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EFFECTS OF CITRIC ACID, EDTA AND TETRACYCLINE HYDROCHLORIDE ON PERIODONTALLY INVOLVED ROOT SURFACES (A SEM STUDY)

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ABSTRACT

Root debridement by scaling and root planing may not completely remove contaminated cementum particularly in more apical areas. The smear layer formed during root planing inhibits cell migration and attachment leading to impaired marginal periodontal healing. In the present study an attempt is made to compare the morphological effects of root surface demineralization using citric acid, TTC and EDTA as root conditioning agents. The findings obtained from this study could form the basis for future in vivo studies using the same agents for demineralization procedures.

KEY WORDS: Root planing, smear layer, Root conditioning

INTRODUCTION

Periodontitis affected cementum presents loss of collagen fibre insertion, may harbour bacterial cells and may be contaminated by endotoxins which may suppress fibroblast migration and proliferation on cementum. Root debridement by scaling and root planing may not completely remove contaminated cementum particularly in more apical areas and the smear layer formed during root planing inhibits cell migration and attachment leading to impaired marginal periodontal healing¹.

There is considerable interest in the use of chemical agents to assist in root preparation. Agents have been proposed to accomplish the following :

- Eliminate cytotoxic material from affected root surfaces
- Clean the exposed dentine surfaces
- Decalcify planed root surfaces exposing dentine or cementum matrix collagen and thus facilitate attachment between the root surface and the healing connective tissue².

In the present study an attempt is made to compare the morphological effects of root surface demineralization using citric acid, TTC and EDTA as root conditioning agents.

Objective of the study

- Efficacy of smear layer removal.
- Appreciation of collagen fibre like structures within intertubular dentine.
- Number of dentinal tubule orifices.
- Diameter of individual dentinal tubules.
- Total surface area occupied by the dentinal tubules.

Materials and Methods

Twenty extracted single rooted human teeth of 12 subjects with advanced periodontitis were collected from the Department of Oral Surgery, SDM College of Dental Sciences, Dharwad.

Criteria for selection of the teeth :

- No history of root planing, scaling and prophylaxis in the previous six months.
- No history of acute pain or swelling necessitating their removal.
- Proximal attachment loss of 5 mm or more
- Bleeding on gentle probing
- Abscence of caries on the proximal surface selected for the study.
- No history of use of antibiotics during the previous 6 months.
- No history of any systemic diseases.

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The extracted teeth were placed in sterile vials containing normal saline solutions which were processed without delay.

Preparation of teeth : The teeth were cleaned of blood and saliva with a soft bristled brush and distilled water. A dissecting microscope was used to determine the level of connective tissue attachment and that was marked with a small dental bur. The reference groove was then accentuated by deepening the groove with a diamond disk in high speed hand piece under continuous water coolant. The proximal surfaces of each tooth were vigorously root planed with Gracey No.1 & 2 hand curette which was re sharpened after each block in an attempt to remove all cementum and achieve a smooth, hard, glass like surface.

The instrumented surfaces were then reexamined to ensure removal of all calculus. Using a high speed hand piece with copious water coolant the crowns were resected at the cemento-enamel junction (CEJ). To provide an experimental surface, each tooth was sectioned longitudinally as 2 parts, and the pulpal side was flattened with a straight bur followed by a small inverted cone to make a horizontal groove on the pulpal surface for identification purpose. Lastly, these specimen were harvested by a horizontal cut in the coronal 1/3rd of each tooth half, to yield dentine slabs about 4 x 4 mm in size. This yielded a total of 40 dentine slabs. Then each tooth section was then scrubbed with a soft brush and distilled water to remove grinding debris.

Preparation of acid solutions : A citric acid solution was prepared by slowly adding 65 g of citric acid crystals into 100 ml of distilled water and stirred constantly. This gave a pH 1 solution, when checked with a pH meter.

A tetracycline hydrochloride solution was prepared by slowly dissolving 500 mg of laboratory grade pure tetracycline hydrochloride in 5 ml of distilled water. The solution was continuously stirred for 10 minutes. This gave a pH of 3.3 solution when checked with pH meter.

