Effects of Tetracycline, EDTA and Citric Acid Application on Fluorosed Dentin and Cementum Surfaces: An *In Vitro* Study

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Abstract: Fluorosis is one of the factors that may bring about mineralization changes in teeth. Routine treatment of root biomodification is commonly followed during periodontal therapy. The purpose of the present study is to compare and evaluate the root surface changes in fluorosed and nonfluorosed teeth subsequent to the application of Tetracycline, EDTA, and Citric acid. Both fluorosed and nonfluorosed teeth comprising of periodontally healthy and diseased, were included in this study. Each of them was grouped into Tetracycline Hydrochloride, EDTA and Citric acid treatment groups. Using Scanning electron microscope (SEM), the photomicrographs of dentin and cementum specimens were obtained. Results showed that the amount of smear layer removal and exposure of collagen matrix in dentin specimens were variable in different groups. The exposure of fibrillar structures on cementum specimens were seen significantly on healthy specimens as compared to diseased specimens. Thus, the root biomodification procedure brings in definite difference between fluorosed and non-fluorosed dentin and cementum specimens.

Keywords: Root biomodification, fluorosed dentin and cementum, scanning electron microscope, tetracycline, EDTA, citric acid.

INTRODUCTION

Adequate removal of plaque, calculus, and cytotoxic substances from the diseased root surface appears to be essential for periodontal regeneration. Mechanical instrumentation of root surface results in the formation of a smear layer of organic and mineralized debris, which has been suggested to act as a physical barrier, inhibiting new attachment and acting as a substrate for bacterial growth.

To overcome the above limitations of using only mechanical root instrumentation, chemical root surface conditioning has been introduced. This conditioning is intended to decontaminate, detoxify and demineralize the root surface, removing the smear layer and exposing collagen matrix, thereby providing a matrix which supports migration and proliferation of cells involved in periodontal wound healing. For chemical root surface treatment, a variety of compounds have been used: Sulphuric acid, Hydrochloric acid, Lactic acid, Maleic acid, Phosphoric acid, Citric acid, Ethylenediaminetetraacetic acid (EDTA), and Tetracycline hydrochloride. Of these, Citric acid, Ethylenediaminetetraacetic acid (EDTA) and Tetracycline hydrochloride have received the most interest.

It has been suggested that the topical application of Citric acid on the exposed root surfaces may prevent the apical migration of dentogingival epithelium, which may be due to early fibrin linkage of the root surface, thereby enhancing new attachment formation [1]. Partial demineralization of the root dentin with Citric acid appears to enhance mesenchymal cell adhesion, possibly by a biochemical mechanism [2].

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The drawback of Citric acid conditioning is that it creates an extremely acidic pH in the surrounding tissues, which may result in unfavorable wound healing responses [3] and also denaturation of the collagen. Thus, its use has been discontinued. Recently, newer agents like Tetracycline and EDTA are preferred due to their moderate pH. Surface demineralizing effect, using Tetracycline hydrochloride, enhances binding of matrix proteins to dentin and stimulates fibroblast attachment and growth [4]. EDTA exerts its demineralizing effect through chelating divalent cations at neutral pH, while phosphoric acid acts through its low pH and dissolves or erodes a mineralized surface.

The American daily news has recently reported the ill effects of high fluoride content on dental tissues [5]. It fails to mention even the freshly reported information on the effects of fluoride on periodontal tissues [6, 7]. Unfortunately, research has not been focused on fluorosis effects on periodontal tissues as it is with dental caries, although fluorosis is endemic. There are scanty studies available discussing about the fluorosis effects on periodontal tissues. The fluorosed population is bound to experience more periodontal disease [6], and a preliminary study reported increased mineralization of periodontal ligament fibers [7]. Fluorosis is not a global problem like smoking. According to the literature from developing countries, where smoking is prevalent malignantly, the relationship between periodontal disease and smoking is dealt abundantly. The designation of risk factor for smoking is much discussed. Similarly, the day is not too far to designate fluorosis as an environmental risk factor.

