SYNERGISTIC EFFECT OF ND:YAG LASER COMBINED WITH FLUORIDE VARNISH ON INHIBITION OF CARIES FORMATION IN DENTAL PITS AND FISSURES *IN VITRO*

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Background and purpose: Although the effectiveness of neodymium yttrium aluminum garnet (Nd:YAG) laser and fluoride anticaries treatment has been established, most previous studies focused on smooth tooth surfaces. We evaluated the anticaries effects of Nd:YAG laser combined with fluoride varnish (Duraphat) on caries-susceptible pit and fissure areas.
Methods: A total of 36 noncarious molars were treated with either a Nd:YAG laser (2.5 W, 6 sec) followed by fluoride varnish, Nd:YAG laser only, fluoride varnish only, or no treatment (control). Artificial carious lesions were created to assess the acid resistance of enamel after treatment. Undecalcified successive tooth slices were made. Percentage lesion formation, lesion length, and lesion depth were evaluated using polarized light microscopy.
Results: The Nd:YAG laser enhanced the resistance of dental enamel to acid challenge. However, Nd:YAG laser alone was not as effective as the Nd:YAG laser combined with fluoride varnish, especially for the treatment of pits and fissures. Nd:YAG laser treatment combined with fluoride varnish inhibited 43% of lesions at pits and fissures and 80% of lesions on smooth surfaces compared to no treatment. Carious lesions had shallower depth and shorter length. No carious lesion extended beyond the dentinoenamel junction in either laser-treatment group.
Conclusions: A synergistic effect on dental caries prevention in pit and fissure areas and on the smooth surfaces of the tooth can be achieved by applying Nd:YAG laser followed by fluoride varnish.

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Key words:
Nd:YAG laser
fluoride varnish
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Fluoride is a well known preventive agent for dental caries. However, some individuals who receive fluoride still develop pit and fissure caries. This finding indicates that fluoride is apparently less effective for pits and fissures than it is for smooth surfaces of teeth [1, 2]. As discussed in our previous report [3], the vulnerable anatomic structures of pits and fissures account for the prevalence of occlusal caries. Pit and fissure sealant has been developed to overcome this problem. However, complete or partial loss of sealant and resulting secondary caries is common [4]. These facts, of particular concern in areas of high caries prevalence such as Taiwan, emphasize the need for optimizing a caries preventive strategy and measures targeted on pit and fissure areas.

Laser treatment is effective in elevating the resistance of enamel to cariogenic challenge, reducing enamel solubility, and inhibiting caries-like lesion formation [5–8]. The combined effects of fluoride and laser treatment have not been widely studied. Combined laser and fluoride treatment has been shown to enhance the resistance of enamel to cariogenic challenge [8] by increasing the uptake of fluoride by enamel and reducing the acid dissolution rate of enamel [9]. A synergistic effect was obtained using a CO_2 laser combined with a modest amount of fluoride [10, 11]. Zhang et al achieved a comparable dental caries inhibition rate for CO_2 laser treatment at pit and fissure areas and fluoride treatment on smooth surfaces [12].

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Previous studies showed that combined neodymium yttrium aluminum garnet (Nd:YAG) laser and fluoride treatment increases the resistance of smooth surface enamel to acid [7, 13].

Fluoride varnish is a well-accepted fluoride regimen that is effective in smooth surface caries prevention [14, 15]. It has the advantages of easy application, fast setting, slow release, and durability. Nonetheless, most previous studies on the combination of laser and fluoride treatment were performed on smooth dental surfaces [8, 10–13], and few were quantitative. Data on the combination of Nd:YAG laser treatment and fluoride varnish on caries prevention at pit and fissure areas are lacking. The purpose of this study was to quantitatively evaluate the effectiveness of Nd:YAG laser combined with a fluoride varnish on dental caries prevention at caries-prone pit and fissure areas of teeth.

Materials and Methods

Extracted impacted third molars or non-carious molars were selected from dental students who had not received previous fluoride treatment, using visual-tactile diagnostic criteria. Questionable teeth were reexamined using a 100 x Hi-Scope magnifier (Compact Micro Vision System Model KH-2200, Tokyo, Japan). Immediately after extraction, the teeth were washed and debrided of attached tissue by curette and ultrasonics. The teeth were then polished with fluoride-free prophylaxis paste. All prepared teeth were stored in deionized water at 4°C until use.