EDTA solution was prepared by slowly adding 24 grams of disodium salt of EDTA to 100 ml of distilled water and stirred constantly. This gave a p^{H} of 7.4 when checked with a p^{H} meter. All the three solutions were prepared immediately before the experimental treatment.

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Out of the 40 dentine slabs, 10 sections were randomly assigned to the following four treatment groups

Group-I :	Hand	instrumentation	and			
	conditioning with normal saline for 5					
	min as control					
Group-II:	Hand	instrumentation	and			
	conditioning	g with citric acid (pH ²	I) for			
	5 min					
Group-III:	Hand	instrumentation	and			
	conditioning	g with laboratory	pure			
	tetracycline	hydrochloride (pH	3.3)			
	for 5 min					
Group-IV :	Hand	instrumentation	and			
	conditioning	g with EDTA (pH -7.4	4) for			
	5 min					

List of Armentarium
Soft bristle brush.
 Dissecting microscope.
 Sterile vials.
 Diamond cutting discs and cone bur.
 Gracey's No I and II Hand curette.
 Tweezer and cotton pellets.
• p ^H Meter.
 Electron microscope.

Chemical treatment of root surfaces: The acid solutions were applied for 5 minutes to the experimental root surfaces with cotton pellets moistened with the agent, and the cotton pellets were changed 2 times per minute. These solutions were applied with light pressure to allow the agent to wet the surface without burnishing the agent into the root surface. Additional tooth sections served as controls and treated with cotton pellets moistened with normal saline solution. After conditioning, specimens were rinsed for 2 minutes with distilled water.

Preparation for SEM study : After treatment of the root surfaces, samples were fixed in 2.5% gluteraldehyde in phosphate buffer (pH 7.3) for 24 hours at 40 C, washed three times for ten minutes each in phosphate buffer, post-fixed in phosphate buffered 1.5% osmium tetroxide for 2 hours and washed three more times in phosphate buffer.

The specimens were then dehydrated in a graded series of aqueous ethanol solutions [50%, 70%, 85%, 95% and 100% ethanol] for ten minutes



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each. After 2 more ten minutes washing in absolute ethanol, the samples were dried overnight in a dessicator jar containing silica gel. They were mounted on SEM stubs with silver paint, and sputter coated with gold(**Fig.1**). The mounted slabs were evaluated using a Jeol 100 CX SEM. (**Fig.2**)

SEM examination : The presence of smear layer, exposure of the collagen fiber like structures, the number of dentinal tubules, diameter of the tubules and the average total surface area occupied by the tubules were measured at a magnification of x 3000 with zero tilt angle

The efficacy of smear layer removal by using different solutions was estimated by the following scores :

- **Score 0**: No patches of smear layer present.
- Score 1 : Small patches of smear layer present
- Score 2: Definite patches of smear layer present.
- Score 3 : Considerable amount of smear layer present.
- Score 4 : Completely covered by smear layer

The appreciation of collagen fibre like structures within the intertubular area were estimated by using the following scores :

Score 1 :	Poor (cannot be appreciated)
Score 2 :	Moderate
Score 3 :	Good

Score 4 : Excellent

The diameter of the dentinal tubule orifices, total surface area occupied by the dentinal tubule orifices were measured directly from the screen of the SEM with caliberated scales. A single investigator performed all morphometric measurements. Total of 100 x 100 m area was measured from each specimen.

Results

The study consisted of 40 specimens, out of which, 10 specimens were randomly assigned to four treatment groups : Group I - Saline, Group II - Citric acid (**CA**), Group III - Tetracycline HCL (**TTC**) and Group IV - EDTA. After the treatment the specimens were immediately prepared for the SEM examination. A single investigator performed

all the morphometric analysis. The mean values obtained for various parameters in the different groups were subjected to statistical analysis by applying students 't' test.

Efficacy of smear layer removal : When the mean efficacy of smear layer removal was compared between Groups I and II, Groups I and III, Groups I and IV, Groups II and III, Groups II and IV, it was found to be statistically significant at 5% level of significance (p < 0.05). However there was no statistical significance between Groups III and IV as shown in Table No.I. (Fig.3, Fig.4, Fig.5 and Fig.6)

Appreciation of collagen fibre like structures: When the mean appreciation of collagen fibre like structures was compared between Groups I and II, Groups I and III, Groups I and IV, Groups II and III, Groups II and IV, it was found to be satistically significant at 5% level of significance. However there was no statistical significance between Groups III and IV as shown in Table No.II.

