Introduction

Enamel structure of tooth is the hardest tissue in our body. It is a complex of mineral and organic material with 85% minerals, 12% water and 3% protein and lipid by volume. Hydroxyapatite is the mineral component of the enamel with hexagonal symmetry and the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Except the trapped organic components, the main difference between hydroxyapatite and enamel structure’s apatite is the presence of about 3% $\text{CO}_3^{2-}$ by weight, i.e. dental enamel apatite is carbonated hydroxyapatite. Despite recent advances in dental caries prevention, it is prevalent and remains a serious health problem. In addition, dental caries is considered the most prevalent disease during human life, with high prevalence in some individuals.

Laser irradiations have been used for many types...
of treatment in dentistry, including removal of caries, inhibiting caries, tooth preparation for restorative dentistry, soft and hard tissue surgery, and for activation of dental bleaching agents. Since the 1960s, it has become increasingly apparent that high-power lasers can be used to decrease the rate of subsurface demineralization of enamel structure, changing its crystalline construction, solubility in various acids and its permeability. Enamel structure can be modified with laser irradiation treatment, causing surface roughness, cracks, fusion and formation of multiple pores and some bubble-like inclusions. A potential preventive effect of laser on healthy enamel structure has been demonstrated; the effect of irradiation on white spot lesions is still unclear. The mechanism(s) underlying caries prevention by laser irradiation treatment remain unclear; knowledge of the chemical composition of irradiated enamel may play a significant role in the field of caries prevention using various laser devices.

Raman spectroscopy is a method for studying the enamel structure, using Raman spectroscopy, the molecular vibrational bands of synthetic or biological materials can be identified. Although FT-Raman spectroscopic method is mostly compared with the better-known FTIR (Fourier transform infrared spectroscopy), the former has some advantages over the latter technique. With Raman spectra, there is little interference with water content of samples, making the technique appropriate for studying several biological samples. In this context, the aim of this study was to evaluate the chemical changes occurring in enamel structure irradiated with Nd:YAG and Er:YAG laser, using Raman spectroscopy analysis.

Methods

Collection and preparation of teeth

The teeth utilized in this study were collected based on the Ethics in Research Committee of the Dental School guidelines. Fifteen freshly extracted human molars were used. We did not use impacted teeth in the present study. After radiographic examination to make sure they were free from any erosion, cracks, caries, or any other defects, 0.1% thymol solution was used to store the teeth at 4°C until use. The required sample sizes were calculated according to previous studies. The crowns of the teeth were removed close to the cemento-enamel junction with double-faced diamond disks in a low-speed handpiece (NSK Nakanishi Inc, Kanuma, Japan). Nail varnish was used to cover the buccal surface of each tooth, but for a 2×2-mm window on the enamel surface of the sample. The tooth samples were divided into 3 groups randomly (n=5).

Experimental design

The sample surfaces were treated as follows: group A: no treatment; group B: Er:YAG laser irradiation; group C: Nd:YAG laser irradiation.

Laser Irradiation

Group A (control group) was untreated. Specimens in group B were irradiated for 10 seconds with Er:YAG laser (US20D, DEKA, Italy) with the following settings: WL (wavelength) = 2,940 nm; Power = 0.5 W; PE (pulse energy) = 50 mJ; RR (repetition rate) = 10 Hz; PD (pulse duration) = 230 µs; irradiation was carried out 4 mm from the tooth surface, and was accompanied by water/air spray. Specimens in group C were irradiated for 10 seconds with Nd:YAG laser (Fotona, FIDELIS, Ljubljana, Slovenia), emitting energy at a wavelength of 1064 nm. The settings used were: power = 0.5 W; pulse duration=100 µsec; fiber 300 µm; irradiation was carried out 1 mm far from the enamel surface, with 300-µm fiber, in sweeping motion.

After treatment, all the specimens were assessed by Raman spectroscopic analysis; all the specimens were also observed for any morphological changes, such as cracks or craters, under a stereomicroscope.

FT-Raman spectroscopic analysis

The surfaces of the specimens were analyzed by Raman spectroscopy at two time intervals: before treatment and after the laser irradiation. Raman spectroscopy was carried out with a Bruker Senterra system (SENTERRA; Bruker Inc., Karlsruhe, Germany) using the 785- and 532-nm lasers. Alterations in mineral and organic enamel contents were analyzed by comparing the Raman peaks centered at 1071 cm⁻¹ (p1) and 2940 cm⁻¹ (p2), to the peak at 961 cm⁻¹ (p3). The areas of the Raman peaks were assessed with Graph 4.4 software (Ivan Johansen).

Statistical analysis

SPSS 17 (SPSS Inc, Chicago, Ill., USA) was used to analyze the results. Paired samples t-test was used to analyze the alterations occurring after laser irradiations. A 95% confidence interval was applied to evaluate the statistical significance.

