



# Effect of Er:YAG Laser Irradiation on Fluorosed and Nonfluorosed Root Surfaces: An In Vitro Study

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**Purpose:** It is well known that fluorosis may be manifested by abnormal mineralization in teeth. The Er:YAG laser has seen increasing use for treatment of root surfaces exposed to periodontitis. However, there is little information regarding the effects of the Er:YAG laser on root cementum of fluorosed teeth. Therefore, this in-vitro study aimed to evaluate and compare root surface changes following Er:YAG laser irradiation of fluorosed and non-fluorosed teeth.

**Materials and Methods:** Thirty periodontally healthy fluorosed and nonfluorosed root specimens were irradiated using an Er:YAG laser (2.94  $\mu\text{m}$  wavelength) at 140 mJ/pulse and 10 Hz under a surface-cooling water spray. Examination by SEM was performed to assess laser-induced ultrastructural changes in the root surfaces. Statistical analysis and dichotomous expression of root surface changes in each of the groups were performed for intra- and intergroup comparisons.

**Results:** Specimens in both treatment groups exhibited evidence of mild thermally induced change, primarily surface melting. Other surface alterations noted in both treatment groups included surface etching, intermittent smear layer, exposure of collagen tufts, and open dentinal tubules. Intergroup comparisons using the dichotomous data indicated that except for melting of root surface, other undesirable morphological changes were found to be more common in nonfluorosed than fluorosed root specimens.

**Conclusion:** Results of the present study suggest that undesirable morphological changes were similar for both the HF and HNF groups. The results also indicate that further in vitro studies are required, using a variety of lower energy settings, before clinical trials can be initiated that would evaluate the use of the Er:YAG laser for treatment of teeth with fluorosed root structure. Also, observation of etched root surfaces with smear layer after laser irradiation may necessitate additional chemical acid etching for composite restoration for treatment of root caries if indicated.

**Keywords:** Er:YAG laser, fluorosis, cementum, scanning microscopy.

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Based on the experimental and clinical data presented in a recent systematic review of the laser literature, it appears that the Er:YAG laser possesses the characteristics most suitable for the nonsurgical treatment of chronic periodontitis.<sup>1</sup> Its ability to ef-

fectively ablate hard tissue and dental calculus without producing undesired thermally induced collateral damage in adjacent tissues has been demonstrated in several studies.<sup>2-6</sup> The lack of thermally induced root surface changes is likely the result of the special optical charac-

teristics of the Er:YAG laser wavelength of 2.94  $\mu\text{m}$  that peaks close to the absorption coefficient of water (3  $\mu\text{m}$ ).<sup>7</sup>

Results from controlled clinical trials and case reports indicate that nonsurgical Er:YAG laser-mediated periodontal therapy results in gains in clinical attachment levels equal to that obtained by traditional therapy.<sup>8-10</sup> In addition, several other desirable effects following use of the Er:YAG laser have been reported in both in vitro and in vivo studies, such as a significant antibacterial effect, elimination of bacterial endotoxins, ability to easily remove plaque and calculus, an irradiation effect limited to an ultra-thin layer of tissue, promotion of comparable or faster bone repair when compared to conventional osteoplasty using a high-speed handpiece, and its use in maintenance of dental implants, make it a promising tool for periodontal treatment.<sup>11</sup>

Dental fluorosis is a common complaint of patients from the Davangere district of Karnataka, India, which has naturally occurring high fluoride levels in the water. Dental fluorosis is known to cause hypomineralization of enamel<sup>12</sup> and dentin.<sup>13</sup> The impact of fluorosis on root cementum and periodontal ligament has not been reported in the dental literature.

The present study arose firstly from our routine clinical observations of moderate to advanced periodontitis in subjects residing in the high fluoride areas of the Davangere district. Indeed, a strong association of periodontal disease with naturally occurring high levels of fluoride in the local water has been reported elsewhere.<sup>14</sup> Secondly, SEM observations have revealed higher globular-shaped mineralized debris and partial/initial mineralization of connective tissue fibers in the periodontal ligament area in healthy fluorosed teeth (HF) as compared to healthy nonfluorosed teeth (HNF).<sup>15</sup>

There are no published papers addressing the effect of laser irradiation on fluorosed teeth. As Er:YAG laser irradiation is known to affect mineralized tissues, the therapeutic benefit provided by lasers on fluorosed root cementum should be studied. Therefore, an initial attempt has been made in this in vitro study to evaluate and compare the root surface changes following Er:YAG laser irradiation on HF and HNF teeth.