Number of dentinal tubule orifices : When the mean number of dentinal tubule orifices was compared between, Groups II and III, Groups II and IV, Groups III and IV, it was found to be statistically significant at 1% level of significance (p < 0.01) as shown in Table No.III.

Diameter of the dentinal tubules : When the mean diameter of the dentinal tubules was compared between Groups II and III, Groups II and IV, it was found to be statistically significant at 1% level of significance. However there was no statistical significance between Groups III and IV as shown in Table No.IV.

Total surface area occupied by the dentinal tubule orifices : When the mean total surface area occupied by the dentinal tubule orifices was compared between Groups II and III, Groups II and IV, it was found to be statistically significant at 1% level of significance. However there was no statistical significance between Groups III and IV as shown in Table No.V. Extensive surface cracking was a frequent finding among all the different Groups.

TABLE No.1 MEAN EFFICACY OF SMEAR LAYER REMOVAL COMPARISON BETWEEN DIFFERENT GROUPS

TABLE No.2 MEAN APPRECIATION OF COLLAGEN LIKE STRUCTURES : COMPARISON BETWEEN DIFFERENT GROUPS

Variable	Mean	SD	t-Value	p-	Signifi	Mean	SD	t-Value	р-	Significa
				Value	cance				Value	nce
Saline	4.00	0.000	04 0000	-0 0E	c	1.00	0.000			
CA	0.3	0.4831	24.2222	<0.05	3	2.24	0.5164	-8.5732	<0.05	S
Saline	4.00	0.000	15 0217	-0 0E	c	1.00	0.000	0.4405	0.05	0
TTC	1.4	0.5162	10.9217	<0.05	3	1.4	0.5164	-2.4495	<0.05	S
Saline	4.00	0.000	21.00	-0.05	e e	1.00	0.000	0.4405	.0.05	c
EDTA	1.2	0.4217	21.00	<0.05	5	1.4	0.5164	-2.4495	<0.05	5
CA	0.3	0.4831	4.04.00	0.05	~	2.4	0.5164	4 2201	-0 0E	0
TTC	1.4	0.5162	-4.9193	<0.05	S	1.4	0.5164	4.3301	<0.05	3
CA	0.3	0.4831	4 4000	0.05	0	2.4	0.5164	1 2201	-0 0E	¢
EDTA	1.2	0.4217	-4.4388	<0.05	5	1.4	0.5164	4.5501	<0.05	3
TTC	1.4	0.5162			NS	1.4	0.5164	0	<u>\0 05</u>	NS
EDTA	1.2	0.4219	0.9487	>0.05		1.4	0.5164	0	20.05	

TABLE No.3 MEAN COMPARISON OF DENTINAL TUBULE ORIFICES BETWEEN DIFFERENT TREATMENT GROUPS

Varia ble	Material	Mean	SD	t-Value	p-Value	Significance
	Tetracycline HCL Citric Acid	23.90 27.40	7.9645 7.0110	-1.0431	>0.01	NS
Tubul e	Tetracycline HCL EDTA	23.90 25.20	7.9645 8.8544	-0.3452	>0.01	NS
	Citric acid EDTA	27.40 25.20	7.0110 8.8544	+0.6159	>0.01	NS

TABLE No.4 MEAN COMPARISON OF DIAMETER OF DENTINAL TUBULE ORIFICES BETWEEN DIFFERENT TREATMENT GROUPS

Variable	Material	Mean	SD	t-Value	p-Value	Significance
Tubule	Tetracycline HCL	1.2030	0.0945	-11 1/2	<0.01	S
	Citric Acid	1.7530	0.1265	-11.142		
	Tetracycline HCL	1.2030	0.0945	0.0256	>0.01	NS
	EDTA	1.241	0.0890	-0.9250		
	Citric acid	1.7530	0.1265	10 4691	-0.01	c
	EDTA	1.241	0.0890	-10.4001	<0.01	3

