# Evaluation of Primary Tooth Enamel Surface Morphology and Microhardness after Nd:YAG Laser Irradiation and APF Gel Treatment—An *in vitro* study

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***Objective***: *Laser irradiation and fluoride has been used as a preventive tool to combat dental caries in permanent teeth, but little has been done for primary teeth which are more prone to caries. The purpose of this study was to evaluate microhardness alterations in the primary tooth enamel after Nd-YAG laser irradiation alone and combined with topical fluoride treatment either before or after Nd-YAG laser irradiation.* ***Method***: *Ten primary molars were sectioned and assigned randomly to: control group, Nd-YAG laser irradiation, Nd-YAG lasing before APF and APF followed by Nd-YAG lasing. The groups were evaluated for microhardness. Surface morphological changes were observed using SEM.* ***Results***: *Statistical comparisons were performed. The control group's SEM showed a relatively smooth enamel surface and lasing group had fine cracks and porosities. In the lasing + fluoride group a homogenous confluent surface was seen. In the fluoride + lasing group an irregular contour with marked crack propagation was noted. There was a significant increase in the microhardness of the treatment groups.* ***Conclusion***: *Nd-YAG laser irradiation and combined APF treatment of the primary tooth enamel gave morphologically hardened enamel surface which can be a protective barrier against a cariogenic attack.*

***Keywords***: *Primary tooth enamel, Nd-YAG laser, fluoride, SEM and Microhardness.*
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## INTRODUCTION

The past five decades has seen dramatic changes in the field of dentistry, especially preventive dentistry with the chance discovery of fluoride and its role played in caries prevention. The primary dentition is at a much higher risk for caries development than the permanent dentition. The major factors contributing for this could be composition and thickness of enamel in primary teeth. This may help to account for the fact that two thirds of the caries in primary teeth occur on the smooth surfaces, where as 10-15% of caries lesion develop in the smooth surfaces of permanent teeth. In contrast pit and fissure caries accounts for 85-90% of the lesion in permanent dentition.[1] Feeding habits, illness in early infancy with possible disruption and disturbance in amelogenesis and enamel maturation, inadequate oral hygiene measures, lack of knowledge regarding the value of primary dentition by parents and unavailability of dental personnel trained in infant oral health, may all contribute in some way to the increased prevalence of caries in primary dentition.

Since Stern and Sognnaes demonstrated that laser exposure by using ruby laser increases acid resistance of tooth enamel, many investigations related to the application of laser in the area of preventive dentistry have been carried out. Among them Yamamoto *et al* indicated that human enamel irradiated with Nd-YAG laser was more resistant to acid decalcification than an unlased control. Suzuki, Morita *et.al* using various types of laser suggested Nd-YAG Lasers were suitable for clinical use.[2] On the other hand topically applied fluorides being used as caries preventive agents, Sodium Fluoride (NaF) and acidulated phosphate fluoride (APF) being the most well known fluorides in dental practice. Another field of interest has been the exploration of combined use of laser and fluoride for the purpose of developing new and more effective procedures for caries prevention. Yamamoto and Sato demonstrated that smooth surface

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of enamel exposed to normal pulsed Nd-YAG laser after application of fluoride, increased fluoride uptake in to the enamel and reduced the subsurface demineralization in permanent teeth. However until now no detailed studies have been reported about the use of Nd-YAG laser on enamel of primary tooth smooth surface along with fluoride.

The purpose of this study was to characterize surface morphological alteration and microhardness variations in primary tooth enamel after *in vitro* low fluence Nd-YAG laser irradiation alone and combined topical fluoride treatment either before or after low fluence Nd-YAG laser irradiation.

## MATERIALS AND METHOD

This study was conducted in the Department of Pedodontics and Preventive Dentistry, College Of Dental Sciences, Davangere. Ethical clearance to conduct the study was obtained from the governing body for ethical issues of the institute. Informed consent was obtained from the parents to use the extracted/exfoliated teeth. The materials of the study comprised of a total number of 10 human healthy extracted retained primary molars/exfoliated primary molars. They were then cleaned using medium/coarse grit pumice and kept in distilled water up to their use in the study. Each tooth was subjected to thorough examination using a magnifying glass and only those teeth which did not show any demineralization areas, mottled areas or cracks were selected for the study.

