The Influence of Laser Irradiation on The Acid Resistance of Demineralized Enamel Grooves in vitro

Daisuke INABA*, Hisazumi IKEDA**, Okiuji TAKAGI***, Masami YONEMITSU* and Joop ARENDS****

Abstract: Demineralized enamel grooves were exposed to an acid gel at pH=5 for 3 weeks after irradiation with a KrF excimer laser with a wavelength of 248 nm (total energy dose=172 J/cm²) or with the second harmonic wave of a Q-switched Nd:YAG laser with a wavelength of 532 nm (total energy dose=100 J/cm²). The mineral distributions were assessed quantitatively by transversal microradiography. Compared with unlased controls, the mineral parameters, i.e., the lesion depth (ls, μm) and the mineral loss value (ΔZ, vol%·μm) at the bottom of the grooves were not really different in the samples irradiated by either the pulsed excimer or the Nd:YAG laser, and a similar result was observed in the outer enamel surfaces near the grooves. This suggest that progression of demineralization in enamel lesions is not inhibited effectively by laser irradiation.

Key words: Laser, Acid resistance, Enamel, Demineralization, Microradiography

Introduction

A number of studies have investigated the effects of laser irradiation on dental tissues1-19. So far as sound enamel is concerned, it was suggested that a certain type of laser, e. g. a normal pulsed Nd:YAG laser, had the possibility to improve the acid resistance in smooth surfaces6-9 as well as in pits and fissures9 under given conditions. It is not well known, however, if such laser effects exist also in early caries lesions of enamel.

In most studies, in contrast to actual intraoral status, the dissolution rate of calcium from enamel by short time acid etching has been employed to assess the acid resistance of lased enamel. Therefore, it is interesting to examine if laser irradiation can prevent early caries lesions in enamel after a long-term acid challenge with an acid gel system, especially in pits and fissures where the susceptibility to caries is much greater than on smooth surfaces.

The aim of this study was to investigate the acid resistance of a demineralized groove in enamel after irradiation with pulsed excimer and Q-switched Nd:YAG lasers.

Materials and Methods

Sample preparation

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— 683 —
We used eight sound human premolars extracted from patients aged 11-14 years old (mean 12.1 years) for orthodontic reasons. After removal of the roots, the crowns were separated into two parts (buccal and lingual) using a water-cooled diamond saw. Each buccal or lingual enamel sample was mounted on an acrylic plate and completely coated with an impression compound (Pericompound, Shofu, Japan) except for the original enamel surface. The enamel surfaces were slightly abraded with wet abrasive paper (grit 800) to provide flat planes of about 15 mm².

To simulate a fissure, a U-shaped groove of about 300 μm in width and 500 μm in depth was formed using a water-cooled thin diamond-coated saw (Isomet, Buhler, U. S. A.). To produce early carieslike lesions, the enamel samples with a groove were demineralized in a 0.1 M lactic acid gel (pH =5) containing 1 wt% carboxymethylcellulose (CMC) at 37°C for 12 hours (h).

**Pretreatment and acid challenge**

After demineralization, the enamel samples were subjected to one of the following 4 pretreatments.

1. irradiation with the excimer laser (Ex group),
2. irradiation with the excimer laser combined with application of 40 μl of a 2% NaF solution (Ex-F),
3. irradiation with the Q-switched Nd:YAG laser (Y), and
4. no laser treatment (control).

They were subsequently all subjected to an acid challenge by immersion in a 0.1 M lactic acid gel (pH=5) containing 6 wt% CMC at 37°C for 3 weeks (w).²

**Laser irradiation**

In the Ex group, the krypton-fluoride (KrF) excimer laser with a wavelength of 248 nm was used as a light source (Project-10, Resonetics, U. S. A.). The samples were irradiated at a flux of 86 mJ/pulse/cm² and a frequency of 10 Hz for 200 seconds (s) (200 shots) resulting in a total energy dose of 172 J/cm². In the Ex-F group, the samples were exposed to the excimer laser in the same way as the Ex group under wet conditions but with 40 μl of a 2% NaF solution.

In the Y group, the second harmonic wave of a Q-switched Nd:YAG laser (Quanta-Ray DCR-3, Spectra-Physics, U. S. A.) with a wavelength of 532 nm was used. After painting the enamel surfaces with India ink, the samples were lased at a rate of 160 mJ/pulse/cm² and a frequency of 10 Hz for 10 s (100 shots) resulting in a total energy dose of 80 J/cm².

In both laser irradiations, the beam was guided...
Table 1 Comparison of microradiographical mineral parameters, i.e., the lesion depth ($l_d$) and mineral loss value ($JZ$), in and near demineralized grooves after laser irradiation followed by a 3-week acid challenge.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bottom of groove</th>
<th>Outer surface near groove</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$l_d$ (μm)</td>
<td>$JZ$, vol%·μm</td>
</tr>
<tr>
<td>Excimer</td>
<td>86 ± 6*</td>
<td>2,549 ± 157</td>
</tr>
<tr>
<td></td>
<td>84 ± 5</td>
<td>2,609 ± 149</td>
</tr>
<tr>
<td>Excimer+F</td>
<td>128 ± 1</td>
<td>4,170 ± 69</td>
</tr>
<tr>
<td>Nd:YAG</td>
<td>139 ± 4</td>
<td>5,101 ± 522</td>
</tr>
<tr>
<td>Untreated</td>
<td>94 ± 12</td>
<td>3,590 ± 124</td>
</tr>
<tr>
<td></td>
<td>83 ± 8</td>
<td>2,088 ± 134</td>
</tr>
<tr>
<td></td>
<td>58 ± 8</td>
<td>2,090 ± 61</td>
</tr>
<tr>
<td></td>
<td>117 ± 28</td>
<td>3,874 ± 569</td>
</tr>
</tbody>
</table>

* Mean and SD values of 3 scans for each sample.

by a quartz prism and projected vertically to the enamel target plane. The light intensities were measured using a power meter (30 A-P, Ophir Optics, Israel).