Dental fluorosis is one of the common complaints of subjects hailing from high water fluoride areas of Davangere district, Karnataka, India. Dental fluorosis is known to cause hypomineralizaton of enamel [8] and dentin [9]; the influ-

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ence of fluorosis on the cementum is not dealt in literature. Also, the present study arose, firstly from our routine clinical observations of moderate to advanced periodontitis in subjects residing from high fluoride belts of Davangere district, wherein, a strong association of periodontal disease with high fluoride water was found, using a Community Periodontal Index of Treatment Needs (CPITN) in a population aged 15- 74 years [6]. Secondly, SEM observations revealed higher globular mineralized debris and partial / initial mineralization of connective tissue fibres (periodontal ligament area) in Fluorosed healthy teeth group as compared to non-Fluorosed group [7].

The routine periodontal treatment in these patients has aroused a doubt, whether the root biomodification effects in fluorosed teeth would remain similar or different as compared to non-fluorosed teeth. Few reports indicate that there are no clinically significant benefits of treating root surfaces with chemical agents. The effects of root conditioning are inconsistent in periodontal literature. However, root conditioning as a treatment modality is not abandoned yet.

Hence, a first attempt is made in this study using Scanning electron microscope for the evaluation of smear layer and collagen exposure in dentin specimens and appearance of fibrillar structures in cementum specimens of fluorosed and non-fluorosed teeth which comprised of periodontally healthy and diseased teeth, subsequent to the application of Tetracycline HCl (TTC), EDTA and Citric acid (CA).

MATERIALS AND METHODOLOGY

Collection of Teeth Specimens

In this study, fluorosed and non-fluorosed teeth, which comprised of periodontally healthy and diseased teeth, were included. The freshly extracted teeth were obtained from the Department of Oral and Maxillofacial Surgery, College of Dental Sciences, Davangere, Karnataka, India, and were used according to a protocol that satisfied the ethical standards of Rajiv Gandhi University of Health Sciences, Karnataka, India.

The extracted teeth were required to meet the following inclusion criteria: (1) were to be fully erupted, (2) extracted non-traumatically due to orthodontic reasons, (3) no history of recent periodontal instrumentation or dental prophylaxis, (4) periodontally diseased teeth with at least 60% attachment loss indicated for extraction, and (5) for fluorosed teeth, the fluorotic enamel stains were 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: (1) teeth with proximal caries extending to the cementum, (2) fillings extending beyond CEJ, and (3) intrinsic stains caused by other reasons such as porphyria, erythroblastosis fetalis, tetracycline therapy etc.

A total of 74 fluorosed and non-fluorosed healthy (n=37) and diseased teeth (n=37) specimens were taken for this study. Teeth Specimens were divided into TTC (n=26), EDTA (n=26), and CA (n=22) treatment groups.

The extracted teeth were immediately washed in sterile saline solution and were stored in bottles containing 0.9% saline.

Sectioning and Preparation of Teeth Specimens

Periodontally Healthy Fluorosed and Non-Fluorosed Teeth

The root surfaces were hand instrumented, using a sharp periodontal curette to remove the remnants of periodontal ligament without resulting in total removal of cementum, by using 12 strokes approximately. Coronal section was then performed 1 mm below the cemento- enamel junction and apical section 3 mm from the root apex, using a sterile diamond disk running at low speed with sterile water coolant. Then a longitudinal section was performed to obtain 2 specimens from each root representing dentin and cementum specimen. The two halves were instrumented, using both hand and rotary (fine diamond tapered bur) instruments to expose the dentine and were washed in water. For cementum specimens, the above procedure was repeated on different teeth group till the mesio-distal sectioning into 2 halves. Dentin and cementum specimens were randomly distributed into TTC, EDTA and CA groups for root conditioning procedures.

Periodontally Diseased Fluorosed and Non-Fluorosed Teeth

A reference groove was marked on the root surface at the level of soft tissue attachment. The root surface was instrumented, using a sharp periodontal curette to remove the remnants of periodontal ligament, calculus and superficial cemental layer coronal to the level of marking, using 50 strokes approximately [10].

The anatomical crown, including 1 mm of the coronal portion of the root, was resected with a high speed diamond disc. Dentin and cementum specimens were taken from the middle one-third of the root of periodontitis-affected human root surfaces. The root portion coronal to the level of groove was retained and the portion apical to the groove was discarded. The preparations of dentin and cementum specimens for periodontally diseased teeth roots were done in the same way as for the periodontally healthy root specimens. Dentin and cementum specimens were randomly distributed into TTC, EDTA and CA groups for root conditioning procedures.

