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## RESEARCH ARTICLE

### DEMINERALIZING AGENTS VERSUS ER: YAG LASER IRRADIATION EFFECT ON ROOT SURFACE DEMINERALIZATION: A SCANNING ELECTRON MICROSCOPIC STUDY.

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#### Abstract

**Context:-** To make root surfaces conducive for periodontal regeneration the use of additional chemical protocols (Root conditioning) after scaling and root planning is important for the removal of smear layer formed after the basic therapy.

**Aims:-** The purpose of present study was to evaluate and compare the effectiveness of Erbium: Yttrium Aluminum Garnet (Er:YAG) laser irradiation with EDTA, Citric acid and Tetracycline hydrochloride for removal of smear layer and exposure of dentinal tubule orifices.

**Methods and Material:-** 75 freshly extracted periodontally involved single rooted teeth were collected. And randomly divided into 5 treatment groups having 15 teeth each: Control group (0.9% Normal saline), EDTA 24% (pH 7.4), Tetracycline hydrochloride (500mg/5ml pH 1.8), Citric acid (pH 1), Er:YAG laser 2.94μm at 80mJ repetition rate of 10 Hz for 250 microsecond (short pulse) and fluency of each pulse 10.3J/cm<sup>2</sup>. Specimens were subjected to scanning electron microscopy (SEM) and photographs were assessed by a single examiner who was blinded to the study. Parameters were assessed by Sampaio et al index (2005) and the results obtained were subjected to statistical analysis.

**Results:-** Er:YAG Laser irradiation showed non-significant results when compared to Control group and significant to Tetracycline hydrochloride. EDTA and Citric acid showed highly significant results compared to other groups but showed non-significant results when compared with each other

**Conclusions:-** In our study Er:YAG laser was not very effective in removal of smear layer and opening of dentinal tubules, EDTA and Citric acid showed similar and better efficacy in root surface biomodification as compared to other groups.

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#### Introduction:-

Periodontitis involves inflammatory process of bacterial origin affecting the periodontal tissues and provoking the destruction of supporting structures of the teeth. It causes pathological alteration in the periodontium. Hence, it has become apparent that if the goal of periodontal regeneration is to be realized, the problem of regeneration needs to be approached from a biological perspective. Scaling and root planning is unable to completely decontaminate root

surface and results in production of a smear layer,<sup>[1]</sup> which is formed by remnants of calculus, plaque and contaminated dental hard tissues.<sup>[2],[3]</sup> It might act as a barrier, preventing blood clot adhesion to the root surface<sup>[1]</sup> and periodontal regeneration.<sup>[2]</sup> To overcome this problem root surface conditioning is done. It exposes the organic dental matrix rendering root surface more biocompatible, which may increase success in regenerative procedures by creating an appropriate surface for cell attachment and eventual development of fiber attachment.<sup>[4]</sup> For root surface treatment, a variety of chemicals are used like Sulphuric acid, Hydrochloric acid, Lactic acid, Ethylenediaminetetraacetic acid (EDTA), Tetracycline hydrochloride etc. Of these, Citric acid, EDTA and Tetracycline hydrochloride have received the most interest.<sup>[5]</sup>

Citric acid has been shown to alter the surface characteristics of treated root surface by removing the smear layer, demineralizing the planed surfaces and eluting bacterial endotoxins from the pathologically altered cementum surfaces.<sup>[6],[7],[8]</sup> It is also capable to partially expose dentin collagen<sup>9</sup> which is important to increase collagen splicing, improve fibrin linkage, and consequently inhibit epithelial down growth.<sup>[10]</sup> This effect stimulates the fibroblast attachment and migration<sup>11</sup> and facilitates new cementum formation<sup>[12]</sup> The drawback of this is that it creates an extremely acidic pH in the surrounding tissues.. Thus its use has been discontinued.<sup>[13]</sup> Tetracyclines are broad spectrum antimicrobials which are used for root conditioning as well. Tetracycline hydrochloride demineralized dentin has been shown to be bacteriostatic, retains more antimicrobial properties than penicillin treated root surfaces and demonstrates substantivity. Tetracycline's anticollagenase activity appears to produce favorable clinical results.<sup>[14]</sup> There are conflicting reports in literature concerning optimal solution concentration and application time.<sup>[13]</sup> Of etchants in clinical use, EDTA appears to promote early cell tissue colonization by promoting a more biocompatible surface for cell and tissue attachment.<sup>[15]</sup> EDTA is not dependent on a low pH.<sup>[16]</sup>