Microradiography

After acid challenge, thin planoparallel sections of about 80-μm thickness were cut transversally from the samples. They were microradiographed together with a reference aluminium step wedge under standardized conditions as established previously. To quantify mineral distribution, 3 microdensitometric scans were made in two locations for each sample, i.e., the enamel surface near a groove and the bottom of the groove (Fig. 1). As shown in Fig. 2, a mineral profile was processed from densitometric data and the two mineral parameters of interest, i.e., the lesion depth ($l_d$, μm) and the mineral loss value ($JZ$, vol%·μm), were measured.

Results

The mean $l_d$ and $JZ$ values and the standard deviations from 3 scans for 2 locations of the 4 groups in each sample are compiled in Table 1. It is obvious that the lased samples show roughly the same range of $l_d$ and $JZ$ values at the bottom of the grooves when compared with the untreated controls, indicating no remarkable inhibitory effects on lesion progression. In the outer surfaces near the grooves, the values of $l_d$ and $JZ$ of the lased samples are also not greatly different from those in the unlased samples. Compared with the parameter values at the bottom of the grooves, those at the outer surfaces near grooves show somewhat greater values in the same samples. The 12-h initial demineralization, before irradiation was assessed separately and showed $l_d$ and $JZ$ values of 32 μm and 1,765 vol%·μm, respectively.

Discussion

From a practical point of view, caries prevention and caries arrest are essential for the most caries susceptible regions in enamel, e.g., occlusal fissures and cervical areas where surfaces are frequently exposed to an acid attack by dental plaque. Therefore, it is reasonable to focus on demineralized enamel samples in laser studies on caries prevention. Under the conditions employed, lased early caries lesions did not show remarkable acid resistance after 3-week challenge by an acidic gel when compared with untreated controls.

The results obtained are different from previous laser studies in which relatively short exposure periods to an acidic solution were employed to assess acid resistance of lased sound enamel. One possible explanation of this difference is the
acid challenge methods used. A short time etching by an acidic solution is suitable to evaluate mainly the acid resistance of sound outermost lased enamel. An advantage of the plaque-like sticky gel system is that it enables us to evaluate the acid resistance of enamel up to a given depth by simulating relatively slow and continuous reaction in a liquid phase between outer environment and tooth mineral.

A question is also why demineralization progressed in the lased caries lesions after application of acid gel system for 3 weeks. The present results indicate at least that lased enamel did not prevent outer hydrogen ions from penetrating into a deeper lesion body. Although the reasons for our results need to be examined further, the mechanisms can be presumed to be as follows.

1. Initial demineralization of enamel surfaces before laser irradiation makes enamel surfaces porous due to mineral loss.

2. Subsequently, a fused layer produced by laser irradiation might not be formed uniformly.

3. Thermal effects of laser irradiation and subsequent cooling may have formed cracks in enamel surfaces resulting in enhanced permeability.

In addition, two important features have been reported in the literature on laser irradiation of enamel. One is that laser irradiation alters only the outermost enamel surfaces up to 1.5 \( \mu m \) or potentially within 100 \( \mu m \) in depth. Furthermore, it was suggested that laser irradiation sizably decreased water, carbonate, and organic components in enamel. There is also a possibility that laser irradiation may influence demineralized enamel more destructively than sound enamel especially due to a greater amplitude of vibration in the second harmonic wave of the Q-switched Nd:YAG laser.

In summary, the susceptibility of the demineralized enamel to an acid challenge was presumably increased by laser irradiation resulting in progression of demineralization even if some mineral was somewhat altered to be locally acid resistant. Thus, a good description of lased enamel lesions would be a porous filter made of acid resistant materials. (The term ‘acid resistance’ does not mean less permeable.)

Because caries prevention or caries arrest is achieved clinically both by increased acid resistance and by remineralization of enamel lesions, it is still questionable whether a single application of laser irradiation for caries prevention is effective or not. In fact, we found in a previous study that acid resistance of enamel lesions did not depend on laser irradiation but on remineralization status. To clarify the practical effectiveness of laser treatments, it is necessary to examine the effect of a single application of laser irradiation on caries arrest under the conditions of plaque deposition by intraoral studies.

From the present experiments in vitro, it can be concluded that irradiation with a pulsed excimer laser or Q-switched Nd:YAG laser did not improve the acid resistance of demineralized enamel samples effectively in or near grooves after exposure to an acidic gel system.

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エナメル質脱灰裂溝の in vitro 耐酸性におよぼす
レーザー照射の影響

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概要：脱灰したヒトエナメル質人工裂溝に、波長248nm の krF エキシマレーザー（総エネルギー密度：172J/cm²）、または波長532nm のQスイッチ Nd: YAG レーザー第2高調波（総エネルギー密度：100J/cm²）を照射後、乳酸ゲル（pH=5）に3週間浸漬する耐酸性試験に供し、マイクロラジオグラフィによりミネラル分布を定量的に評価した。裂溝底部の脱灰深さ l₃(μm) とミネラル喪失量 JZ (vol%・μm) は、エキシマレーザー照射試料、Nd: YAG レーザー照射試料とも処理試料と比較して明らかな違いを示さなかった。また、裂溝に近接するエナメル質表層部でも同様の傾向が認められた。すなわち、エナメル質初期陥没のさらなる脱灰の進行はレーザー照射によって抑制されない可能性が示唆された。

索引用語：レーザー、耐酸性、エナメル質、脱灰、マイクロラジオグラフィ

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