Root Surface Treatment

In this study, a concentration of 500mg/5ml (100mg/ml), pH 1.8 of Tetracycline HCl [11, 12] 24% EDTA pH 7.4 [13], and Citric acid pH 1 [14] were used. The specimens were burnished with solution-saturated cotton pellet with respective agents in each group for 3 minutes [15]. Pellets were changed at every 30-second intervals, and specimens were then rinsed under running tap water.

Preparation for SEM

The specimens were placed in 2.5% Glutaraldehyde in 0.1 M Phosphate buffer (pH 7.4), for a minimum of 24 hours. Following washing and dehydration through a graded alcohol series (25% to 100%), they were mounted on SEM stubs. Mounted specimens were air-dried for 48 hours and sputter coated with 30 to 40nm of gold. Finally, specimens were examined by using a scanning electron microscope (JEOL-JSM-840A, operating at an accelerating voltage of 20kV). Representative photomicrographs were obtained at x3500 and x1500 for dentin and cemental surfaces.

SEM photomicrographs were assessed for following findings: (1) for Dentinal specimens: presence / absence of smear layer at x 3500 and x1500 and (2) for Cemental specimens: appearance of fibrillar structures.

The collection and preparation of tooth specimens were done by a single Periodontist (K.S) while the SEM examination was done by C.M.C.

RESULTS

A total number of 37 healthy fluorosed and non-fluorosed teeth (FH and NFH, respectively) and 37 diseased fluorosed and nonfluorosed teeth (FD & NFD respectively) were randomly divided into Tetracycline HCl (TTC), Ethylenediaminetetraacetic acid (EDTA), and Citric acid (CA) groups. All the specimens were evaluated for dentinal and cemental changes after the root biomodification procedure using SEM.

The dentinal and cemental SEM photographs were scored for smear layer using following scoring criteria:

0 None

1 Smear layer involving random areas of surface that totals between 1-32% of total surface area.

2 Smear layer involving random areas of surface that totals between 33-65% of total surface area.

3 Smear layer involving > 66% of total surface area.

Assessment of degree of demineralization of Dentinal specimens:

0 None

1 (Slight) Localized exposure of matrix collagen with an evidence of acid denaturing and possible widening of a few dentinal tubule orifices.

2 (Moderate) Localized or generalized exposure of matrix collagen with an evidence of acid denaturing, obvious widening of dentinal tubule orifices, and evidence of peritubular dentin demineralization.

3 (Severe) Generalized exposure of matrix collagen with severe acid denaturing, gross widening of dentinal tubule orifices, and obvious peritubular dentin demineralization.

Assessment of degree of demineralization of Cemental specimens:

0 None

1 (Slight) Localized exposure of matrix collagen with an evidence of acid denaturing and possible widening of Periodontal ligament fibres (PDL) / Gingival fibres (GF) insertion sites.

2 (Moderate) Generalized exposure of matrix collagen with an evidence of acid denaturing and obvious widening of PDL/GF insertion sites.

3 (Severe) Generalized exposure of matrix collagen with severe acid denaturing and gross widening of PDL/GF insertion sites.

The results of the study are interpreted in Tables **1-6** and Figs. (**1-6**).

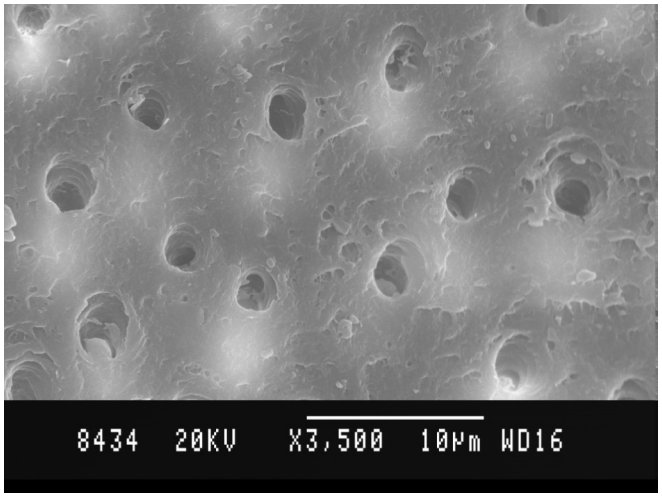

Fig. (1). Tetracycline treated dentin specimens (FLH).