## MATERIALS AND METHODS

### Tooth Specimens

A total of 15 HF and 15 HNF teeth was included in this study. 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 set forth by the Research Ethics Committee of Rajiv Gandhi University of Health Sciences, Karnataka, India.

The extracted teeth were required to meet the following inclusion criteria: (1) fully erupted and belonging to systemically healthy subjects, (2) premolars extracted nontraumatically for orthodontic reasons, (3) no history of recent periodontal instrumentation or dental prophylaxis, and (4) for fluorosed teeth, the fluorotic enamel stains were confirmed by the clinical examination and history of the subjects from natural high water-fluoride areas in and around Davangere (fluoride concentration 1.5 to 3.0 ppm). The exclusion criteria were: (1) teeth with proximal caries extending to the cementum, (2) impacted teeth, (3) restorations extending beyond the cemento-enamel junction, and (4) intrinsic stains from other causes, such as porphyria, erythroblastosis fetalis, tetracycline therapy, etc.

The extracted teeth were immediately washed in running tap water and then stored in bottles containing 0.9% saline. Using a sterile diamond disk running at low speed with sterile water coolant, the tooth specimens were sectioned at the cemento-enamel junction to separate the crown from the root. This was followed by sectioning of root bucco-lingually into halves. This resulted in 30 root specimens in each group. Out of these 30 specimens, 15 root specimens served as the experimental group (lased) and 4 root specimens served as control group (nonlased).

### Laser Treatment

Experimental group (lased) specimens were irradiated with an Er:YAG laser (Fidelis Plus III, Fotona, Germany). The model Er:YAG laser emits a pulsed infrared radiation at a wavelength of 2.94  $\mu\text{m}$ , with maximum fluence of 48  $\text{J}/\text{cm}^2$ , 1.5 J maximum pulse energy, a maximum frequency of 50 Hz, and a maximum power of 20 W delivered through a 7-mirror articulated arm.

The laser beam was directed onto the root surfaces under water irrigation<sup>16</sup> using an R02 handpiece and a quartz fiber delivery tip.<sup>6,17</sup> Laser parameters were set at 140 mJ/pulse and 10 pulses/s (control panel setting). The treatment was performed in a coronal-apical direction using parallel passes with the delivery tip angled at approximately 15 to 20 degrees to the target surface.<sup>18</sup> The root samples were irradiated for 1 min<sup>2</sup> in a focused and contact mode.<sup>16</sup> The final selection of laser parameters was based on reports

**Table 1 Characteristics of experimental (lased) group**

Group characteristics	Experimental group	
	Healthy fluorosed (HF)	Healthy nonfluorosed (HNF)
No. of samples	15	15
2.94 $\mu\text{m}$ Er:YAG laser irradiation parameters	140 mJ/pulse, 10 Hz, 15-20 degree angulation, focused, contact mode, under water irrigation for 1 min	140 mJ/pulse, 10 Hz, 15-20 degree angulation, focused, contact mode, under water irrigation for 1 min

**Table 2 Explanation of grading scale used for morphological changes**

Morphological feature	Assigned score			
	0	+	++	+++
Surface etching	None	Surface etching involving between 1% and 32% of total surface area	Surface etching involving between 33% and 65% of total surface area	Surface etching involving > 66% of total surface area
Surface melting	None	Surface melting involving between 1% and 32% of total surface area	Surface melting involving between 33% and 65% of total surface area	Surface melting involving > 66% of total surface area
Smear layer	None	Smear layer involving between 1% and 32% of total surface area	Smear layer involving between 33% and 65% of total surface area	Smear layer involving > 66% of total surface area
Number of patent dentinal tubules	None	1-5	6-10	> 11

by several different groups of investigators who used a variety of settings ranging from 60 to 300 mJ/pulse and 10 Hz without evidence of thermal damage to root surfaces.<sup>19-21</sup> The characteristics of the experimental groups are summarized in Table 1.

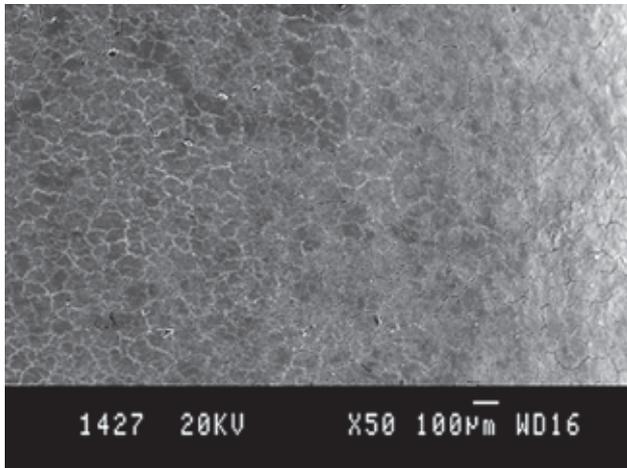
### Scanning Electron Microscope Analysis

