Effects of CO_2 laser and fluorides application on root demineralization

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Purpose: The effects of CO_2 laser irradiation and fluoride application on the acid resistance of tooth root surfaces were evaluated.
Materials and Methods: A total of 35 extracted human premolar roots were completely coated with acid-resistant varnish except for a window (2x3 mm) on the proximal surface. The roots were assigned to one of seven treatment groups as follows: CO_2 laser irradiation at 17 J/cm^2 (Group 1); irradiation at 25 J/cm^2 (Group 2); irradiation at 41 J/cm^2 (Group 3); 200-minute soak in 0.05% NaF solution (Group 4); 50-minute soak in 0.2% NaF solution (Group 5); 5-minute application of 2.0% NaF solution (Group 6); and no treatment (control). The specimens were subjected to a 2-day pH-controlled cyclic demineralization/remineralization process. The lesion depth on each sample was measured using polarized-light microscopy.
Results: The lesions in groups 3, 4, 5, and 6 were significantly shallower than the lesions in the control specimens ($p<0.01$), but there were no significant differences between groups 1 and 2 and the control, or between groups 1, 2, 3, and 4 ($p>0.05$). The lesions in groups 5 and 6 were significantly shallower. Group 3 exhibited significantly reduced lesion depth compared to the control.
Conclusion: Fluoride application significantly improved the acid resistance of the root surface. CO_2 laser irradiation at an energy density of 41 J/cm^2 improved the acid resistance of the root surface, while CO_2 laser irradiation at energy densities of 15 and 27 J/cm^2 had no beneficial effect.
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Key Words: fluoride, laser, tooth demineralization/remineralization, tooth root

Introduction

The elderly population has been steadily increasing, and development of periodontal treatments and dental home-care techniques has contributed to ensure that individuals retain their natural teeth as they age.[1] During aging, the root surfaces of teeth gradually become exposed to the oral environment due to gingival recession and consequently become susceptible to dental caries.[2,3] The critical pH at which decalcification occurs on root surface is the same as for deciduous teeth, and tooth decay in this area is a common problem in older patients.

The use of CO_2 laser irradiation to prevent enamel or dentin caries has been well described,[4-13] and argon lasers have also been reported to be effective in enhancing the demineralization resistance of root surfaces.[14,15] However, few studies have demonstrated the effectiveness of CO_2 laser irradiation in preventing root surface demineralization.[16] Furthermore, the effect of energy density in CO_2 laser irradiation on root demineralization has not been confirmed in previous studies.

It is well known that the application of fluoride to enamel surfaces increases resistance to demineralization,[17-23] and several studies have described the effect of fluoride application on root surface demineralization.[24-29] One in vitro study examined the demineralization of human molar roots following exposure to pH 5.5 buffered solutions containing various concentrations of fluoride, calcium, and phosphate. The rate of demineralization decreased with the increase of fluoride concentration.[23] Acidulated phosphate fluoride (APF) treatment significantly enhanced the resistance to demineralization of root surfaces during *in vitro* acidogenic exposure.[24] The fluoride concentration and application period may influence the efficacy of root surface treatments. Although these aspects are important, they have not been investigated in previous studies.

Therefore, the present study examined the effects of energy density of CO_2 laser and fluoride concentration on the depth of root surface lesions occurring after pH cycling. The null hypothesis was that the CO_2 laser energy density and the fluoride concentration would not affect the demineralization resistance on root surface.

Materials and Methods

This study was approved by the Ethics Committee of The Nippon Dental University School of Life Dentistry at Niigata (#ECNG-H-40, 2010). The study was undertaken using 35 extracted human premolars with previously unexposed, caries-free root surfaces. Informed consent was obtained from the patients under a protocol approved by the institutional review board of The Nippon Dental University School of Life Dentistry at Niigata. The teeth were cleaned and stored in 0.01% thymol solution at 4˚C until use. The crown was removed approximately 2 mm below the cemento-enamel junction, and the root was longitudinally sectioned at the center of the buccal surface. The root surface was lightly planed immediately before carrying out the experiment.