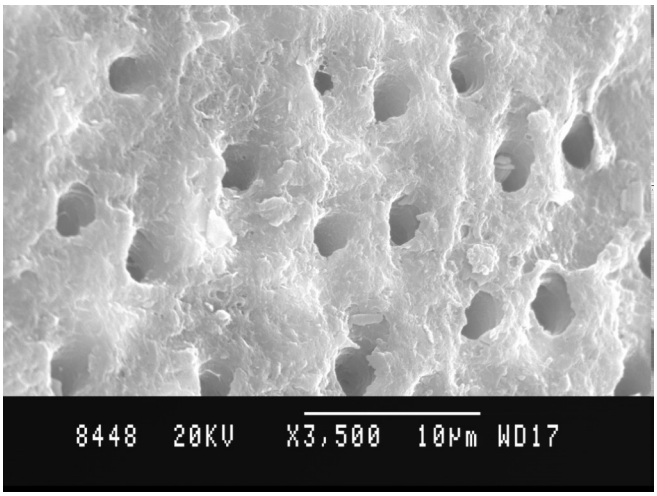

Fig. (2). EDTA treated dentin specimens (FLH).

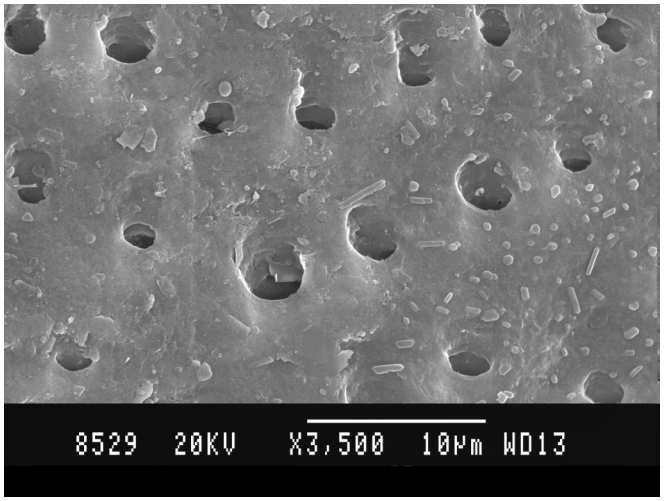

Fig. (3). Citric acid treated dentin specimens (NFLH).

Smear Layer and Exposure of Dentin Collagen Matrix: (Tables 1-3 and Figs. 1-3)

Smear layer is representative of the degree of demineralization. In the TTC treated dentin specimens group, FH group showed the maximum smear layer scores (1.55), followed by NFH (1.2), FD (1.0), and NFD (0.11). Also, FH group was

the most susceptible to demineralization (1.33), followed by NFH (1.2), NFD (1.1), and FD (0.88).

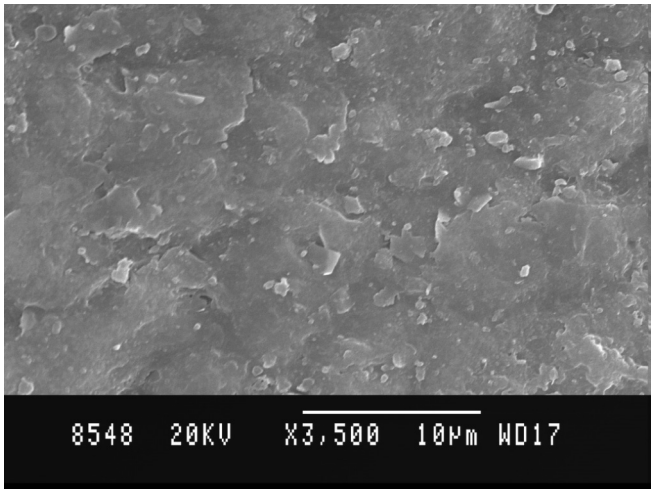

Fig. (4). Tetracycline treated cementum specimens (NFLD).