Following irradiation, the specimens were placed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for a minimum of 24 h. The specimens were then rinsed using three irrigation cycles with 10 ml of a 0.1 M buffered phosphate solution. They were then dehydrated by 10-min immersions in increasing concentrations of ethyl alcohol, ie, 70%, 80%, 90%, and 100%. Following dehydration, the specimens were mounted on aluminum SEM stubs. Mounted specimens

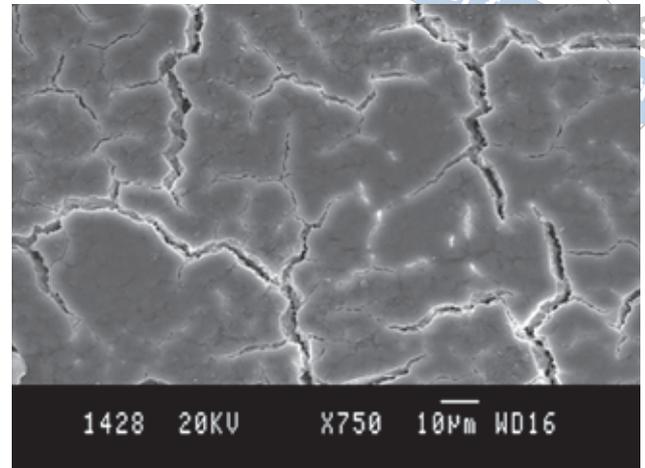
were air dried for 48 h and sputter coated with 30 to 40 nm of gold. Specimens were examined by scanning electron microscopy (JEOL-JSM-840A, operating at an accelerating voltage of 20 kV). Representative photomicrographs were obtained at 500X and 2500X magnifications for all lased specimens and at 50X and 750X for control specimens. One investigator, an experienced electron microscopist, was blind to specimen treatment and evaluated and scored all photographs.

The SEM photomicrographs were assessed for evidence of: (1) surface etching, (2) surface melting, (3) smear layer, (4) surface charring, (5) heat-induced surface cracking, (6) exposure of collagen tufts, and (7) patent dentinal tubules.

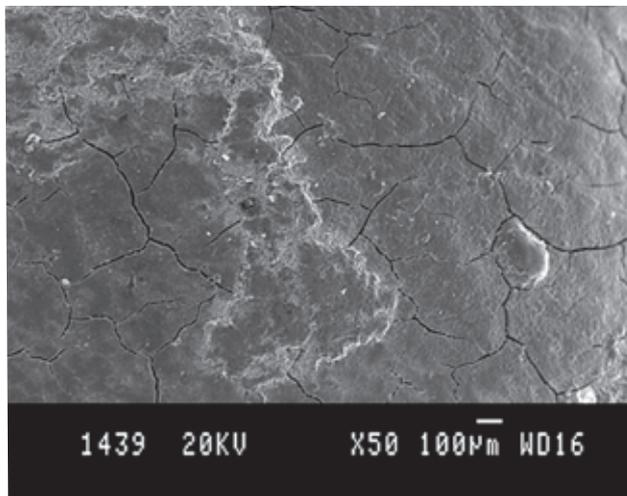
Surface etching, surface melting, smear layer, and presence of patent dentinal tubules were scored using an arbitrary numeric scale (Table 2). Other morphological changes, ie, surface charring, heat-induced



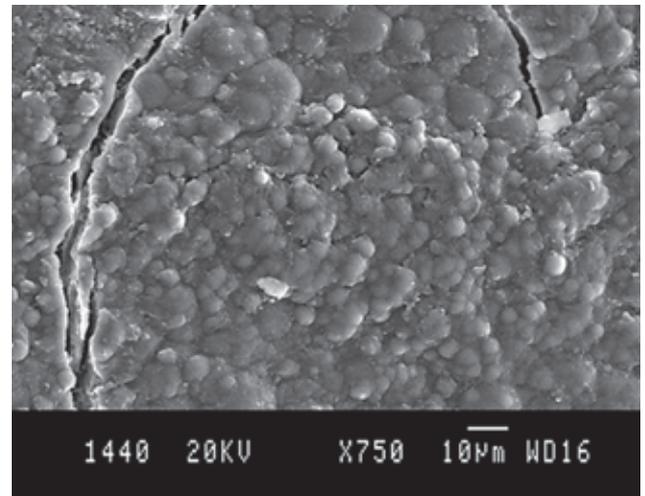
**Fig 1** Untreated HF control specimen showing a smooth surface topography. Bar = 100 µm at an original magnification of 50X.