The roots were assigned to one of seven treatment groups as follows: CO_2 laser irradiation at 17 J/cm^2 (Group 1); irradiation at 25 J/cm^2 (Group 2); irradiation at 41 J/cm^2 (Group 3); 200-minute soak in 0.05% NaF solution (Group 4); 50-minute soak in 0.2% NaF solution (Group 5); 5-minute application of 2.0% NaF solution (Group 6); and no treatment (control). Exposure was limited to a rectangular area (2x3 mm) on the root surface created by coating the remaining surfaces with acid-resistant varnish (Protect varnish, Kuraray Noritake Dental, Tokyo, Japan). The exposed area was irradiated using a CO_2 laser (Opelaser 03SIISP, Yoshida, Tokyo, Japan) whose output was in the form of a continuous series of pulses. The 10.6 μm-wavelength, 0.5 W beam was defocused to a 1 mm spot size. The irradiation cycle consisted of a pause time of 10 ms and an irradiation time of 5 ms (Group 1), 10 ms (Group 2), or 50 ms (Group 3). A computer-controlled X-Y stage (Shot-602, Sigma-koki, Tokyo, Japan) was used to ensure uniform exposure. The root was fixed to the stage, which was traversed at 1 mm/s through the laser beam. Groups 5 and 6 were treated with NaF in a room temperature (23˚C) bath stirred at 120 rpm.

The specimens were subjected to a 2-day pH-cycling process in which five roots from each group were immersed daily in a demineralizing solution (pH 4.7, containing 0.05 M acetic acid, 2.2 mM calcium, and 2.2 mM phosphate ions) for 18 hours, and then in a remineralizing solution (pH 7.0, containing 0.15 M potassium chloride, 1.5 mM calcium, and 0.9 mM phosphate ions) for 6 hours. The solutions were maintained at 37˚C and stirred at 120 rpm. The roots were irrigated with deionized water for 5 minutes during transfer between solutions and at the conclusion of the cycling process.

The samples were sectioned perpendicular to the root surfaces and through the center of each window using a hard-tissue microtome (Isomet, Buehler, Lake Bluff, IL, USA). Three sections with a thickness of approximately 200 μm were obtained from each window. Each section was ground to a thickness of approximately 100 μm using a whetstone (#2,000).

The sections were examined at 200x magnification using a polarized light microscope (Eclipse LV100POL, Nikon, Tokyo, Japan). Digital photomicrographs were obtained using a CCD camera (DS-L2, Nikon, Tokyo, Japan). The lesion depth in each section was determined by measuring the width between the original root surface and the deepest position of the lesion using the camera control software.

The experimental results were analyzed using ANOVA and Tukey's multiple comparison tests for post hoc analysis ($p<0.05$). All analyses were carried out using the SPSS 12.0 software package for Windows (SPSS, Chicago, IL, USA)

Results

The mean and standard deviation of the lesion depths for each experimental group are presented in Table 1. ANOVA testing indicated significant differences between the mean lesion depths in the experimental groups and the control. The lesions in groups 3, 4, 5, and 6 were significantly shallower than the lesions in the control specimens ($p<0.002$). The lesions in groups 5 and 6 were significantly shallower than the lesions in groups 1, 2, and 3 ($p<0.05$). Group 3 exhibited significantly reduced lesion depth compared to the control, but slight carbonization was observed on the irradiated root surface. However, there were no significant differences among groups 1, 2 and the control, or among groups 1, 2, 3, and 4 ($p>0.05$).

Table 1. Results of lesion depths under various conditions of laser irradiation and fluoride application

Specimen groups	Conditions of laser irradiation				Fluoride application	Lesion depth (μm) (Mean ± S.D.)*
	Power	Duration	Interval	Energy		
1	0.5 W	5 ms	10 ms	17 J/cm^2	None	$41.0 \pm 3.4^{a,b}$
2	0.5 W	10 ms	10 ms	25 J/cm^2	None	$42.3 \pm 2.9^{a,b}$
3	0.5 W	50 ms	10 ms	41 J/cm^2	None	38.4 ± 10.8^{b}
4	None				0.05% NaF	$37.9 \pm 4.2^{b,c}$
5	None				0.2% NaF	$32.6 \pm 3.4^{c,d}$
6	None				2.0% NaF	28.3 ± 3.2^{d}
Control	None				None	45.9 ± 2.7^{a}

* The same superscript letters indicate no significant difference ($p>0.05$).