Recently, laser therapy has been investigated for a wide range of dental applications. It has been shown that the Er:YAG laser can be safely used to condition root surfaces effectively.<sup>[17]</sup> Irradiation with this produced root surface changes that might be expected from acid etching i.e. removal of smear layer and exposure of the collagen matrix. Furthermore, this laser is believed to eliminate bacteria and inactivate bacterial toxins diffused within root cementum.<sup>[18]</sup> It has been suggested that Er:YAG laser irradiation effectively and rapidly eliminated most of the lipopolysaccharide on the extracted root surfaces and might be useful for root conditioning in periodontal therapy. This laser when used at lower energy densities shows sufficient potential for root surface modification to warrant further investigation.<sup>[17]</sup>

Hence the present study was designed to evaluate the effects of Er:YAG laser on the periodontally involved root surface and to compare its efficacy with Citric acid, Ethylenediamine tetra-acetic acid (EDTA) and Tetracycline hydrochloride in removal of root surface smear layer and opening of the dentinal tubules after root planing.

## Materials and Methods:-

This in-vitro study was conducted in Department of Periodontics, College of Dental Sciences Davangere, Karnataka.

75 periodontally involved single rooted teeth were selected and the sample preparation was done by scaling and root planning with hand curets followed by crown sectioning using a water cooled high speed diamond disk. The specimens obtained were then washed and cleaned with normal saline. A test area was marked on the proximal surface of the root 3mm from the cervical area, approximately 5mm wide from coronal to apical direction. (FIG1) Only 1 trained operator performed all the procedures. The specimens were randomly divided into 5 groups: Control group (0.9% Normal saline), EDTA 24 % (pH 7.4) for 3 minutes, Tetracycline hydrochloride (500mg/5ml pH 1.8), Citric acid (pH 1) for 3 minutes, Er:YAG laser 2.94µm at 80mJ repetition rate of 10 Hz for 250 microsecond (short pulse) and fluency of each pulse 10.3J/cm<sup>2</sup>. (FIG2,3,4) Immediately after the application of the reagents the specimens were rinsed with normal saline (10 ml). Specimens were then subjected to scanning electron microscopy (SEM-JOEL JSM 840 A operating at 15kv). The entire test surface of each specimen was scanned initially to obtain a general overview of the surface topography of each specimen. Standardized photomicrographs of the selected sites were obtained at magnification of X500 & X3000 for each specimen. (FIG5a-5e)

The study parameters included were removal of the smear layer and opening of the dentinal tubules as assessed by Sampaio et al index (2005)<sup>[19]</sup>

1. Root surface without smear layer, with the dentinal tubules completely opened without evidence of smear layer in the dentinal tubules
2. Root surface without smear layer with the dentinal tubules completely opened, but with some evidence of smear layer in the dentinal tubules entrance
3. Root surface without smear layer with the dentinal tubules partially opened
4. Root surface covered by a uniform smear layer with evidence of dentinal tubule opening
5. Root surface covered by a uniform smear layer without evidence of opening of the dentinal tubules
6. Root surface covered by an irregular smear layer, with the presence of grooves and or scattered debris.