The study was divided into two parts:

Step 1: evaluation of the effects of Nd:YAG lasers on the surface topography of the primary tooth enamel.
Step 2: evaluation and comparison of the surface micro hardness of the normal, lased only, lased + fluoridated and fluoridated + lased primary tooth surface.

The selected 10 teeth were taken and sectioned using a straight hand piece using a diamond disc (Rapidocut disc, DFS Co Ltd.,Germany) onto the mandrill. The (1/4th) quarter enamel slabs of each tooth were assigned randomly to below mention 4 groups. The samples were assigned to 4 groups: Group1 (control) .Group 2 (lasing only) .Group 3 (lasing + fluoride) .Group 4 (fluoride + lasing).

Once this grouping was completed the enamel surface of group 2 and 3 were coated with Chinese black ink using a fine camel hair brush (no 2) to make absorption of laser more effective, each sample was lased using Quanta Ray Nd:Yag Laser (Newport corp. USA )for a duration of 10 seconds. Once the lasing is completed the enamel surface is cleansed of the ink using methanol. The 3rd group was then subjected to 4 minute APF gel treatment followed by thorough rinsing with double de-ionized distilled water. The 4th group was first subjected to APF gel for 4 minutes and then rinsed in double de ionized water followed by blotting it dry with a blotting paper. This is followed by application of Chinese black ink on the enamel surface and then subjected to lasing for 10 seconds. All the groups were then evaluated for micro hardness variations using ZWICK/ROELL indenter and surface morphological changes using Scanning Electronic Microscopy after the respective treatments. The obtained results of micro hardness were subjected to statistical analysis by ANOVA, Mann-Whitney and Wilcoxon's analysis for inter and intra group comparisons.

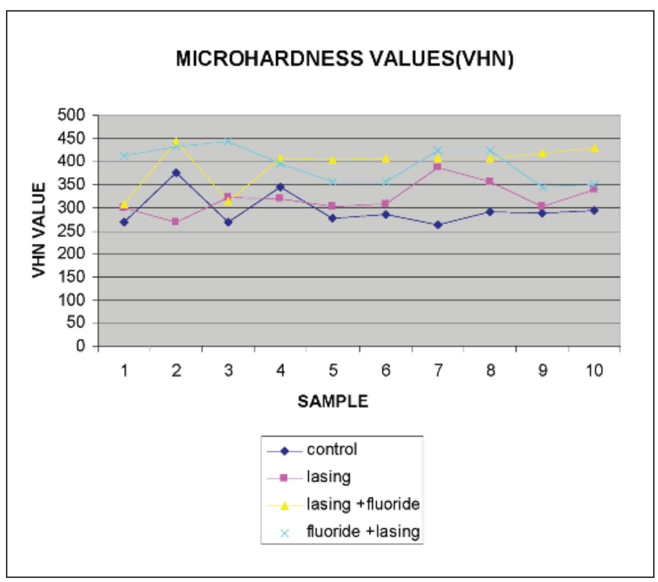
## RESULTS

The primary tooth samples showed an interesting pattern of increase in microhardness Vickers hardness number (VHN) in comparison to control and treated groups. The control group which was untreated had a range of 264-376 (VHN) with a mean value of 295.4.The lasing group showed a range of 269-387 (VHN) with a mean of 320.3. The lasing + fluoride group showed a range of 307- 444 (VHN) with a mean of 394.6. The fluoride + lasing group showed a range of 344-444 with a mean of 394 (Table 1 and Graph1). Table 1 shows a marked increase in the microhardness (VHN) from control to the treatment groups. A one way ANOVA was carried out for the intergroup variance of the microhardness values for the four groups and a P value of less than 0.01 indicated a

**Table 1. Comparision of Range, Mean and Standard Deviation of Each Group (VHN)**

| | CONTROL | LASING | LASING + FLUORIDE | FLUORIDE + LASING |
|---|---|---|---|---|
| RANGE (Min-Max) | 264-376 | 269-387 | 307-444 | 344-444 |
| MEAN | 295.4 | 320.3 | 394.6 | 394.0 |
| STANDARD DEVATION | 36.4 | 33.6 | 46.3 | 38.4 |

