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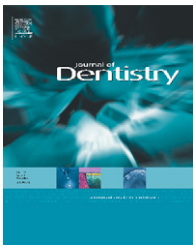

Laser-induced compositional changes on enamel: A FT-Raman study

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ABSTRACT

Preventive effects of lasers on enamel caries have been documented in the past few decades. However, its specific mechanism remains uncertain.

Objectives: To evaluate the laser-induced change of enamel compositions, including carbonate and organic matters using Fourier-transform Raman spectroscopy (FT-Raman).

Methods: Twelve windows (1 mm × 1 mm), created on six defect-free primary incisors, were characterized by FT-Raman microscopy (1024 nm) before and after Er:YAG laser treatment (Fidelis®) with 5.1 J/cm^2–2 Hz–5s. To assess the statistical significance of laser effects, Raman peaks assigned to ν_1 phosphate, type-A/B carbonates, and organic matters were evaluated with the paired-samples *t*-test.

Results: The standardized intensity of type B carbonate decreased significantly from 0.117 to 0.106 ($p = 0.029$), whereas the standardized intensity of carbonate A remained unchanged ($p = 0.467$). Related to organic matters, the standardized intensity of peaks at 2940 cm^{-1} and in the ranges of 1200–1600 cm^{-1} decreased significantly, with $p = 0.005$ and $p < 0.001$, respectively. Revealing enamel crystallinity, the bandwidth of ν_1 phosphate on lased surfaces appeared to be unaltered after laser treatment ($p = 0.477$).

Conclusions: Laser treatment may provide caries-preventive effect on enamel through reduction of carbonate and modification of organic matters.

1. Introduction

It has been consistently demonstrated that lasers under certain conditions can significantly increase the acid resistance of enamel, by altering crystallinity, acid solubility, and permeability of enamel.[1,2] The mechanism of laser-induced caries prevention has been investigated by various chemical and physical methods but the methodologies remain to be verified in some areas.[1,2]

To characterize the structural and compositional change in enamel caused by laser therapies, our research team has employed polarized light microscopy (PLM),[2] microradiography (MRG),[3] scanning electron microscopy (SEM),[4] Fourier transform infrared spectroscopy (FT-IR),[5] thermogravimetric analysis (TGA),[6] X-ray diffraction analysis (XRD)[5] and secondary ion mass spectrometry (SIMS).[4,7] However, in preparation for these analyses, the sample tissue may be altered or damaged.

Raman spectroscopy, as a versatile and non-destructive spectroscopic technique, circumvents most of these problems and allows for simultaneous characterization of the inorganic and organic phases of tooth. Furthermore, Raman spectra exhibit little interference with water, making Raman spectroscopy advantageous for the study of many biological specimens.[8] Applications of Raman spectroscopy in dental researches have included preliminary studies of enamel

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powder,[9] artificial apatite, synthetic carbonated apatite[10,11] and synthetic fluorohydroxyapatite.[12] The relative orientation of single crystal in human dental enamel, and the deposition of CaF_2-like crystals after fluoride treatment have been investigated.[13,14] Spectra of irradiated enamel and dentine were recorded to evaluate the compositional alteration after Nd:YAG, Er:YAG and CO_2 laser treatment for cavity preparation.[15,16] However, the effect of Er:YAG laser on chemical composition and crystal structure of enamel pertaining to caries prevention has not been investigated using Raman spectroscopy.

Therefore, the objective of this study was to characterize the structural, chemical and crystallographic changes after laser treatment, in particular the carbonate and organic concentration changes after laser treatment, so as to further investigate the cariostatic mechanism of the Er:YAG laser therapy.

2. Materials and methods

2.1. Tooth selecting and grouping

Six carious-free human deciduous incisors, stored in 0.1% thymol solution, were obtained from the National University Hospital, Singapore. The procedure used for collection of teeth was approved to fall under the exemption category of the Institutional Review Board of the National University of Singapore (NUS-IRB reference code: 04-106E). Four non-defect windows, all about 1 mm × 1 mm in size were created on each tooth with a black permanent marker.