### Results:-

The mean scores for all the groups are shown (Table1). On intergroup comparison between the control group and EDTA and between control group and citric acid the difference of the mean scores was found to be highly significant( $p=0$ ). On comparing EDTA with Tetracycline and Laser group, it was found to be highly significant( $p=0$ ) and significant ( $p=0.001$ ) respectively. On comparing Tetracycline group with Citric acid and Laser it was found to be highly significant ( $p=0$ ) and significant( $p=0.011$ ) respectively. When Citric Acid group was compared to Laser group it was found to be significant( $p=0.001$ ). The percentage distribution of specimens with corresponding scores are shown (Table2). In Group I(Control), Out of 15 specimens, 46.7 % specimens showed a score of 5 and 53.3% specimens showed a score of 6 on Sampaio et al index. In Group II(EDTA), out of 15 specimens, 33.3% specimens showed a score of 3, 60% specimens showed score of 4 and 6.7% specimen showed score of 6. In Group III(Tetracycline Hydrochloride), Smear layer removal and opening of the dentinal tubules was not evident in specimens belonging to this group. Out of these 15 specimens all the samples (100%) showed a score of 6. In group IV(Citric Acid), Smear layer removal and opening of the dentinal tubules was evident in this group. Out of 15 specimens, 40% specimens showed a score of 3, 33.3% of specimens showed a score of 4, 13.3% specimens showed a score of 5 and 6 each. In group V(Er:YAG Laser), Smear layer removal and opening of the dentinal tubules was not very evident in this group. Out of 15 specimens, 33.3% of specimens showed a score of 4, 20% of specimens showed a score of 5, 46.7% of specimens showed a score of 6. On intergroup comparison between Control group and EDTA and Citric acid, EDTA and Citric acid was found to be highly significant ( $p<0.0$ ). On comparison of EDTA with Tetracycline hydrochloride and Laser group, EDTA was highly significant ( $p=0.0$ ) with tetracycline group and significant with laser group ( $p<0.001$ ). Tetracycline hydrochloride group on comparison with Citric acid and Laser group, showed that Citric acid was found to be highly significant. ( $p<0.0$ ) and Laser group significant ( $p<0.011$ ). On comparing Citric acid group with Laser group, Citric acid was found to be significant ( $p<0.001$ ).

### Discussion:-

Root conditioning has been recommended as an adjunct to mechanical root surface debridement to remove smear layer and root associated endotoxins and to expose collagen fibers on the dentin surface.<sup>[20]</sup> The option of the adjunctive use of chemical root treatments appears to have had minimal impact on clinical outcomes.<sup>[6], [21]</sup> Therefore, development of novel systems for scaling and root planing, as well as further improvement of currently used mechanical instruments, is required. As lasers can achieve excellent tissue ablation with strong bactericidal and detoxification effects, they are one of the most promising new technical modalities for nonsurgical periodontal treatment. Another advantage of lasers is that they can reach sites that conventional mechanical instrumentation cannot. The adjunctive or alternative use of lasers with conventional tools may facilitate treatment, and has the potential to improve healing.<sup>[22]</sup>

Among all lasers used in the field of dentistry, Er:YAG laser has been reported to be the most promising laser for root surface treatment as it exhibits bactericidal and detoxification effects which provides favorable conditions for the attachment of periodontal tissue.<sup>[23]</sup>

In the present study the effect of Er:YAG laser ( $2.94\mu\text{m}$ ) with EDTA 24% (pH 7.4), Citric acid (pH 1) and Tetracycline Hydrochloride (pH 1.8) were compared for removal of smear layer and opening of the dentinal tubules. Root planing was done in order to enhance the action of root conditioning agents which were burnished (Active method) onto the prepared specimens with the help of a cotton pellet for 3 minutes. There is no consensus regarding the mode of application of the reagent to the root surface and thus, it varies among clinicians. Srirangarajan 2012 found that smear removal by active application was better than passive application.<sup>[24]</sup>

In this study, the diseased cementum was removed and root conditioning was done to mimic the dental clinical picture and to make the periodontitis affected root surface biologically hospitable to epithelial and connective tissue cell adherence and attachment.<sup>[25]</sup>

Here, in vitro test model was used to facilitate the selection of comparable test surfaces and to standardize experimental position for direct access to the test surface. These are aspects that have to be considered when clinical consequences of the study are drawn.<sup>[26]</sup>

The SEM has a greater depth of focus than the transmission and light electron microscope and can resolve about 150Å. Thus all parts of a rough surface can be in focus. Secondly, the surface of a bulk specimen can be viewed directly, thus eliminating the need for thin section or replication procedure<sup>[27]</sup> and giving more exacting information regarding the structure of root surface. In this study, all the procedures were done by one operator in order to eliminate inter-operator variability and minimize the variables such as stroke length, force and pressure applied during instrumentation.