One way ANOVA, F=17.1, P<0.01, S

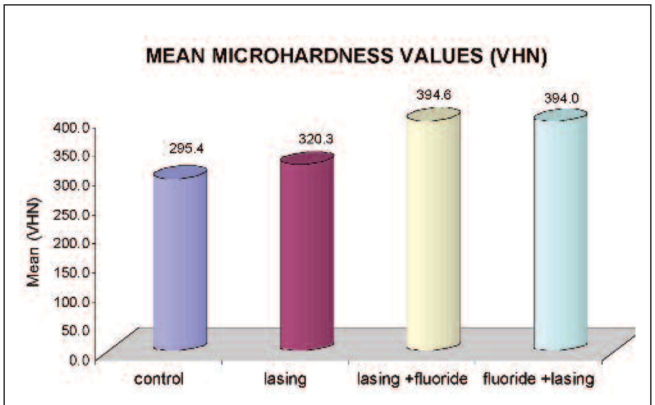



**Graph 1.**

**Table 2.** Difference in Microhardness of All Treatment Groups (VHN)

| | CONTROL | LASING | | LASING + FLUORIDE | | FLUORIDE + LASING | |
|---|---|---|---|---|---|---|---|
| | | | DIFF | | DIFF | | DIFF |
| MEAN | 295.4 | 320.3 | 24.9 | 394.6 | 99.2 | 394.0 | 98.6 |
| STANDARD DEVIATION | 36.4 | 33.6 | 60.4 | 46.3 | 40.2 | 38.4 | 49.7 |
| %INCREASE (from control) | _ | _ | 8.4 | _ | 33.6 | _ | 33.4 |
| Z Value* | _ | _ | 1.58 | _ | 2.80 | _ | 2.81 |
| P - value | _ | _ | 0.11,NS | _ | <0.01,S | _ | <0.01,S |

*Wilcoxon's signed rank test (intra group comparison) P<0.05, P<0.01, significant>0.05 Not significant.

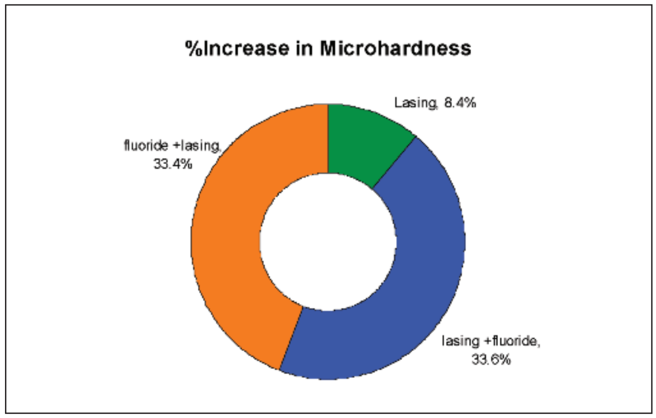



**Graph 2.**

**Table 3.** Comparison & % Increase of Microhardness Between Treatment Groups

| | Microhardness(VHN) (Difference from control) | | | GROUP COMPARISIONS | |
|---|---|---|---|---|---|
| | MEAN | ±SD | %INCREASE | GROUPS COMPARED | P- VALUE* |
| LASING | 24.9 | ± 60.4 | 8.4 | LASING Vs. LASING + FLUORIDE | <0.01,S |
| LASING + FLUORIDE | 99.2 | ± 40.2 | 33.6 | LASING Vs. FLUORIDE + LASING | <0.01,S |
| FLUORIDE + LASING | 98.6 | ± 49.7 | 33.4 | LASING + FLUORIDE Vs. FLUORIDE + LASING | 0.82,NS |

*Mann-Whitney test (inter group comparison)




**Graph 3.**

significant increase in microhardness from control to treatment groups. The mean and percentage increase of the difference in microhardness of treated groups to that of the control group are as follows: (Table 2 and Graph 2,3) for lasing group a mean difference of 24.9 with a percentage (%) increase of 8.4 was seen in comparison to control which was not significant. The lasing + fluoride group showed a significant increase in mean difference from control by 99.2 and a % increase of 33.6.The fluoride +lasing group showed a similar significant increase in mean difference of 98.6 and a % increase of 33.4.The percentage increase in microhardness of the treatment groups on intergroup comparison showed statistically significant variation between lasing alone to lasing+ fluoride and fluoride+ lasing (Table 3). It was not significant statistically on comparing lasing + fluoride to fluoride + lasing groups.

