authors. In our study, each tooth was embedded in the acrylic blocks and processed for hard tissue microtome [5]. Mesio-distal sections about 100 μm thick were cut parallel to the longitudinal axis of each tooth [6].

In the present study, light microscope was used for the histologic assessment. The research pertaining to this has evolved from rabbits and rodents which has few drawbacks. Few studies only contribute to fluorosis related projects. Imaging methods that may be of use for examining this tissue include light microscopy, scanning electron microscopy [2,12], and transmission electron microscopy, as well as other approaches such as micro-computerized tomography (microCT), that may be limited by resolution and animal model used (i.e., size of cementum and ability to detect it) [3].

In our study, parameters assessed were as follows thickness of acellular cementum, number of cells in cellular cementum, presence or absence of cellular cementum.

The results of current study are discussed here. Thickness of acellular cementum in nonfluorosed and fluorosed teeth: In the nonfluorosed premolars of 18 to 25 years, acellular cementum thickness was higher (23.88±11.77 microns) as compared to fluorosed teeth (17.69 ±8.98 microns). As per the authors knowledge this study presents the comparative report on acellular cementum thickness in nonfluorosed and fluorosed cementum for the first time in literature.

Zander and Hurzeler in 1958 studied the thickness of cementum and showed that thickness varied directly with the age of the tooth in a straight-line relationship, having an average thickness of 0.1 mm at age 20 and increasing to 0.2 mm at age 55[21]. Ratiola CA, Craig RG in 1961 conducted a study on freshly extracted 18 human teeth to evaluate the microhardness of cementum of normal teeth and teeth exposed to periodontal disease. It was reported that cementum thickness ranged from 5 to 150 microns. Most of the cementum was from 15 to 30 microns thick except for the extremely thin cervical area and the thicker apical area.

Nakagaki et al in 1988 conducted a study on 30 human canines from 10 subjects of age range 40 to 66 years to determine the histological structure of human cementum using haematoxylin staining method. It was observed that each subject had his or

her own individual pattern of cemental structure, as well as fluoride distribution. Although the width of the cementum varied from place to place in the same tooth and from one tooth to another in the same subject, the proportion of cellular to acellular cementum in the teeth of any one individual was almost constant. Acellular cementum is the first to be formed and covers approximately the cervical third or half of the root; it does not contain cells. This cementum is formed before the tooth reaches the occlusal plane, and its thickness ranges from 30 to 230 µm [22]. Nakagaki et al in 1988 conducted a study to assess fluoride distribution and histological structure of human cementum. Parameters assessed were, pattern of histologic structure, relationship between fluoride concentration and density of cementocytes, acellular and cellular cementum and the distribution of fluoride [16].

The cemental thickness assessment is clinically important as the width (thickness) tends to influence the fluoride concentration as suggested by Yoon et al in 1960 who reported that fluoride concentration is higher in outer cementum layer than any of other mineralized tissue and because of its thin cementum it tends to have higher fluoride concentration. Although the width of the cementum varied from place to place in the same tooth and from one tooth to another in the same subject, the proportion of cellular to acellular cementum in the teeth of any one individual was almost constant. Thus, it appeared that each subject had his or her own individual pattern of cemental structure, as well as Fluoride distribution [16]. The fluoride is known to interfere with mineralization in terms of causing hypomineralization and if the cemental thickness influences the fluoride levels, the periodontal disease initiation may be faster due to hypomineralization than the nonfluorosed teeth. This possibility requires to be studied. However, the higher occurrence of periodontal disease in fluorosed teeth than in nonfluorosed subjects has been reported by vandana et al.in 2014.

The studies related to the objective of our study is not comparable directly as their subjects age, sex, water fluoride exposure and methodology vary and differ from this study.

The histologic assessment of nonfluorosed and fluorosed bone has been done (unpublished data. Dissertation submitted to RGUHS, to study the mechanical, histlogical properties and mineral content of fluorosed and nonfluorosed bone and cementum"-an in vitro study.2016) which reported Cellularity of cortical and cancellous bone was found to be statistically significant in nonfluorosed group (10.72±4.10, 8.74±2.34) when compared to fluorosed group (6.61 ±3.31, 5.69±1.31) respectively. Trabecular density of bone: Trabecular density was same in both nonfluorosed and fluorosed bone [statistically non-significant, p= 0.615]. However, trabeculae were thick in nonfluorosed bone and short and thin in fluorosed bone. Resting and reversal lines were more prominent in nonfluorosed bone than in fluorosed bone. Marrow content was fatty in both the groups. Osteoclasts were present in all subjects of nonfluorosed bone whereas osteoclasts were very few to absent in fluorosed bone. The observation of the current study reported that the thickness of acellular cementum in nonfluorosed teeth (23.88±11.77 microns) was found to be more than in fluorosed teeth (17.69 ±8.98 microns) but was statistically non-significant. Histologically, density of cells in cellular cementum of nonfluorosed teeth (4.36±1.27) was found to be statistically highly significant than in fluorosed teeth (1.60±1.01). The increased cemental thickness and cellularity may provide a reason to initiation of periodontal disease in nonfluorosed than in fluorosed cementum which is thinner with less cellular density. The observed histologic changes such as decreased cemental thickness and cellularity of fluorosed cementum would influence the pathogenesis of periodontal disease and /or outcome of periodontal treatment. This may the reason of higher occurrence of periodontitis in the study by K.L.Vandana. Dental fluorosis may soon be designated as environmental risk factor in endemic fluorosed area. Clinicians have to pay attention to treatment of fluorosed and nonfluorosed roots i.e, periodontal therapy (during scaling and root planning), endodontic treatment (during root canal treatment) and orthodontic treatment (alteration of orthodontic forces).

Conflict of interest and source of funding statement

The authors have stated explicitly that there are no conflicts of interest in connection with this article. This study was self-funded

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