**Fig 2** Higher magnification view of an untreated HF control specimen showing a smooth surface topography. Cracks are artifacts induced by dehydration during tissue processing. Bar = 10 µm at an original magnification of 750X.



**Fig 3** Low magnification view of an untreated HNF control specimen showing a relatively smooth surface. Bar = 100 µm at an original magnification of 50X.



**Fig 4** High magnification view of an untreated HNF control specimen showing a surface topography of evenly distributed globular mounds of cementum of varying diameters. Bar = 10 µm at an original magnification of 750X.

surface cracking, and exposure of collagen tufts were graded using a dichotomous scale of Yes / No.

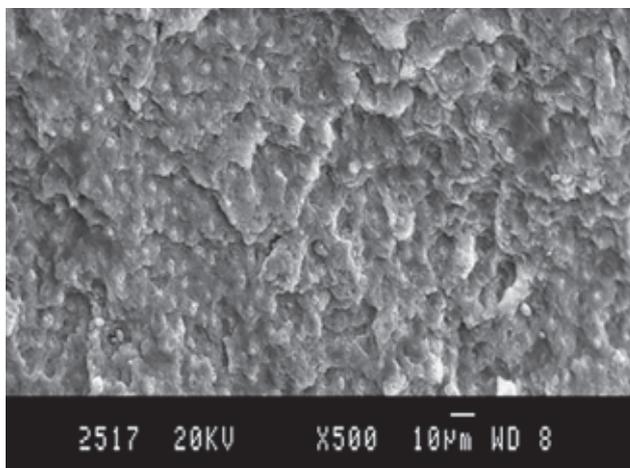
### Statistical Analysis

Using the arbitrary numeric scale, a mean score and standard deviation was calculated for all specimens, at both the magnification of 500X and 2500X. In addition, the dichotomous data was ascertained for each group. Since the data were in scores, nonparametric tests were used for intra- (Wilcoxon's signed rank test) and

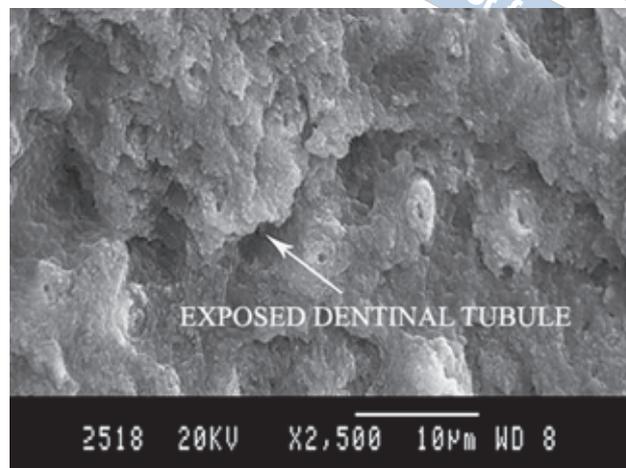
inter- (Mann-Whitney test) group comparisons. Categorical data were analyzed by Fisher's exact test. For all the tests, a p-value of 0.05 or less was considered statistically significant.

### RESULTS

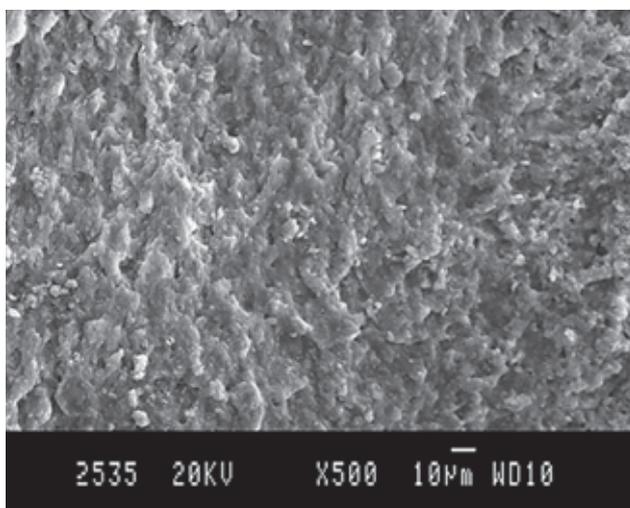
Untreated HF control specimens presented an undulating but smooth topography (Figs 1 and 2), whereas the untreated HNF controls featured a relatively rough