3. Laser treatment

All the experimental windows received an Er:YAG laser treatment (Fontana Fidelis®, Solvenia) at a very short pulse (VSP) mode with a pulse width of 100 ms and energy density of 5.1 J/cm^2, under a cooling water flow of 10 ml/min. The spot size is 1 mm and each spot was irradiated for 5 s at 2 Hz. The tip was clamped to prevent movement and each experimental window was irradiated for one spot in the center. All the windows were then rinsed with de-ionized water for 10 min after laser treatment.

3.1. Stereomicroscopy characterization

The teeth were characterized at 40× magnification with stereomicroscope (SZ4045TR, Olympus®, Tokyo, Japan) and fiber optic illuminator (Olympus LGPS, Olympus®, Tokyo, Japan) before and after treatments to evaluate the treatment effects on surface morphologies.

3.2. Raman characterization

Before and after laser treatment, the center of each examined window was aligned with incident light (1024 nm) and scanned by a FT-Raman (Bruker IFS 66, Bruker optics, Germany) instrument with a FRA 106 attachment collecting 500 scans at a spectral resolution of 4 cm^{-1}. The spot size was approximately 1 mm and therefore the spectra obtained are from the whole lased zone.

3.3. Data collection

Spectrum was deconvoluted by curve fitting using the OPUS 4.2 software (Bruker, Germany), and normalized using the intensity value of 960 cm^{-1} PO_4^{3-} band.[17] To evaluate the treatment effect, three parameters were extracted from spectra: (a) peak intensity, such as the maximum intensity of the phosphate peak at 960 cm^{-1}, (b) peak integrated intensity (area under the curve)[18] and (c) band full width at half maximum (FWHM). For partially overlapped bands, a preliminary deconvolution was processed before spectrum acquisition. Mixed Gaussian–Lorenzian shapes were used in all band analyses.[18] Some windows were removed from the sample pool due to low signal to noise ratio, which is monitored by this software. Twelve data set, with sufficient quality for curve-fitting analysis, were used for final analysis.

3.4. Statistical analysis

A paired-samples t-test was used to analyze the change of peak (integrated) intensity, and half peak-width, and relative carbonate content before and after treatment. A 95% confidence interval was used to evaluate the statistical significance.

4. Results

No apparent morphological change, such as crack or craters, has been observed for all the samples characterized with stereomicroscope.

Fig. 1 shows typical Raman spectra obtained from normal and irradiated enamel surface. Spectra showed strong PO_4^{3-} ν_1 at 960 cm^{-1} in the normal and lased surfaces.[13,19] It is also evident for ν_3 phosphate at 1028–1075 cm^{-1} and ν_4 at 591 cm^{-1} and ν_2 at 430 cm^{-1}.[20] All the spectra of lased surface appear very similar to those of their untreated counterparts.

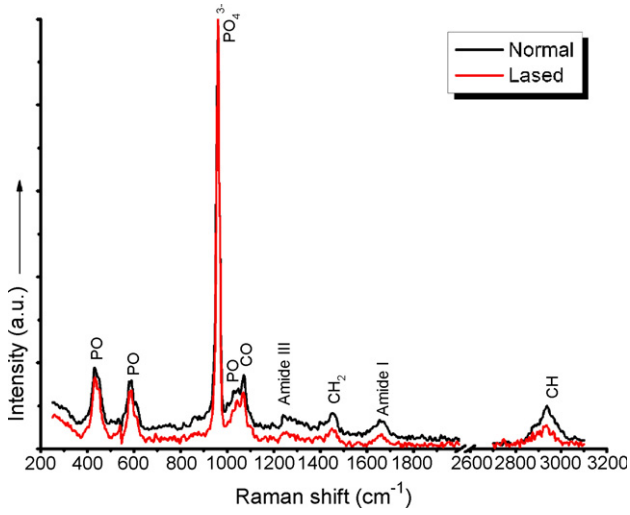

Fig. 1 – Raman spectra of natural incisor surface (upper curve) and lased incisor surface (lower curve).