## SCANNING ELECTRON MICROGRAPHS (×1000)

The sound enamel surfaces from the control group were relatively smooth with frequent enamel prism ends present on the surface. The surface showed shallow depressions and fine porosities within these depressions (Figure 1). The enamel surfaces are devoid of any surface deposits. The micrograph of lased group showed irregular roughened surfaces with occasional areas of fine surface cracking and discontinuities (white arrow, Figure 2). The surfaces were peppered with cavitations and craters leading to an irregular undulated surface. The surface did not show any of the usual enamel prism ends seen in control group. The micrograph of lasing + fluoride group, showed a relatively homogeneous and confluent surface coatings that masked the under lying enamel surface (white arrow, Figure 3). Loosely adherent surface granules and globules were present (black arrow,

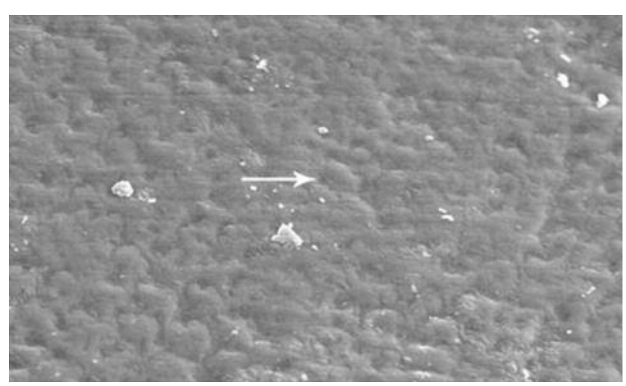


**Figure 1.** Control group's surface morphology SEM (x1000). White arrow-surface showing shallow depressions and fine porosities.

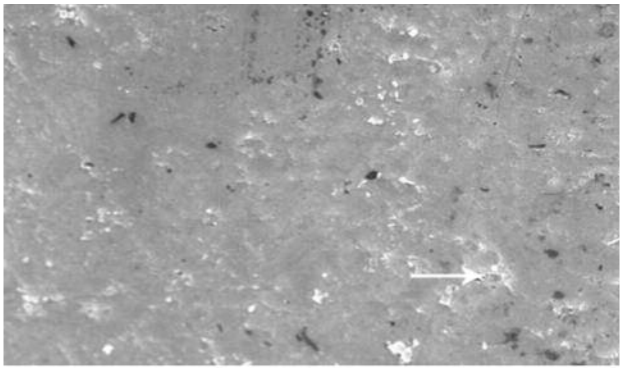


**Figure 2.** Lasing alone group's surface morphology SEM (x1000). White arrow – cavitations and craters leading to irregular surface

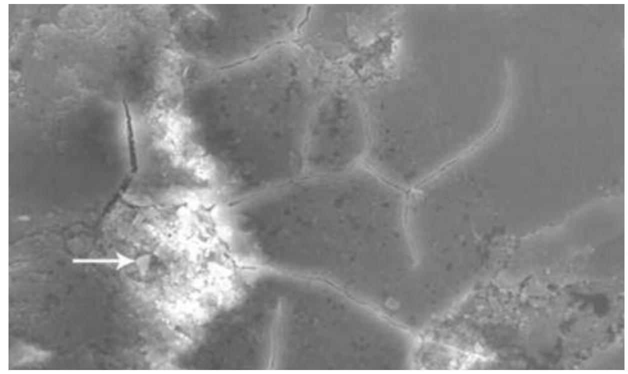


**Figure 4.** Fluoride+lasing group's surface morphology SEM (x1000). White arrow – cracked surface coating due to lasing