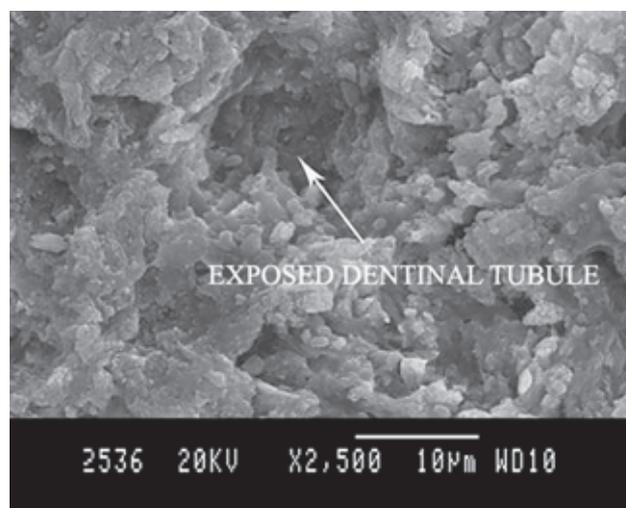
**Fig 5** Lased HF specimen showing typical surface etching pattern. Bar = 10 µm at an original magnification of 500X.



**Fig 6** High magnification view of lased HF specimen showing surface etching and patent dentinal tubules. Bar = 10 µm at an original magnification of 2500X.



**Fig 7** Lased HNF specimen featuring a uniform pattern of surface etching. Bar = 10 µm at an original magnification of 500X.



**Fig 8** Higher magnification view of a lased HNF specimen with the characteristic pattern of surface etching and patent dentinal tubules. Bar = 10 µm at an original magnification of 2500X.

topography consisting of evenly distributed globular mounds of cementum of varying diameters (Figs 3 and 4). In both laser-treated HF and HNF specimens, there was no evidence of charring or heat-induced surface cracking. However, both sets of specimens occasionally exhibited small areas of surface melting, smear layer formation, patent dentinal tubules, and exposure of collagen tufts (Figs 5 to 8). In addition, all laser treated specimens, regardless of group, exhibited a uniform surface etching (Figs 6 and 8).

Intra- and intergroup comparisons of laser-induced surface etching using both 500X and 2500X magnifica-

tions revealed no significant differences between HF and HNF specimens, ie,  $p = 1.00$  and  $p = 0.32$ ;  $p = 0.31$  and  $p = 0.14$ , respectively (Table 3). In a similar manner, there was no significant difference in intra- and intergroup comparisons for laser-induced surface melting between HF and HNF specimens, regardless of magnification:  $p = 1.00$  and  $p = 1.00$ ;  $p = 0.41$  and  $p = 0.42$ , respectively (Table 4).

Smear layer scores were predominately a "0", regardless of group. Consequently, the mean score  $\pm$  SD could not be calculated; instead, dichotomous scores (0 and +) were assigned to each group and the rela-

**Table 3 Groupwise data of etched surface following Er:YAG laser irradiation**

Magnification	Group	Etched surface score				Mean score ± SD	Median	HF vs HNF *
		0	+	++	+++			
500X	HF	1	5	2	7	2.0 ± 1.1	2.0	p = 0.31, NS
	HNF	1	1	4	9	2.4 ± 0.9	3.0	
2500X	HF	2	3	3	7	2.0 ± 1.0	2.0	p = 0.14, NS
	HNF	-	1	4	10	2.6 ± 0.6	3.0	
HF: 500X vs 2500X **		p = 1.00, NS						
HNF: 500X vs 2500X **		p = 0.32, NS						

\* Mann-Whitney test. \*\* Wilcoxon's signed rank test. NS = non-significant. p ≤ 0.05 statistically significant.

**Table 4 Groupwise data of surface melting following Er:YAG laser irradiation**

Magnification	Groups	Surface melting score				Mean score ± SD	Median	HF vs HNF *
		0	+	++	+++			
500X	HF	5	2	3	5	1.6 ± 1.3	2.0	p = 0.41, NS
	HNF	6	1	7	1	1.2 ± 1.1	2.0	
2500X	HF	4	3	4	4	1.5 ± 1.2	2.0	p = 0.42, NS
	HNF	5	3	6	1	1.2 ± 1.0	1.0	
HF: 500X vs 2500X **		p = 1.00, NS						
HNF: 500X vs 2500X **		p = 1.00, NS						

\* Mann-Whitney test. \*\* Wilcoxon's signed rank test. NS = nonsignificant; p ≤ 0.05 statistically significant.

tionship between these scores was compared using Fisher's exact test (dichotomized). An intra-group comparison between 500X and 2500X magnifications in the HF and HNF group was not significant (p = 0.33 and p = 0.07, respectively). The intergroup comparison between the 500X magnification of HF and HNF groups was significant (p = 0.03), whereas intergroup comparison between the 2500X magnification of HF and HNF groups was not significant (p = 0.17; Table 5).