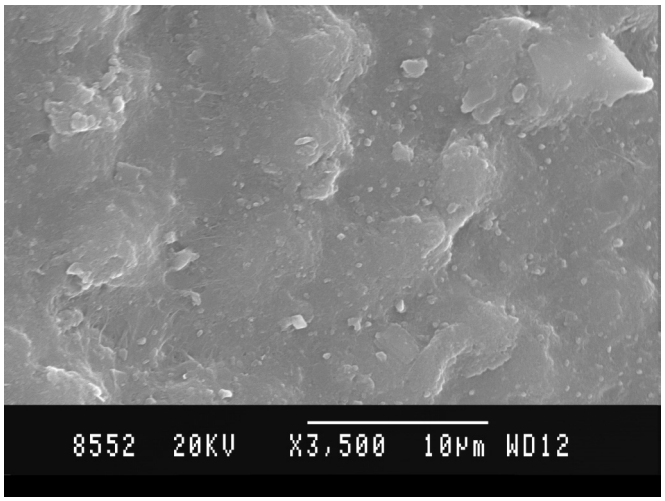

Fig. (5). EDTA treated cementum specimens (NFLD).

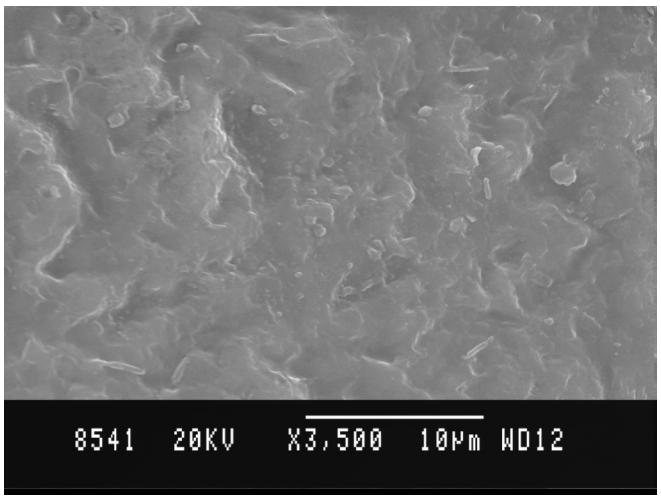

Fig. (6). Citric acid treated Cementum specimens (FLD).

In the EDTA treated dentin specimens group, NFD group showed the maximum smear layer scores (1.11), followed by FH (1.0), NFH (0.88), and FD (0.55). Also, FH group was the most susceptible to demineralization (0.5) followed by NFH (0.33), FD (0.33), and NFD (0.22).

Table 1. Number of Dentin Specimens in Each Group that Exhibits a Smear Layer and Evidence of TTC Induced Demineralization

	FH	FD	NFH	NFD
Smear Layer Scores				
0 (no smear layer)	1	3	2	**8**
1 (1-33% of area)	4	3	4	**1**
2 (34-65% of area)	2	3	4	**0**
3 (>66% of area)	2	0	0	**0**
Total Score	**14**	**9**	**12**	**1**
Average	**1.55**	**1.0**	**1.2**	**0.11**
Evidence of Demineralization Scores				
0 (None)	2	3	1	**1**
1 (Slight)	3	4	6	**5**
2 (Moderate)	3	2	3	**3**
3 (Severe)	1	0	0	**0**
Total Score	**12**	**8**	**12**	**11**
Average	**1.33**	**0.88**	**1.2**	**1.1**

Table 2. Number of Dentin Specimens in Each Group that Exhibits a Smear Layer and Evidence of EDTA Induced Demineralization

	FH	FD	NFH	NFD
Smear Layer Scores				
0 (no smear layer)	2	5	2	**0**
1 (1-33% of area)	5	3	6	**8**
2 (34-65% of area)	0	1	1	**1**
3 (> 66% of area)	1	0	0	**0**
Total Score	**8**	**5**	**8**	**10**
Average	**1.0**	**0.55**	**0.88**	**1.11**
Evidence of Demineralization Scores				
0 (None)	4	6	6	**7**
1 (Slight)	4	3	3	**2**
2 (Moderate)	0	0	0	**0**
3 (Severe)	0	0	0	**0**
Total Score	**4**	**3**	**3**	**2**
Average	**0.5**	**0.33**	**0.33**	**0.22**

In the CA treated dentin specimens group, NFD group showed the maximum smear layer scores (1.29), followed by NFH (1.0), FH (0.86), and FD (0.57). Also, NFD group was the most susceptible to demineralization (0.57), followed by FH (0.29), NFH (0), and FD (0).