Results

The FT-Raman spectra of the inorganic and organic ingredients of the dental enamel are displayed in
Figures 1-4. The spectra of irradiated surface appear very homogeneous compared to those without any irradiation. Figures 1 and 2 depict the FT-Raman spectra of an untreated enamel surface, an enamel surface irradiated with Nd:YAG laser, and an enamel surface irradiated with Er:YAG laser, respectively, at a range of 300–1200 cm⁻¹. The most intense Raman peak, at 960 cm⁻¹, is considered by the symmetrical stretching mode of PO₄³⁻ groups in the mineral apatite component of enamel. The spectra showed a strong peak for PO₄³⁻ in both normal and irradiated samples. The peak at 430 cm⁻¹ and 591 cm⁻¹ are subjected to the ν2 vibration of PO₄³⁻ groups and ν4 vibration of PO₄³⁻ groups. As might be seen in these figures, the mineral content, which is indicated by the intensity ratio of the peak at 960 cm⁻¹, decreased after laser irradiation. Although the peak integrated intensity of the phosphates content (960 cm⁻¹) changed after laser treatment, the analysis showed no statistically significant decrease in the area ratio, either in the group irradiated with Nd:YAG laser (P = 0.241) or in that irradiated with Er:YAG laser (P = 0.429).

The peak at 1065 cm⁻¹ is attributed to the ν1 vibration of B-type carbonate (CO₃²⁻) of the mineral. As may be seen, after irradiation with either Nd:YAG or Er:YAG lasers, the intensity of the peak at 1065 ± 10 cm⁻¹ decreased. The area ratios of intensity of CO₃²⁻ to that of 960 cm⁻¹ PO₄³⁻ peak are listed in Table 1. Analysis of the area subjected to the carbonate content (1065/960 cm⁻¹) depicted a significant decrease in the area ratio, both in specimens lased with Nd:YAG laser (P = 0.025) and in those irradiated with Er:YAG laser (P = 0.027). The results also revealed that the effect of Nd:YAG laser on the carbonate concentration was not significantly different from that of the Er:YAG laser (P = 0.196). Organic materials have vibrational bands at a range of 1200–1700 cm⁻¹; the relatively weak vibrational bands of amide I and amide III are in this range. CH₂ stretching vibration produces a strong peak at 2935 ± 10 cm⁻¹. Figures 3 and 4 illustrate the FT-Raman spectra of an un-treated enamel surface and those irradiated by the types of laser, at a range of 2900–3000 cm⁻¹. The CH₂ vibrational bands (2935 ± 10 cm⁻¹) lie in this range. As it can be seen in Figures 3 and 4, the intensity of CH₂ band at 2935 ± 10 cm⁻¹ is lower in the case of the irradiated surfaces, due to a considerable decrease in the concentration of organic materials. The analysis of the area subjected to organic content (2935/960 cm⁻¹) exhibited a significant decrease in the area ratio after laser irradiation with either type of laser: Nd:YAG laser (P <
0.001) and Er:YAG laser (P = 0.003). These results also reveal that the effect of Nd:YAG laser on the intensity of the CH2 band at 2935 ± 10 cm⁻¹ was not significantly different from that of the Er:YAG laser (P = 0.350).

Discussion

The 2935 ± 10 cm⁻¹ band has been used in a number of studies to semi-quantify organic changes, although this band is both clearer and stronger than the amides bands. The organic peaks at 1200–1700 cm⁻¹ show broader properties because many materials may remain in partially amorphous and a hybrid phase. Dental enamel structure contains very low concentrations (1%) of organic matrix. Organic materials might have an important role in controlling enamel diffusion rate. The 2940 cm⁻¹ band has been subjected to semi-quantification of organic changes since it is clearer than amides bands. In our study, a decrease in the band intensity was noticed after irradiating the enamel surface with Nd:YAG or Er:YAG laser, indicating a reduction in the organic matrix. These results have been confirmed with other reports. It has been concluded that laser irradiation might afford changes and decomposition of both the organic matrix and the carbonated enamel structure’s apatite. As a result, it might have a great role in preventing enamel diffusion and reducing its dissolution, thus preventing enamel caries.

Conclusion

The significant reduction of carbonate and organic matrix that occurs after applying Nd:YAG or Er:YAG lasers might indicate that this treatment is a suitable strategy for caries prevention. The results show that the Raman technique might be appropriate to survey changes in composition and structure of irradiated enamel.

Table 1. Nd:YAG and Er:YAG laser effect on the mean and standard deviation of carbonate/PO₄ and organic/PO₄ content in natural enamel

<table>
<thead>
<tr>
<th>Bands</th>
<th>Mean (un-lased)</th>
<th>Mean (lased via Nd:YAG)</th>
<th>P*</th>
<th>Mean (un-lased)</th>
<th>Mean (lased via Er:YAG)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonate/PO₄</td>
<td>0.069 (0.017)</td>
<td>0.048 (0.009)</td>
<td>0.025</td>
<td>0.107 (0.019)</td>
<td>0.049 (0.008)</td>
<td>0.027</td>
</tr>
<tr>
<td>Organic/PO₄</td>
<td>0.101 (0.010)</td>
<td>0.051 (0.001)</td>
<td>&lt;0.001</td>
<td>0.047 (0.007)</td>
<td>0.057 (0.000)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* All the data analyzed by paired-samples t-test.
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**Authors’ contributions**

SS, RF and MJ contributed to the concept and design of the study. SS, MJ, NC and YR contributed to data acquisition and interpretation, and drafted the manuscript. SS, MJ and YR contributed to critically revising the manuscript. All the authors have read and approved the final manuscript.

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**Competing interests**

The authors declare no competing interests with regards to the authorship and/or publication of this article.

**Ethics approval**

The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences.

**References**