Collagen tuft exposure in lased specimens determined that the intergroup comparison between the HF and HNF groups was not significant (p = 0.46; Table 6). Analysis of the presence of patent dentinal tubules showed that an intragroup comparison between 500X and 2500X magnifications in the HF and HNF groups was significant (p = 0.05 and p = 0.02, respectively). In contrast, the intergroup comparison between the 500X magnification of HF and HNF groups and also between 2500X magnification of HF and HNF groups

**Table 5 Groupwise data of smear layer following Er:YAG laser irradiation**

Magnification	Group	Smear layer score				HF vs HNF*
		0	+	++	+++	
500X	HF	12	3	-	-	p = 0.03, S
	HNF	6	3	5	1	
2500X	HF	14	-	1	-	p = 0.17, NS
	HNF	11	4	-	-	
HF - 500X vs 2500X *		p = 0.33, NS				
HNF - 500X vs 2500X *		p = 0.07, NS				

\* Fisher's exact test (dichotomized). S = significant, NS = nonsignificant, p ≤ 0.05 statistically significant.

**Table 6 Groupwise data of exposure of collagen tufts following Er:YAG laser irradiation**

Magnification	Groups	Exposure of collagen tufts		HF vs HNF*
		YES	NO	
2500X	HF	4	11	p = 0.46, NS
	HN	6	9	

\* Fisher's exact test (dichotomized). NS = nonsignificant. p ≤ 0.05 statistically significant.

were not significant ( $p = 0.57$  and  $p = 0.13$ , respectively; Table 7).

In addition to the above statistical analysis of intra- and intergroup comparisons, all the grading scores of specimen surface changes for each of the laser-treated groups were converted into dichotomous criteria of Present/Absent (expressed as a percentage) and then further intergroup comparisons were conducted (Table 8). All comparisons were made using photographs at 2500X magnification. Results revealed that surface etching in the HNF group occurred with more frequency than in the HF group (100% vs 86.67%). In a similar manner, specimen surface melting was noted in the HF group more often than in the HNF group (73.3% vs 66.67%); smear layer formation was noted more often in the HNF group (26.67%) than in the HF

group (26.7% vs 6.7%); exposure of collagen tufts was noted more frequently in the HNF group than in the HF group (40% vs 26.7%); and lastly, the presence of patent dentinal tubules occurred more frequently in the HNF group than in the HF group (86.7% vs 80%).

## DISCUSSION

The present in vitro study represents an initial attempt to evaluate and compare the root surface changes on root structure of periodontally healthy fluorosed and nonfluorosed teeth following irradiation with a 2.94- $\mu\text{m}$  wavelength Er:YAG laser. The majority of studies that report on the effects of Er:YAG laser irradiation

**Table 7 Groupwise data of open dentinal tubules following Er:YAG laser irradiation**

Magnification	Groups	Open dentinal tubules score				Mean score $\pm$ SD	Median	HF vs. HNF**
		0	+	++	+++			
500X	HF	1	5	3	6	1.9 $\pm$ 1.0	2.0	p=0.57, NS
	HNF	1	5	6	3	1.7 $\pm$ 0.9	2.0	
2500X	HF	3	5	5	2	1.4 $\pm$ 1.0	1.0	p=0.13, NS
	HNF	2	12	1	-	0.9 $\pm$ 0.5	1.0	
HF: 500X vs 2500X **		p = 0.05, S						
HNF: 500X vs 2500X **		p = 0.02, S						

\* Mann-Whitney test. \*\* Wilcoxon's signed rank test. S = significant, NS = nonsignificant. p  $\leq$  0.05 statistically significant.

**Table 8 Groupwise data of dichotomous expression of root surface changes following Er:YAG laser irradiation**

Laser group		Root surface changes				
		Etched surface	Melting of surface	Smear layer	Exposure of collagen tufts	Patent dentinal tubules
Healthy fluorosed	2500X	13 / 15 (86.67%)	11 / 15 (73.33%)	1 / 15 (6.67%)	4 / 15 (26.67%)	12 / 15 (80%)
Healthy nonfluorosed	2500X	15 / 15 (100%)	10 / 15 (66.67%)	4 / 15 (26.67%)	6 / 15 (40%)	13 / 15 (86.67%)

on tooth structure have used periodontally diseased teeth. A few studies have used periodontally healthy teeth extracted for orthodontic<sup>6,17,22,23</sup> or prosthetic reasons,<sup>17</sup> or due to pericoronitis.<sup>6,23</sup>