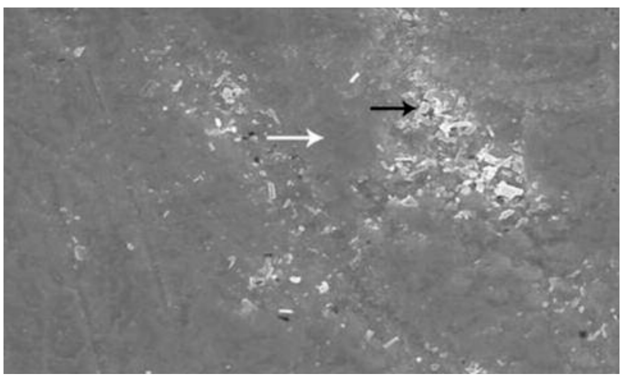


**Figure 3.** Lasing+fluoride group's surface morphology SEM (x1000). White arrow – homogeneous and confluent surface coating Black arrow – loosely adherent granules and globules

Figure 3). There are also infrequent small isolated porosities without fracturing of the surface coatings. The micrographs of the fluoride + lasing group had irregular contours with numerous granular to globular irregularities protruding from the surface coating. The surface coatings were quiet porous and had a prominent fracturing pattern with fine cracking of surface coating. Numerous cracked surface coatings covered almost the entire surface (white arrow, Figure 4).

## DISCUSSION

The role of laser irradiation on dental enamel has been studied in a multi-pronged manner using different lasers and techniques. Yamamoto and Sato indicated that human enamel irradiated with acousto-optically Q-switched Nd-YAG laser was more resistant to acid decalcification than an unlased control.[3] Tagomori and Morika in there review suggested that the normal pulsed and acousto-optically Q-switched Nd-YAG lasers were suitable for clinical use.[2] Normal pulse was found to be more suitable than acousto-optically Q-switched lasers because of the higher acid resistance and lower degree of damage of the enamel surface. Hence the laser used in this study was a normal pulsed Nd-YAG laser. Delbem *et al* [4] have shown that APF gel application was more effective than neutral gel on reduction of enamel loss by microhardness evaluation Goodman and Kaufman[5] qualitatively demonstrated that enamel exposed to an Argon laser in the presence of NaF, had increased the fluoride up take and decreased the rate of dissolution in acidic solution.

### *Vickers Microhardness Evaluation*

Vickers microhardness analysis was done in accordance to the studies done by Gutierrez-Salazar and Reyes-Gasga,[6] Tagomori and Iwase[7] and Kuramoto.[8] Surface microhardness has been accepted to evaluate mineral loss or gain by the enamel.[9] The mean value of microhardness obtained for control group in our study is 295.4 VHN which was lower than to that obtained in the study by Westerman et al., which gave a mean of 316 HK. This slight increase can be attributed to use of permanent teeth and using Knoop system of hardness.[10]

The mean microhardness value obtained for lasing group in our study was 320.3 VHN. The microhardness of the lasing + fluoride (394.6) group showed highest increase in our study which was not statistically significant in comparison to fluoride + lasing (394.0) but in the Westerman et al.[9] study only a marginal increase was seen between lasing alone and lasing + fluoride .This fact can be attributed to the difference in the samples used i.e., primary enamel and lasing source parameters in our study. Several possible mechanisms have been proposed for the irradiation-induced caries resistance.[9] Irradiation reduced the lattice strain of hydroxyapatite and decreased solubility by alterations in carbonate, water and organic content of the tooth mineral phases. It led to microseive creation allowing re-precipitation of mobilized calcium, phosphate and fluoride during demineralization. Lasing reduced the permeability of mineral structure because of protein denaturation and protein swelling. It Increased uptake of fluoride, calcium, and phosphate from exogenous sources. It created surface coating reservoirs for calcium, phosphate and fluoride; in high risk conditions it had bacteriostatic or bactericidal effect on plaque microorganisms.