Exposure of Fibrillar Structures on Cemental Specimens: (Tables 4-6 and Figs. 4-6)

In the TTC treated cemental specimens group, FH group showed the maximum smear layer scores (1.75), followed by FD (1.25), NFH (0.5), and NFD (0.5). Also, FH group was

the most susceptible to demineralization (1.5) followed by NFH (1.2), FD (1.25), and NFD (0.5).

Table 3. Number of Dentin Specimens in Each Group that Exhibits a Smear Layer and Evidence of CA Induced Demineralization

	FH	FD	NFH	NFD
Smear Layer Scores				
0 (no smear layer)	1	3	0	**0**
1 (1-33% of area)	6	4	7	**5**
2 (34-65% of area)	0	0	0	**2**
3 (> 66% of area)	0	0	0	**0**
Total Score	**6**	**4**	**7**	**9**
Average	**0.86**	**0.57**	**1.0**	**1.29**
Evidence of Demineralization Scores				
0 (None)	5	7	7	**5**
1 (Slight)	2	0	0	**0**
2 (Moderate)	0	0	0	**2**
3 (Severe)	0	0	0	**0**
Total Score	**2**	**0**	**1**	**4**
Average	**0.29**	**0**	**0.2**	**0.57**

Table 4. Number of Cementum Specimens in Each Group that Exhibits a Smear Layer and Evidence of TTC Induced Demineralization

	FH	FD	NFH	NFD
Smear Layer Scores				
0 (no smear layer)	1	2	3	**3**
1 (1-33% of area)	1	0	0	**0**
2 (34-65% of area)	0	1	1	**1**
3 (> 66% of area)	2	1	0	**0**
Total Score	**7**	**5**	**2**	**2**
Average	**1.75**	**1.25**	**0.5**	**0.5**
Evidence of Demineralization Scores				
0 (None)	1	2	2	**3**
1 (Slight)	1	0	0	**0**
2 (Moderate)	1	1	0	**1**
3 (Severe)	1	1	2	**0**
Total Score	**6**	**5**	**6**	**2**
Average	**1.5**	**1.25**	**1.5**	**0.5**

In the EDTA treated cemental specimens group, NFH group showed the maximum smear layer scores (0.75), followed by FH (0.25), NFD (0.25), and FD (0.25). Also, FD and NFD groups were the most susceptible to demineralization (1.0) followed by NFH (0.75), and FH (0.5).

In the case of CA treated dentin specimens group, FH group showed the maximum smear layer scores (1.25), followed by NFD (1.0), NFH (0.25), and FD (0.0). Also, NFD group was the most susceptible to demineralization (1.25), followed by FD (1.0), FH (0.75), and NFH (0).

Table 5. Number of Cementum Specimens in Each Group that Exhibit a Smear Layer and Evidence of EDTA Induced Demineralization

	FH	FD	NFH	NFD
Smear Layer Scores				
0 (no smear layer)	3	3	1	**3**
1 (1-33% of area)	1	1	3	**1**
2 (34-65% of area)	0	0	0	**0**
3 (> 66% of area)	0	0	0	**0**
Total Score	**1**	**1**	**3**	**1**
Average	**0.25**	**0.25**	**0.75**	**0.25**
Evidence of Demineralization Scores				
0 (None)	2	1	1	**2**
1 (Slight)	2	2	3	**1**
2 (Moderate)	0	1	0	**0**
3 (Severe)	0	0	0	**1**
Total Score	**2**	**4**	**3**	**4**
Average	**0.5**	**1.0**	**0.75**	**1.0**

Table 6. Number of Cementum Specimens in Each Group that Exhibits a Smear Layer and Evidence of CA Induced Demineralization

	FH	FD	NFH	NFD
Smear Layer Scores				
0 (no smear layer)	1	0	3	**2**
1 (1-33% of area)	1	0	1	**0**
2 (34-65% of area)	2	0	0	**2**
3 (> 66% of area)	0	0	0	**0**
Total Score	**5**	**0**	**1**	**4**
Average	**1.25**	**0**	**0.25**	**1.0**
Evidence of Demineralization Scores				
0 (None)	1	2	4	**1**
1 (Slight)	3	1	0	**1**
2 (Moderate)	0	0	0	**2**
3 (Severe)	0	1	0	**0**
Total Score	**3**	**4**	**0**	**5**
Average	**0.75**	**1.0**	**0**	**1.25**