Published research concerning the use of lasers for removal of smear layers and cementum from tooth root surfaces is contradictory. In the present study Fidelis Plus III (FOTONA, Germany) laser unit was used. The energy output and other parameters were determined based on the results of previous studies. The laser beam was focused in a contact mode.<sup>[28],[29]</sup> In the case of contact ablation the procedure is quicker and the energy fluency needed is lower as compared to non contact ablation.

Within the limitations of our study, it was found that root conditioning in the group treated by Er:YAG laser irradiation was not effective compared to EDTA and Citric acid groups, however it was effective on comparison with Tetracycline hydrochloride group. EDTA and Citric acid showed similar efficacy of root surface biomodification and were consistently better when compared to other groups. Tetracycline hydrochloride group on the other hand showed no evidence of smear layer removal and opening of the dentinal tubules but it did show exposure of the collagen fibres.

In this study 24% EDTA was used which according to Blomlof et al<sup>[30]</sup> demonstrated to effectively remove smear layer, however in another study using 24 % EDTA it has been shown to hyperdemineralize dental surface producing a 'Chemical dissolution' smear layer suggesting that overdemineralization leads to complete dissolution of tooth surface, which could be accounted for the presence of smear layer in few samples used in this study. These findings concur with those of Sampaio et al,<sup>[31]</sup>

In majority of the samples treated with Citric Acid, specimens were smooth, undulating in appearance with numerous round to oval dentinal tubule orifices. Tubule orifices were regular in shape, being funnel shaped, indicating better smear layer removal properties. These findings were similar to those obtained by Garrett et al,<sup>[32]</sup> Polson AM et al.<sup>[33]</sup>

The findings obtained in this study in tetracycline group were consistent with Soares PBF et al<sup>[34]</sup> who showed that the dentinal tubules were completely covered by Tetracycline residues and failed to effectively remove the smear layer and Fontanari LA et al<sup>[35]</sup> who showed Tetracycline had a poor capacity to remove smear layer. In this study, majority of the specimens showed evidence of exposure of collagen fibres which were consistent to findings of Nadir Babay et al.<sup>[25]</sup>

Although lasers were not very effective in root surface smear layer and opening of the smear layer in our study, it did cause irregularities on the root surface. Despite the presence of the irregularities, carbonization and craters and fractures were not observed in accordance with studies done by Aoki A et al<sup>[36]</sup> and Folwaczny M et al.<sup>[37]</sup>

**Table 1:-** Showing mean scores of Sampaio et al index (2005) for the five groups.

Groups	Mean	Median	Range
I (Control)	5.5±0.5	6	5 - 6
II (EDTA)	3.8±0.8	4	3 - 6
III (Tetracycline hydrochloride)	6.0±0.0	6	6
IV (Citric acid)	4.0± 1.1	4	3 - 6
V (Er: YAG Laser)	5.1±0.9	5	4 - 6
K- W ANOVA*, H=42.39, p<0.001 significant			
Difference between groups**	I – II	p = 0	HS
	I – III	p = 0.069	NS <sup>#</sup>
	I – IV	P = 0	HS
	I – V	P = 0.31	NS <sup>#</sup>
	II – III	P = 0	HS
	II – IV	p = 0.77	NS <sup>#</sup>
	II – V	p = 0.001	S
	III - IV	P = 0	HS
	III – V	p = 0.011	S
	IV – V	P = 0.001	S

K-W ANOVA– Kruskal – Wallis Analysis of Variance

\*\* Mann-Whitney test,  $p \leq 0.05$ ,  $p < 0.01$  Significant(S),  $p < 0.001$ , Highly significant (HS),  $p > 0.05$  Not significant (NS<sup>#</sup>)