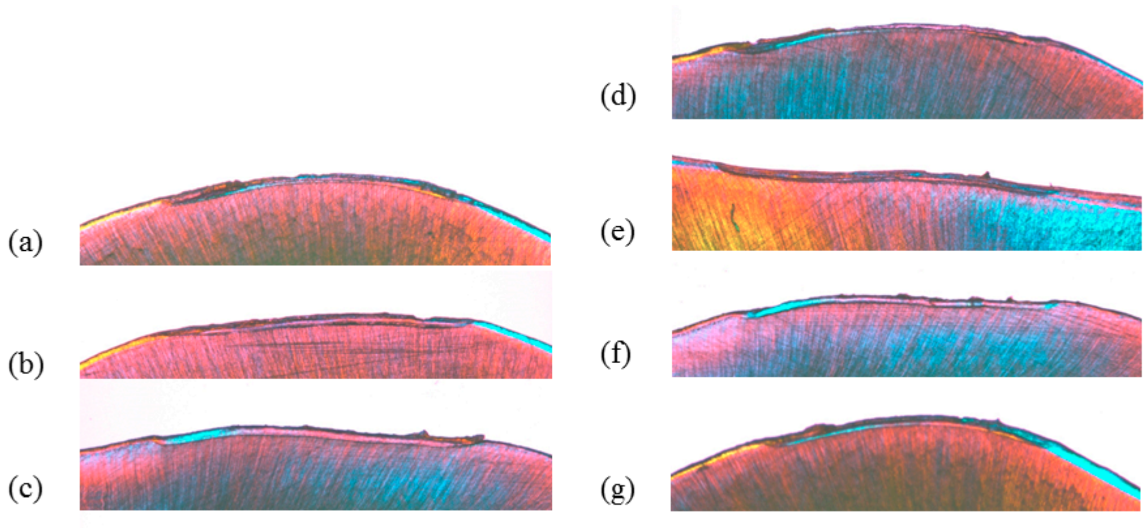

Fig. 1 Representative polarized light microphotographs of experimental groups
(a) Group 1, (b) Group 2, (c) Group 3, (d) Group 4, (e) Group 5, (f) Group 6, and (g) Control

Representative PLM images of demineralized lesions are provided in Fig. 1. The cementum was intact on the root surfaces coated with acid-resistant varnish. In all groups, stripes parallel to the bottom of the lesion were observed over most of the lesion, indicating remineralization during the pH cycling procedure. The widths of lesions in groups 1, 2, 3, and 4 were approximately equal to the mean lesion width of the control samples, while lesions in groups 5 and 6 were smaller.

Discussion

In previous studies, tooth surface acid resistance has generally been evaluated using transverse microradiography (TMR).[11,27,28] We assessed the acid resistance of root surfaces in terms of the lesion depth measured using digital polarized light microphotography (PLM). The PLM method is easier than TMR but is incapable of measuring mineral loss. However, the PLM method is sufficient to evaluate the acid resistance of root surface after pH cycling test. The design of the pH cycling test was based on tests carried out in a previous study.[16] A two-day cycle was conveniently brief, and may be useful to detect significant differences among the experimental groups. In this study, the laser output was in the form of a continuous series of pulses, and 0.5 W laser beam was defocused to a 1 mm spot size. The irradiation cycle consisted of an interval time of 10 ms, and a duration time of Group 1, 2 and 3 were 5, 10, and 50 ms, respectively. A computer-controlled X-Y stage was used to ensure uniform exposure, and the specimen fixed to the stage was moved at 1 mm/s during the laser irradiation. Therefore, the laser energy density of Group 1, 2, and 3 were 17 J/cm^2 (0.25 J/cm^2 per pulse), 25 J/cm^2 (0.5 J/cm^2 per pulse) and 41 J/cm^2 (2.5 J/cm^2 per pulse), respectively.

There are many articles described about effects of fluoride application and laser irradiation on the prevention of tooth root-surface caries.[14-16,24-29] Although these previous reports suggested that fluoride application and laser irradiation were effective in prevention of root caries, their results did not clearly show the effects of energy density of CO_2 laser and fluoride concentration on the root-surface demineralization. Therefore, this study focused to examine how energy density of CO_2 laser and fluoride concentration influence demineralization of root-surface after pH cycling. This study simultaneously examined the effects of a fluoride application of various concentrations and a laser irradiation of various energy densities on tooth root demineralization. From the results of this study, the lesion in the group applied 2.0% NaF (group 6) was significantly shallower than the lesions in the group applied 0.05% NaF (group 4), but there were no significant differences between the lesions of groups irradiated various energy densities of laser (groups 1, 2, and 3). Thus, the high concentration fluoride application demonstrated greater demineralization resistance of root-surface compared to the laser irradiation. The null hypothesis that the fluoride concentration would not affect the root surface demineralization resistance was rejected; however, the null hypothesis about CO_2 laser energy density was accepted.