DISCUSSION

In this study, both fluorosed and non-fluorosed, periodontally healthy and diseased teeth were included. In periodontally healthy teeth, 12 strokes were given to remove the remnants of periodontal ligament fibers by using sharp curette [16], and in diseased teeth approximately 50 strokes were given to remove remnants of periodontal ligament fibres, calculus and superficial cemental layer [10]. Root planing was done in order to enhance the action of root conditioning agents. For the dentin specimens, the tooth was vigorously root planed with hand curettes and finishing burs in high speed hand pieces, in an attempt to remove all the cementum and to achieve a smooth hard glass-like surface of dentin [12].

The formation of smear layer appears to be influenced by the degree of demineralization which in turn is affected by the disease process or by the environmental factors like fluoride. Root surface exposed by periodontitis have showed higher mineral content than normal root surfaces having a higher content of calcium, phosphorus and fluoride; this hypermineralization has been reported to be located in the superficial 40μm – 100 μm of the root surface [17]. The d iseased root surface also demonstrates zone of demineralization, caused by enzymatic activities in the pocket [18]. Whether the diseased root feature is restricted to cementum in terms of hypo and hypermineralization or even the dentin affected is required to be explored.

In case of TTC treated root specimens, most of the non-fluorosed healthy dentin specimens retained smear layer and also showed more evidence of demineralization, while most of the cementum specimens showed no smear layer and only half of the specimens showed evidence of demineralization. A study reported smooth and regular dentinal surface with wide, funnel shaped tubular openings and disclosed a fibrillar network of collagen matrix in non- diseased dentin surfaces, while healthy cementum surfaces were characterized by a non- homogenous fibrillar appearance with the amount of fibrillar material varied among specimens. Some areas showed an undulating surface with a finely granular texture and only scattered discrete structures, resembling intrinsic collagen fibrils [18]. However, a study utilizing dentin specimens from impacted third molars demonstrated the removal of smear layer and a homogenous surface with patent tubular openings of variable shape and size, using TTC solution at different (50- 150 mg/ml) concentrations in 3 (1, 3 and 5 minutes) application periods [15].

Also, most of the non- fluorosed diseased dentin specimens showed the presence of smear layer (score 0) and few of the specimens did not reveal the evidence of demineralization, while the cementum specimens revealed no smear layer and no evidence of demineralization. Another study reported TTC (100 mg/ml) conditioning of the dentin that successfully removed the smear layer and exposed dentin tubule openings in periodontitis affected teeth. Peritubular and intertubular areas of a matted collagen matrix were evident, following the 4 minute treatment, while cementum specimens revealed exposing of fibrillar mat like texture. A dense fibrillar network of interconnecting fibers surrounded and interwined by a mesh of smaller fibrils was evident [19]. Another study revealed removal of smear layer, open dentin tubules, exposed tubules, and exposed collagen fibres on the demineralized root surface, using saturated TTC solution for 5 minutes in diseased root dentin specimens [12].

In case of EDTA treated root specimens, most of them exhibited complete removal of smear layer, while few specimens showed tubules which were partially plugged by the smear layer remnants, indicating variable removal of smear layer. Few specimens demonstrated fibrillar surfaces, with the amount of fibrillar material varying between specimens. While in a study by Blomlof *et al*. [20] on periodontally diseased teeth, none of the different EDTA concentrations could remove all smear from the tubuli openings. However, the supersaturated (24%) solution was significantly more effective than all the lower concentrations of the EDTA, regarding smear removing capacity. Collagen was more readily exposed following etching with a supersaturated EDTA solution as compared to the lower concentrations in the present study. Another study by Blomlof *et al*. [21] on diseased teeth showed that root surface associated smear layer was removed and collagen fibers were exposed with an EDTA gel preparation, used in conjunction with non- surgical therapy. In a study by Lasho *et al*. [22], diseased root planed specimens treated with EDTA had numerous exposed fibers on their surfaces and within the tubules.