Grouping and treatment

Thirty-six teeth were randomly divided into four treatment groups as follows: group 1, Nd:YAG laser treatment followed by fluoride varnish; group 2, Nd:YAG laser treatment only; group 3, fluoride varnish only; and the control group, no treatment. There were nine teeth in each group. An acid-resistant fingernail varnish was applied on the buccal surface of the tooth with a 2 x 5-mm window exposed for laser irradiation. The acid-resistant varnish was also painted on the occlusal surface and a 1.0-mm rim on two sides surrounding all pits and fissures was left unpainted for experimental treatments. A thin layer of black ink was painted on pits and fissures before Nd:YAG laser irradiation to facilitate the absorption of the laser beam. An Nd:YAG laser (SLT Contact Laser, DCL-8, Tokyo, Japan) with an energy output of 2.5 W and a total energy of 15 J was used to irradiate the tooth surfaces for 6 seconds.

Laser irradiation was applied in a sweeping motion along all pits and fissures in a similar way to that used to apply sealant on pits and fissures. All procedures followed strict laser safety guidelines. For the teeth assigned to groups 1 and 3, 0.5 mL Duraphat (Woelm, Eschwege, Germany; 22,600 ppm F) fluoride varnish was applied to the pit and fissure area and on the smooth surface of each tooth.

Artificial caries creation

After treatment, teeth were immersed in acetate buffer ($CaCl_2$ 2.2 mmol/L, KH_2PO_4 2.2 mmol/L, KF 16.6 μmol/L, acetic acid 50 mmol/L) for 48 hours at 37°C. The pH of the buffer solution was adjusted to 4.6 with 1 mol/L KOH to produce artificial carious lesions [16, 17]. The artificial caries lesions present at the end of immersion had a chalky white appearance with an intact enamel surface.

Tooth sections

After carious lesions were created, undecalcified successive tooth slices were obtained by using a microtome (Isomate low speed saw, Buehler, Lake Bluff, IL, USA). The sections were made bucco-lingually across the major fissure from the mesial to the distal end. The deep and narrow base of the fissure was exposed. Approximately 10 to 12 tooth slices were obtained from each tooth block. The sections were approximately 150 μm thick, and were then ground to about 120 μm in thickness using a grinding stone. The tooth slices were polished with abrasive paper (400 grid, Buehler) and cleaned by ultrasound, then immersed in deionized water for 24 hours before polarized light microscopy.

Polarized light microscopy

The tooth section was examined under a polarized light microscope (Olympus Polarized Light Microscope, BH-2, Tokyo, Japan). Lesion characteristics were observed on both smooth surfaces and pits and fissures for each group (Fig. 1). The following parameters were measured at pit and fissure areas: percentage caries formation, defined as the proportion of artificial lesions present or not in all specimens; percentage of carious lesions extending to the dentinoenamel junction (DEJ), defined as the proportion of specimens for which the advancing front of the carious lesion extended beyond the DEJ; depth of lesions (μm), measured from the widest vertical distance from the tooth surface to the deepest border of the lesion; and length of lesions, defined as the total distance of the involved lesion from the beginning point on the slope of the fissure to the farthest extending front of the lesion (Fig. 1). Distance was measured using the built-in scale of the microscope. On smooth surfaces, percentage caries formation, lesion depth (μm), and percentage

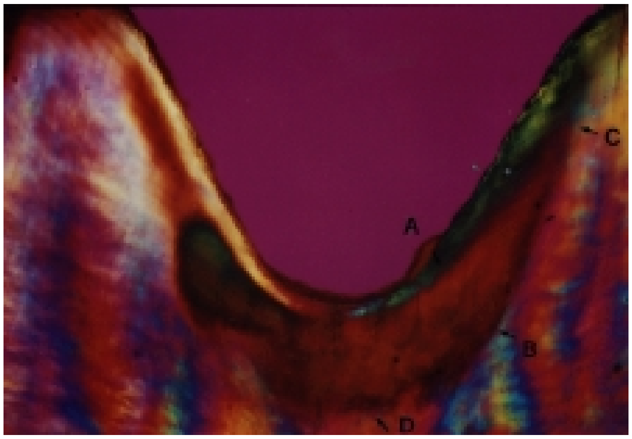

Fig. 1. *Polarized light microscopic photograph showing a pit and fissure area. Parameters measured were depth of lesion (AB), the greatest vertical distance from the tooth surface (A) to the deepest border of the lesion (B); length of lesion (CD), the total distance of lesion involved from the beginning point (C) on the slope of the fissure to the farthest extending end of the lesion (D) (x100).*