Marquez *et al*[11] used a 5 kHz Q-switched Nd-YAG noted an increase in microhardness (KHN) attributed results by

SEM to the fusion of enamel surface. Shimuzi[8] in his study found a similar value of microhardness to that of unlased enamel. Where as Kuramoto,[8] Tagomori *et al*,[7] and Jannet *et al,*[12] have shown a decrease in the microhardness values of the lased enamel. According to Kuramoto[8] the samples lased with low energy (3-21j) showed similar microhardness values to that of control and those subjected to the higher energy (30-100j) showed lesser microhardness values to the control. This was attributed to the fact that higher energy produced bigger cracks and made the enamel brittle leading to decreased microhardness. Here in our study we have used laser parameters of 0.67j as the pulse energy with a pulse energy density of 11.5 j/cm$^2$. The enamel was lased for a duration of 10 sec, which has 10 nano second pulses. These parameters are in accordance to the parameters used by Tagomori and Iwase[7] study. The increase in micro hardness in our study can be attributed to the fact that low fluence energy on primary tooth enamel has been used along with treatment of the lased groups to fluoride application where in the former studies fluoride has not been used.

### *Scanning Electron Micrograph*

The micrographs of the control and lased group respectively revealed the typical structure of the normal enamel and minute cracks and crazings with occasional craters as seen in the study done by Hicks *et al*.[1] But Tagomori and Iwase *et al*[7] reported melted enamel and coalescence of globules, with apparent irregularities and holes in the outer most layer of the enamel.

The SEM micrographs of both the lasing + fluoride and that of fluoride +lasing group were in accordance to the Hicks study.[1] The surface coating of lasing + fluoride group may provide a reservoir of mineral phases during a cariogenic attack and thus the surface layer contains high amount of fluoride in the form of calcium fluoride obtained by the reaction of the fluoride in the APF gel with enamel and making it resistant to caries and also increase in the microhardness of the surface enamel.[13,14] In the last group of our study i.e., fluoride + lasing the surface features can be attributed to two things one is Nd-YAG lasing of the fluoride coating and the second one may be due to surface desiccation during specimen preparation of the SEM. Even though the microhardness of this group was significantly high in comparison to the control group because of the irregular surface coating it need to be evaluated for the effective anti-cariogenic potential using an acidic attack.

The presence of adherent calcium, phosphate, fluoride rich phases[1,14,15,16,17] on low fluences argon lased teeth would help to explain the effects of lasing on in-vitro caries lesion formation which resulted in a caries reduction of 25%-30% in comparison to controls which were unlased. This can hold good even in our study also. The addition of APF treatment prior to and after the Argon laser irradiation of the primary enamel resulted in surface coating and globular and granular material[1,15,16,17,18] is due to the acidic nature of the APF and its effect on enamel solubility. During APF treatment, a thin layer of enamel may become solubilized and the resultant mobilized mineral phases undergo re precipitation on the enamel surface as fluoride rich calcium and phosphate mineral phases (fluoridated di calcium phosphate di hydrate, fluoridated octa calcium phosphate, fluoridated hydroxyapatite).[1,17,18] In previous *in vitro* studies[1,19,20,21] lasing + APF led to 40%-60% reduction in caries. It has not shown any difference in caries resistance on application of APF before or after lasing.[1,19,20,21,22] It has been suggested that the combination of laser + APF affects the critical pH at which enamel undergoes dissolution. The pH of sound enamel is 5.5 is reduced 5 fold due to lasing to 4.8 and by further 6 fold when fluoride is used in combination with lasing to 4.3.[1,23,24] Thus the benefit of combination of fluoride treatment and lasing is readily apparent and can be applied to our study.

## CONCLUSION

Lasing of the enamel surface has resulted in a marked increase in the microhardness. Lasing followed by 1.23% APF gel application has resulted in the highest attainment of microhardness (33.6) followed by fluoride + lasing (33.4), then by lasing alone (8.4%) in comparison to that of the control. The scanning electron micrograph showed a typical topography of the enamel rods, the lasing group showed surface roughening with crazing .the lasing + fluoride group showed a homogenous surface coating of fluoride covering the enamel. The fluoride + lasing group displayed a severely cracked surface coating on the enamel. Lasing followed by fluoride application can be advocated as one of the preventive measure in the fight against caries in primary teeth. Further studies are needed to evaluate the acid resistance of these treated primary teeth to have an in-depth knowledge regarding the preventive potential of lasers.

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