Complex inflammatory, enzymatic, and other biological influences which accompany periodontal disease produce physical or chemical alterations which are particularly apparent in the root cementum.<sup>24</sup> Periodontitis-affected root surfaces exhibit several changes, such as a surface layer of hypermineralized cementum, loss of collagen fiber insertion, contamination by endotoxins, and bacterial penetration of the root surface.<sup>25,26</sup> Because such physical and biological root surface alterations may vary from tooth to tooth,

there is likely some variability in the degree and type of surface change following laser application. In this context, in some studies, histological and SEM examinations have shown that under in vitro conditions, the Er:YAG laser may ablate not only dental calculus but also the superficial portion of the underlying cementum. In such studies, the resulting surface topography was similar to microscopic acid etching.<sup>4,5,17</sup> In contrast, SEM observations from more recent studies have shown that the clinical use of an Er:YAG laser can produce a smooth root surface morphology, even at higher energy settings.<sup>3,16,27</sup>

In the present study, the use of an arbitrary numeric scale provided an indexed grading to the morpho-

logical feature for each laser-treated specimen. This approach may be considered as an alternative for quantifying the root surface changes following laser irradiation, as the majority of published studies reporting SEM observations use a dichotomous description based on consistency of treatment effect across specimens from treatment groups.<sup>2,4,6,16,17,20-22,28-32</sup> In other studies, grading of Er:YAG laser-induced changes in root surfaces was done based on increasing severity of change.<sup>3,33</sup>

As reported in other *in vitro* studies about irradiated root surfaces with the Er:YAG laser, the present study also did not observe thermally induced surface charring and heat crazing.<sup>6,17,22</sup> The lack of thermally induced change on root surfaces is likely the result of two different characteristics inherent to the use of the Er:YAG laser. First, the wavelength of 2.94  $\mu\text{m}$  lies within the mid-range infrared spectrum and is highly absorbed by tissue water. The laser irradiation increases the vibratory motion of water molecules, which in turn rapidly increases temperature and pressures within the tissues, resulting in a rapid phase transformation from liquid to gas. The sequence of these events is so compressed in time that microexplosions occur, resulting in tissue ablation and the typical etched appearance of the surface.<sup>17</sup> Thus, the lack of charring and cracking of the root surface are the result of minimal heat absorption by collateral tissues and are consistent with microexplosive events.<sup>17</sup> Second, the use of a water spray surface coolant, which is standard protocol for the Er:YAG laser when applied to biological tissue, appears important in suppressing heat-induced surface alterations.<sup>3,6,29,34</sup>

In contrast to previous studies, the present investigation observed localized melting of specimen surfaces and smear layer formation.<sup>6,17,22</sup> This difference in reported results, as regards surface smear layer and melting, is best attributed to the higher energy parameters used in the current study as compared to previous investigations.

The observation of exposed collagen tufts – representing the collagen matrix of dentin – in the present study agrees with Israel et al,<sup>17</sup> who reported the exposure of collagen bundles, but conflicts with the results by Sasaki et al<sup>6</sup> and Yamaguchi et al.<sup>22</sup> An additional inconsistency between studies concerns the exposure of dentinal tubules following laser irradiation. As previously noted, the present study consistently observed patent dentinal tubules, which agrees with Sasaki et al,<sup>6</sup> but disagrees with the results of Israel et al<sup>17</sup> and Yamaguchi et al.<sup>22</sup> One must assume the observed inconsistencies are the result of differences in

laser parameters and, therefore, differences in applied energy, and are not the result of differences in character of the root surfaces, as all studies used healthy specimens. This seems particularly relevant, since the present study used both HF and HNF specimens and reported similar results for both specimen groups following laser irradiation.

Similar to the etching of surfaces observed after Er:YAG laser irradiation in our study, a previous *in vitro* study on effect of Er:YAG laser irradiation (with different laser parameters than in the present study) on periodontally healthy root surface by Israel et al<sup>17</sup> reported an acid-etched appearance of the root surface, as observed with SEM.

This observation of acid-like etching patterns with current laser parameters (140 mJ/pulse and 10 Hz, 15 to 20 degrees in contact mode) may have important clinical implications while restoring root-surface caries (if indicated) with a composite restoration. In this context, the evaluation of Er:YAG laser (180 mJ/pulse, 10 Hz, 90-degree angle with contact probe) for root caries treatment compared with conventional bur treatment in an *in vitro* study by Aoki et al<sup>35</sup> revealed that Er:YAG laser ablated carious dentin effectively with minimal thermal damage to the surrounding intact dentin, and removed infected and softened carious dentin to the same degree as the bur treatment. Furthermore, there were characteristic micro-irregularities of the lased dentin surface without smear layer, which appeared to be an advantageous substrate for adhesive luting of resin composites.