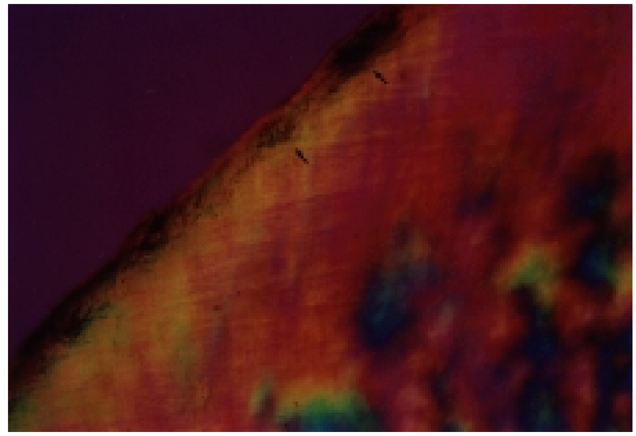

Fig. 2. *Discontinuous, separated small bands of lesions or island-like lesions formed on the surface of the enamel (x100).*

of continuous lesions were measured. A continuous lesion was defined as a non-discontinuous thick lesion formed along the smooth surface. A discontinuous lesion was defined as a separate small band of lesion or island-like lesion (Fig. 2).

Statistical analysis

The depth and length of lesions were calculated and compared. Differences between the four treatment groups were assessed using one-way analysis of variance (ANOVA). Following this, pairwise comparisons were carried out between each experimental group and the control group using paired *t*-test. A p value of 0.05 or less was considered statistically significant.

Results

The Table shows the characteristics of artificial caries formation in the four treatment groups. In terms of the percentage of carious lesions formed, pits and fissures were more vulnerable to acid challenge. In group 1, 52% of specimens developed lesions at the pit and fissure areas, while 15% developed lesions on smooth surfaces. In the control group, 91% of specimens developed lesions in pit and fissure areas, and 72% developed lesions on smooth surfaces.

In the pit and fissure areas, no lesion extended beyond the DEJ in the laser-treatment groups. Com-

Table. Characteristics of artificial caries formation in the four treatment groups

	Group 1	Group 2	Group 3	Control group
Pits and fissures				
Lesion formation (%)	52	77	87	91
Lesions involving DEJ (%)	0	0	17	30
Lesion depth (mm)	112 ± 10*	116 ± 12*	181 ± 20	151 ± 13
Lesion length (mm)	627 ± 56*	747 ± 71	763 ± 69	1,027 ± 98
Smooth surfaces				
Lesion formation(%)	15	39	52	72
Continuous lesions (%)	0	50	62	71
Lesion depth (mm)	77 ± 7*†	100 ± 9*	102 ± 9*	152 ± 10

Group 1 = Nd:YAG laser followed by fluoride varnish; group 2 = Nd:YAG laser only; group 3 = fluoride varnish only; DEJ = dentinoenamel junction. *$p < 0.05$; †$p < 0.01$.

pared to the control group, the depth of lesion reduction was 27% in group 1, 23% in group 2, and 20% in group 3. The length of lesion reduction compared to the control group was 39% in group 1, 27% in group 2, and 29% in group 3.

On smooth surfaces, the lesion depths measured in the four experimental groups were 77 ± 7, 100 ± 9, 102 ± 9 and 152 ± 10 μm, respectively. There was no continuous lesion formation in group 1. Nonetheless, 50%, 62%, and 71% of lesions were continuous in group 2, group 3, and the control group, respectively. These results demonstrate that enamel treated with the Nd:YAG laser followed by fluoride varnish gained stronger acid resistance than enamel receiving other treatments. A pronounced inhibitory effect in group 1 was evident. Group 2 demonstrated less of an inhibitory effect as evidenced by discontinuous, separated small bands of lesions or island-like lesion formation, while other groups exhibited wider and continuous lesions. Compared with the control group, the lesion depth reduction was 49% in group 1, 34% in group 2, and 33% in group 3.