II &lt; IV &lt; V &lt; I &lt; III

**Table 2:-** Distribution Of Specimens With Corresponding Scores And Percentages.

Group wise cross tabulation						
Group	No of specime ns (n)		Specimen Score (SS)			
			SS-3	SS-4	SS-5	SS- 6
I (Normal saline)	15	Count	0	0	7	8
		% within Group	0.00 %	0.00%	46.70%	53.30%
II (EDTA)	15	Count	5	9	0	1
		% within Group	33.30 %	60.00 %	0.00%	6.70%
III (Tetracycline hydrochloride)	15	Count	0	0	0	15
		% within Group	0.00 %	0.00%	0.00%	100.00%
IV (Citric acid)	15	Count	6	5	2	2
		% within Group	40.00 %	33.30 %	13.30%	13.30%
V ( Er: YAG Laser)	15	Count	0	5	3	7
		% within Group	0.00 %	33.30 %	20.00%	46.70%
Total		Count	11	19	12	33
		% within Group	14.70 %	25.30 %	16.00%	44.00%



**Figure Legends:-**

Fig 1: prepared specimens

Fig 2: reagents used in the study

Fig3: application of reagent

Fig 4: laser irradiation

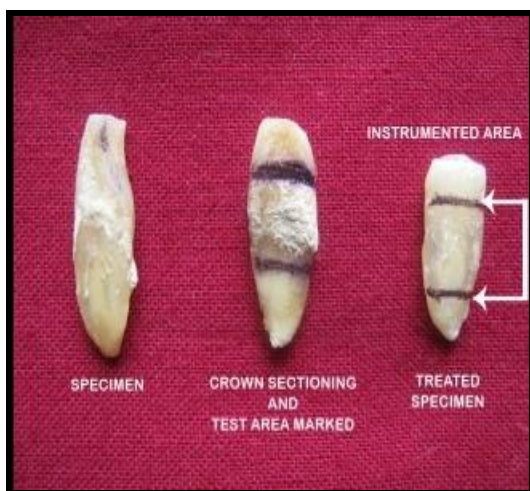
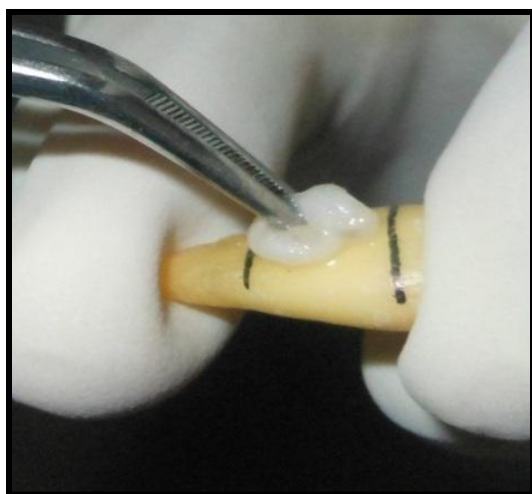
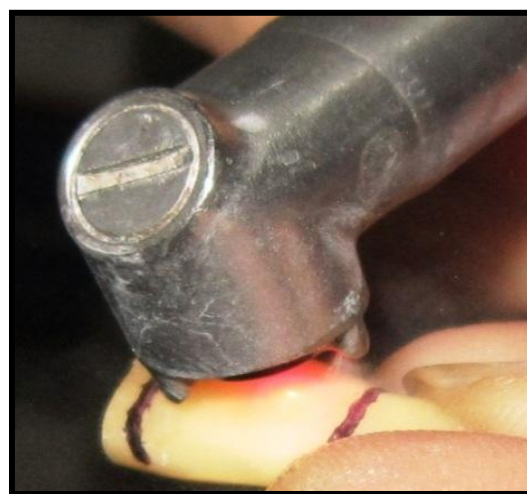
Fig5a.sem photographs(3000x)showing smear layer removal and opening of the dentinal tubules(group i)