The results concerning laser irradiation showed that the lesion depth in group 3 which used CO_2 laser irradiation at an energy density of 41 J/cm^2 was significantly lower than that in the control. On the other hand, the other experimental groups which used the laser irradiation at an energy density of lower than 27 J/cm^2 showed lower lesion depth compared to the control, but there were no significant differences among them. From these results, it was speculated that CO_2 laser irradiation at high energy density might be effective in increasing resistance for demineralization of root-surface, but CO_2 laser irradiation at low energy density might produce no beneficial effect on root-surface.

However, since a carbonization may occur on the irradiated root-surface at high energy level, appropriate energy density of CO_2 laser should be establish when using the laser for prevention of tooth root caries. A series of previous studies demonstrated reduced enamel and dentin demineralization following laser treatment in a caries model.[4-13] In the case of enamel, a decrease in carbonate and phosphate after laser application appears to be the cause of the reduced demineralization. On the other hand, the mechanism of demineralization inhibition in cementum and dentin of tooth root following laser irradiation is yet clarified. Nelson et al.[4] reported that fusion and melting occurred on the root dentin surface after CO_2 laser irradiation at a power density of 50 J/cm^2. Additionally, an increase in inorganic content occurring on the laser-irradiated dentin surface was related to increased dentin resistance to demineralization. However, chemical changes in the dentin substrate resulting from elevated temperatures during laser irradiation are responsible for the decrease in carbonate content and protein deposition. Therefore, CO_2 laser irradiation levels that are sufficiently high to cause carbonization in dentin would not be suitable for application to root surfaces.

Previous studies have reported that fluoride exerts a beneficial effect on demineralization/remineralization of the root-surface even at low concentrations.[25,27,28] Similar results were obtained in the present study. It is well-known that the mechanisms of protection against tooth root caries depend on the deposition of fluoride on the root surfaces, and the deposition of fluoride enhance tooth root demineralization resistance by creating a fairly amount of fluoridated hydroxyapatite.[25] Petersson et al.[28] examined remineralization of root caries lesions after in vitro treatment with various concentration fluorides, and concluded that daily application of a dentifrice slurry containing 1,400 ppm fluoride combined with twice daily rinsing with a 250 ppm fluoride solution significantly remineralize tooth root caries lesions in vitro. Hong et al. reported that the effect of a fluoride varnish on root surface demineralization/remineralization was dependent upon the exposure period and independent of the concentration.[29] In this study, three fluoride application procedures were decided from points of view as follows: 5-minute application of 2.0% NaF solution (9,000 ppm) was assumed as an application of fluoride varnish in clinic (group 6), 50-minute soak in 0.2% NaF solution (900 ppm) was assumed as a tooth brushing with fluoride paste (group 2), and 200-minute soak in 0.05% NaF solution (225 ppm) was assumed as a mouth washing using fluoride rinse (group 3). The results of this study showed that each mean lesion depth in groups 4, 5, and 6 was significantly shallower than that in the control group. Furthermore, the mean lesion depth tended to decrease with increasing fluoride concentration regardless of application period. Accordingly, these results suggested that the fluoride application was effective in increasing demineralization resistance of root-surface, and the efficacy was dependent upon the concentration rather than the application period. Therefore, it is speculated that an application of fluoride varnish such as 2.0% NaF solution in clinic may be more effective in preventing root caries than tooth brushing and mouth washing with fluoride at home. A periodic application of a fluoride varnish in clinic is recommended for preventing root caries.

Effectiveness of the combination of fluoride and laser treatment has been reported for the inhibition of root caries.[16] However, the combination details such as factors of fluoride concentration and laser energy density have not investigated in the previous study. Further researches evaluating effects of combined various concentration fluoride and various energy density CO_2 laser on demineralization resistance of root surface are promising.

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