The depth of lesions at pit and fissure areas was significantly less in groups 1 and 2 than in the control group ($p < 0.05$). However, when the difference in the length of lesions was compared, only group 1 was significantly different from the control group ($p < 0.05$) in pit and fissure areas, while a significant difference in length was found between all experimental groups and the control group on smooth surfaces.

From the parameters measured, the rank order of anticaries potential of the treatments was Nd:YAG laser combined with fluoride varnish, Nd:YAG laser alone, and fluoride varnish only.

Discussion

During our evaluation of experimental carious lesions under a polarized light microscope, it seemed that the laser corrected the anatomic defects of pits and fissures by cleansing and odontoplasty-like effects. This finding was also observed by Bahar and Tagomori [7]. Physical sealing of pits and fissures by laser irradiation has also been observed [3], and is another possible mechanism of caries inhibition. It is achieved by melting and resolidification of the enamel surface crystal at the pit and fissure area [6, 18].

Flaitz et al postulated that argon laser irradiation produces a microsieve network with entrapment and reprecipitation of minerals released during the demineralization process in teeth [8]. The ions released by acid demineralization would be trapped in the microsieve network in lased enamel, whereas such ions diffuse into the surrounding solution in unlased enamel. Argon laser at relatively low fluences produces surface coatings that may provide a certain degree of protection against acid challenge [19].

During Nd:YAG laser irradiation, many partially coalescent globular granules are also produced by the process of melting and the subsequent resolidification of enamel crystals [13]. In this situation, fluoride can easily penetrate into the spaces between the granules. The microspaces formed by laser irradiation may trap the demineralized ions and provide space to allow them to combine with fluoride.

Previous studies have shown that prolonged contact of fluoride varnish on tooth surfaces results in greater deposition of CaF_2-like material on the enamel surface, which may function as a fluoride reservoir [20, 21]. The availability of fluoride ions from CaF_2-like material in the liquid phase around the apatite crystallites is thought to be more important in decreasing dissolution of crystallites than in incorporating fluoride into the crystal lattice [22, 23]. The fluoride from enamel treated with combined laser and fluoride varnish undergoes prolonged release, resulting in greater deposition of CaF_2-like material, which facilitates remineralization of the lesion. Moreover, a less permeable enamel surface for ion diffusion results in inhibition of artificial lesion formation. These facts help explain the remarkable synergism achieved by laser combined with fluoride over either respective treatment modality [10–13].

Fox et al showed that the synergistic effect of combined continuous-wave CO_2 laser treatment and a chemical inhibitor, such as fluoride, in demineralization solution results in almost complete inhibition of enamel dissolution [10]. Laser irradiation alone at a higher energy dose was found to be more effective in increasing the acid resistance of enamel than a lower energy dose. However, in the presence of fluoride, the energy density of the laser applied can be reduced [10, 11]. In our experiment, we used a reduced-energy Nd:YAG laser immediately followed by high-concentration fluoride treatment. The caries formation inhibition rates in our study were comparable to those of Fox et al [10]. Thus, our results also suggest that the clinically detectable damage to teeth, such as brown discoloration and friability, can be avoided with this treatment.

Tagomori and Morioka demonstrated maximal inhibition of calcium dissolution from the enamel surface by prolonged fluoride treatment followed by Nd: YAG laser treatment [13]. Our experimental procedures were reversed, but the lesion inhibition rate on smooth surfaces was consistent with theirs. In our study, fluoride varnish was able to adhere to the enamel for a longer period of time and slowly release the fluoride into the teeth. The retentive and slow-release effects of this procedure prolonged the time of tooth exposure to fluoride. Nd:YAG laser treatment followed by fluoride varnish application inhibited the extension of carious lesions beyond the DEJ within the experimental period, suggesting its value for clinical application.

In conclusion, Nd:YAG laser irradiation followed by fluoride varnish application to either pit and fissure areas or smooth surface areas achieved a synergistic effect preventing caries formation compared to either respective treatment alone, especially in pit and fissure areas where fluoride treatment is unfavorable. These results suggest the usefulness of Nd:YAG laser treatment combined with fluoride varnish as an effective measure for preventing dental caries at pit and fissure areas. However, demonstration of similar results in a clinical situation, as well as determination of safety of this treatment, is needed.

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