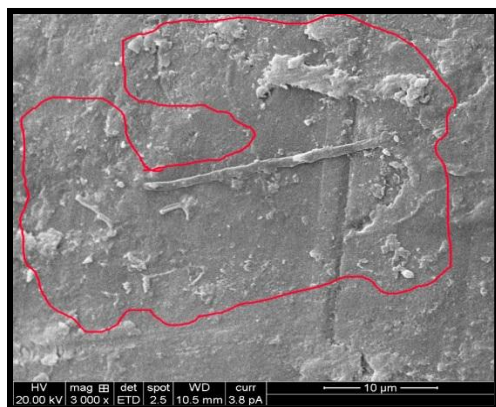
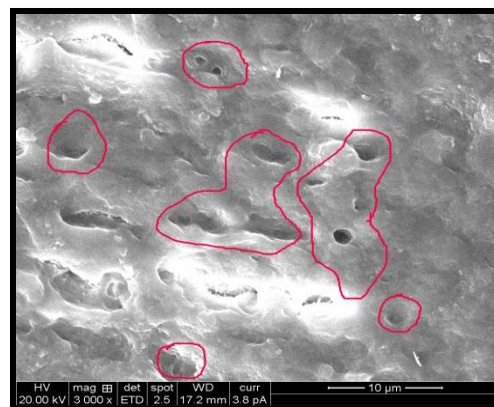
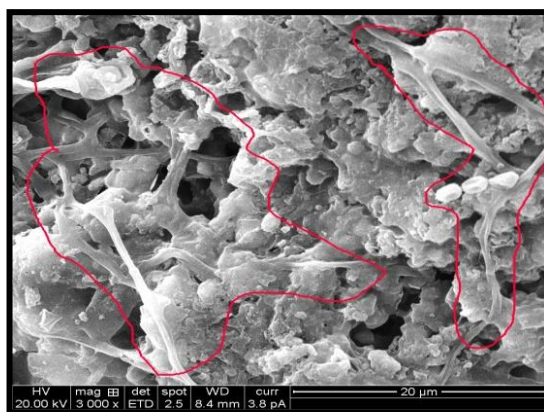
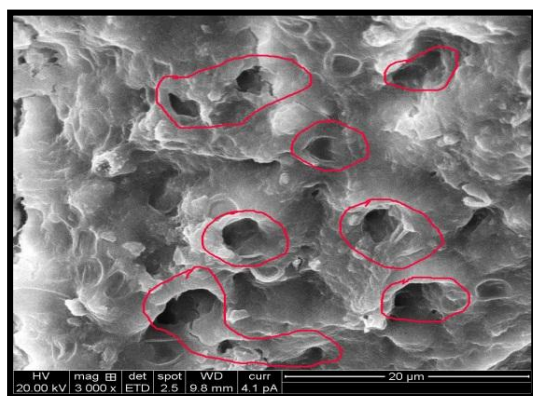
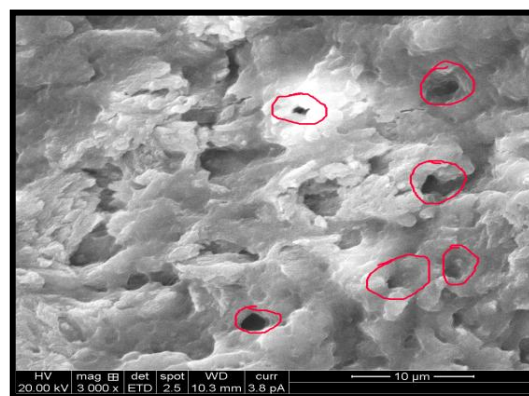
Fig5b.sem photographs(3000x) showing smear layer removal and opening of the dentinal tubules(group ii)

Fig5c.sem photographs (3000x)showing smear layer removal and opening of the dentinal tubules(groupiii)

Fig5d.sem photographs (3000x)showing smear layer removal and opening of the dentinal tubules(group iv)

Fig5e.sem photographs (3000x)showing smear layer removal and opening of the dentinal tubules(group v)

**Figures:-****Fig 1:** prepared specimens .**Fig 2:-** reagents used in The study**Fig3:-** application of reagent.**fig 4:-** laser irradiation.

**Fig5:-sem photographs showing smear layer removal and opening of the dentinal tubules****Fig5:-a-group 1 (control)****3000x****group ii- edta(24%,ph 7.4)****3000x****Group iii:- tetracycline hydrochloride(ph 1.8)****3000x****Groupiv:- citric acid (ph 1)****3000X****groupv:- er:yag laser.****3000X**

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