However, in our study, along with the presence of etched surfaces resembling that achieved with acid application, there was melting and smear layer formation on the root surface (periodontally healthy, caries free), which may weaken the bond strength of the composite to the exposed dentin (if such a restoration is indicated). Thus, an additional acid-etching step would be required after the laser irradiation to remove the smear layer and facilitate adequate bond strength for the composite restoration.

The intergroup comparisons between the HF and HNF groups using dichotomous data indicated that undesirable surface alterations, such as smear layer and open dentinal tubules, as well as changes that could be deemed desirable, such as surface etching and exposure of collagen tufts, were noted with greater frequency in HNF than HF specimens. Interestingly, surface melting was seen more often in HF specimens. Although a conclusive interpretation of such results would be invalid, one might conjecture that fluorosed specimens are more resistant to the energy levels used

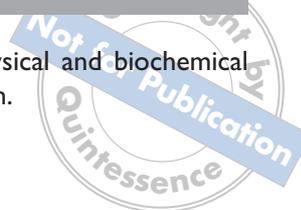
in the current study. Further, the higher content of fluoroapatite in fluorosed specimens may render them more susceptible to melting of the mineral component.

The variability of effect in HF and HNF specimens following Er:YAG laser irradiation is analogous to the variable response to acid etching reported by Vandana et al.<sup>36</sup> The authors studied the effects of acid-induced surface etching on HF and HNF specimens using tetracycline hydrochloride (TCH), ethylene diamine tetra acetic acid (EDTA), and citric acid (CA). They reported that the HF group specimens exhibited more etching and smear layer formation when treated with TCH and CA than did HNF specimens. In contrast, when EDTA was used, the HNF specimens exhibited more surface etching and smear layer formation. Thus, one can only conclude that variability between HF and HNF specimens following treatment by either the Er:YAG laser or mild acidic solutions indicates the need for a more refined research approach in order to determine possible clinical implications.

A search of the current literature fails to reveal any studies comparable to the present investigation, i.e., effect of the Er:YAG laser on fluorosed vs nonfluorosed tooth root structure. Consequently, meaningful comparisons are not possible. In this regard, it is well recognized that a major dilemma in the dental laser literature is the lack of standardization between studies which, in turn, prevents comparison of results. Published studies may differ with respect to laser wavelengths; parameters may vary widely or be insufficiently reported, which in turn does not allow calculation of energy density. In addition, there are differences in experimental design, and often a lack of proper controls.<sup>37</sup>

There are several obvious limits of the present study, eg, its small sample size. Having noted that, the sample size in the present study is similar to earlier investigations by Crespi et al,<sup>16</sup> Eberhard et al,<sup>31</sup> and Moghare et al,<sup>33</sup> and larger than that of other investigations reporting on the effects of the Er:YAG laser on dental tissues.<sup>2,4,6,17,21,22,29,30</sup> A second shortcoming is the use of the SEM for evaluation of root surface changes. The SEM is a surface-scanning microscope and does not detect subsurface damage.<sup>37</sup> In this context, a study by Folwaczny et al<sup>7</sup> noted subsurface alterations in dentin following ablation of cementum by the Er:YAG laser at power settings of 60, 100, and 180 mJ by using light microscopy. Such subsurface alterations were not reported in their previous studies, in which SEM was the instrument of choice for specimen evaluation.<sup>21</sup> Thus, one may suggest that future studies comparing HF and HNF specimens should access

both surface and subsurface physical and biochemical changes following laser irradiation.



## CONCLUSION

Comparative SEM evaluation of HF and HNF specimens following treatment with an Er:YAG laser failed to demonstrate any significant differences in response within or between the specimen groups. In the present study, as the observed undesirable morphological changes were similar in HF and HNF groups using the Er:YAG laser energy settings of 140 mJ/pulse and 10 Hz, further in vitro studies are required at lower energy settings, followed by clinical trials to elucidate and validate the use of Er:YAG laser on the periodontally healthy fluorosed root surface. However, the effects and subsequent application of Er:YAG laser on the periodontally diseased fluorosed root surface needs to be studied. Also, the laser parameters used in this study caused an acid-etched appearance, but with the presence of smear layer and melting of the root surface. This may hamper an adequate bond strength of the composite restoration (if indicated for restoring root caries); thus, an additional chemical acid etching may be needed to remove this smear layer and facilitate the bonding of the composite restoration to the exposed dentin surface.

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