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TABLE No.5
MEAN COMPARISON OF TOTAL SURFACE AREA OCCUPIED BY THE DENTINAL TUBULE
ORIFICES BETWEEN DIFFERENT TREATMENT GROUPS

Variable	Material	Mean	SD	t-Value	p-Value	Significance
Tubule	Tetracycline HCL	108.0038	37.1606	7 4527	<0.01	S
	Citric Acid	261.2067	53.3280	-7.4557		
	Tetracycline HCL	108.0038	37.1606	1 0121	>0.01	NS
	EDTA	126.3222	43.5304	-1.0121		
	Citric acid	261.2067	53.3280	16 1062	<0.01	c
	EDTA	126.3222	43.5304	+0.1902		5

S : Significant; NS : Non Significant

Discussion

The surface appearance of the root planed specimens in this study resemble those in previous investigations and thus may represent a smear layer ^{2,3,4}. Smear layer is formed irrespective of the treatment modality used [i.e., ultrasonic, curette or diamond bur] ³. In this study all the acids (citric acid, TTC and EDTA) used were effective in removing the smear layer than the saline treated group. Out of these, citric acid had better efficacy in smear removal than tetracycline HCL and EDTA. However there is no statistically significant difference between tetracycline HCL and EDTA.

The agents used in this study either diminished or completely removed the smear layer and exposed the dentinal tubule. This study is in agreement with the study done by Hanes P. J et al4 who had observed some amount of surface debris on specimens treated with different acids.

The method of application has varied among the clinicians i.e., placement of the agent either passively or burnishing. In the present study application with light pressure is preferred over burnishing technique as the later may itself form smear layer which may partially or completely obliterate the dentinal tubule openings. Furthermore both the techniques were found to be equally effective in removing the smear layer in a study Rosa wen C et al ⁵.

The results of this study indicate that citric acid causes greater degree of collagen fibre exposure than tetracycline HCL and EDTA. However, there is no statistically significant difference in degree of collagen exposure between tetracycline HCL and EDTA treated groups.

Counting of the dentinal tubule orifices in saline group was not possible as the root surface was covered by smear layer. Hence comparison was made between the acids used. Comparing the mean number of dentinal tubule orifices exposed there was no statistically significant difference between all the three acid groups. This indicated that all the three acids were effective in exposing the dentinal tubules almost to the same extent. This study is in agreement with the study done by Hanes P.J et al⁶ who compared between citric acid and tetracycline HCL.

Inter group comparison showed citric acid had greater mean diameter (1.75 m) than tetracycline HCL [1.20 m] and EDTA (1.24 m] but when compared between tetracycline HCL and EDTA there was no statistical significant difference. However it appears that the mean diameter of the acid treated groups are higher than the mean diameter of the control specimens [1.05 m] in a study done by Labahn R et al⁷. This widening of dentinal tubule orifices was due to the preferential demineralization of peritubular dentine. The results of this study were similar to the studies done by Hanes P.J and Labahn R ^{6,7}. However, this study is in contrast to studies done by Lafferty T.A and Lasho D. J where the dentinal tubules and the

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dentine between the tubules were affected to the same degree by either of the acid solutions ^{2,8}.

Inter group comparison showed citric acid had greater mean total surface area occupied by the dentinal tubules than tetracycline HCL and EDTA. This was due to the greater diameter of the dentinal tubules obtained in citric acid groups. This finding was in agreement with the study done by Hanes P.J et al⁶ who compared between citric acid and EDTA.

The results of this study are limited to the physical finding of root surface changes and does not present in vivo differences that may result from the physiologic effect of these acids. Differences between our results and those of other studies may be related to the disease status of the dentine specimens utilized, the demineralizing agent or a combination of these variables. Additional studies of these variables with better standardization are needed for better understanding.

CONCLUSION

All the three acids are effective in removing the smear layer and exposing the collagen fibers. Citric acid is more effective in removing the smear layer and exposing the root collagen compared to tetracycline HCL and EDTA. Citric acid causes greater degree of morphological alterations (mean diameter, mean total surface area occupied by dentinal tubules) than tetracycline HCL and EDTA. Citric acid is considered to be a better root conditioning agent. However, the use of tetracycline HCL and EDTA cannot be ruled out.

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