In this study, the CA treated surfaces were smooth, undulating in appearance with numerous round to oval dentinal tubule orifices. Tubule orifices were regular in shape, several being funnel shaped, indicating better smear layer removal properties compared to others. Smooth mat-like appearance and structures resembling collagen fibrils were observed in few specimens; similar result was seen using citric acid pH 1 for 5 minutes by immersion technique in bovine dentin specimens [23]. By using citric acid pH 1 and oxytetracycline 500mg/5ml (pH 1.3) for 5minutes by passive application, it was reported that the smear layer in oxytetracycline treated specimens was not as effective as in citric acid treated specimens, and it was varied from specimen to specimen [24].

The CA treated root planed healthy cemental surfaces showed minimal changes, they exhibited a faint mat-like structure. In another study, Citric acid pH 1 for 3 minutes by immersion technique in root planed normal cementum surfaces showed an over all surface morphology of undulating mounds, with a fibrillar, mat-like surface texture [25].

The CA treated root planed diseased cemental surfaces showed numerous particles of mineralized debris and the surface was also devoid of insertion sites for periodontal ligament fibers, whereas in another study by Hanes P *et al*. [25], undulating mounds, with a fibrillar, mat-like surface texture were seen by using CA pH 1 for 3minutes by immersion tech. in root planed normal cementum surfaces.

Medline search does not reveal any comparative studies between fluorosed & non-fluorosed dentin and cementum root surface changes, following conditioning. The differences between the present results and those of other studies may be related to the specimens utilized, the extent of instrumentation, the concentration of the conditioning agents, or a combination of these variables. Also, the biochemical and morphological changes in the root surface produced by the various mechanical techniques and conditioning agents are yet to be understood. Further experimental and clinical studies are needed to evaluate the significance of these differences.

CONCLUSION

The removal of smear layer and exposure of collagen matrix in Dentin specimens were variable in different groups, using TTC, EDTA, and CA. FH, NFH and FD groups were found to be more susceptible to demineralization by TTC followed by EDTA and CA while NFD group was more susceptible to TTC followed by CA and EDTA.

The exposure of fibrillar structures on Cementum specimens was seen to a variable degree in both fluorosed & non-fluorosed, healthy and diseased specimens. FH, NFH and FD groups were found to be more susceptible to demineraliza-

tion by TTC followed by EDTA and CA while NFD group was more susceptible to CA followed by EDTA and TTC.

This variability in demineralization of dentin & cementum specimens by various agents in case of periodontally diseased teeth specimens can be attributed to type, pH and mechanism of action of agent, and also hypermineralization of root in periodontitis, whereas in case of periodontally Healthy teeth, it can be attributed to only type, pH and mechanism of action of agent.

CONFLICT OF INTEREST AND SOURCE OF FUNDING STATEMENT

The authors declare that they have no conflict of interest. The study was self-funded by the authors and their institution.

ABBREVIATIONS AND TERMS USED

Enamel	=	Hard mineralized tissue that covers the anatomical crowns of the teeth
Dentin	=	Mineralized connective tissue that surrounds and encloses pulp. It contains dentinal tubules, which radiate from the pulp cavity to the outer surface of dentin
Cementum	=	Mineralized connective tissue that surrounds the anatomical roots of the teeth
Periodontal Ligament	=	Specialized connective tissue that surrounds, supports and protects teeth by its attachment to the roots and surrounding alveolar bone
Fluorosis	=	Endemic disease in geographic areas where the content of fluoride ion in the drinking water exceeds 2 ppm. Dental fluorosis is the specific disturbance of tooth formation caused by excessive fluoride intake.
Root Planing/ Mechanical instrumentation	=	Constitutes the use of mechanical instruments like curettes on the root surface to free it from the deposits like debris, plaque and calculus
Smear layer	=	Produced on the root surface after mechanical debridement and contains inorganic and organic material like calculus, plaque and cementum and even microbes
Chemical root conditioning	=	Constitutes treatment of root surface with chemicals to free it from smear layer and to make the root surface compatible for periodontal tissue regeneration.
TTC	=	Tetracycline hydrochloride
EDTA	=	Ethylenediaminetetraacetic acid
CA	=	Citric acid
SEM	=	Scanning electron microscope
FH	=	Fluorosed healthy
NFH	=	Non-fluorosed healthy
FD	=	Fluorosed diseased
NFD	=	Non-fluorosed diseased
CEJ	=	Cemento-enamel junction

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