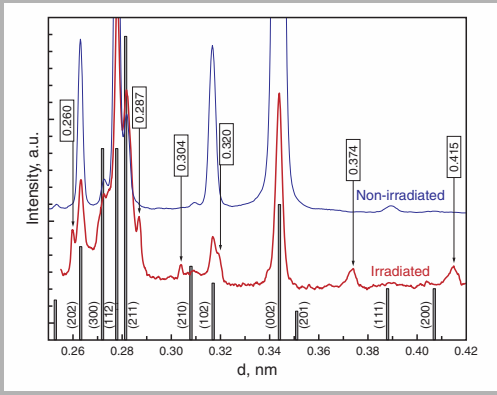
Abstract: The Er,Cr:YSGG system is commonly employed in tissue removal, but recently it has also been clinically evaluated for caries prevention. The present work explains the clinical and pre-clinical observations on the basis of the crystallographic changes that this laser can produce in the dental enamel. The analyzed samples were obtained from sound human third molar teeth. The laser irradiation was conducted with a Er,Cr:YSGG laser with 12.5 mJ/pulse, 0.25 W, and 2.8 J/cm^2. The laser device operates at a wavelength of 2.79 μm, and the pulse width duration is 140 μs, with a repetition rate of 20 Hz of spot size of 750 μm. The crystalline structure of the samples was evaluated by X-ray diffraction at a synchrotron beamline The X-ray beam was configured at a grazing angle, to maximize the surface diffraction signal and to better detect the possible new crystallographic phase produced after the laser irradiation. It was observed that the crystallographic structure tetracalcium phosphate (TetCP, JCPDF 25-1137) exhibits several peaks that match more precisely with the new experimental peaks of the irradiated enamel. The present results suggesting the coexistence of tetracalcium phosphate with hydroxyapatite in enamel irradiated with Er,Cr:YSGG laser and can be the answer to the clinical and pre-clinical observations reported in the literature.



X-ray diffraction patterns of non-irradiated and Er,Cr:YSGG laser-irradiated human enamel

Crystalline structure of human enamel irradiated with Er,Cr:YSGG laser

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1. Introduction

The thermal action of laser irradiation can modify the crystalline structure of the mineral matrix of human enamel leading to a recrystallization process (melting and crystal growth) [1], changes in lattice parameters [2], and/or formation of new compounds, such as tricalcium phosphate in the enamel alpha phase (α-TCP) $\{\alpha$-$Ca_3(PO_4)_2\}$ [1,3–6] and beta phase (β-TCP) $\{\beta$-$Ca_3(PO_4)_2\}$ [7,8], as well

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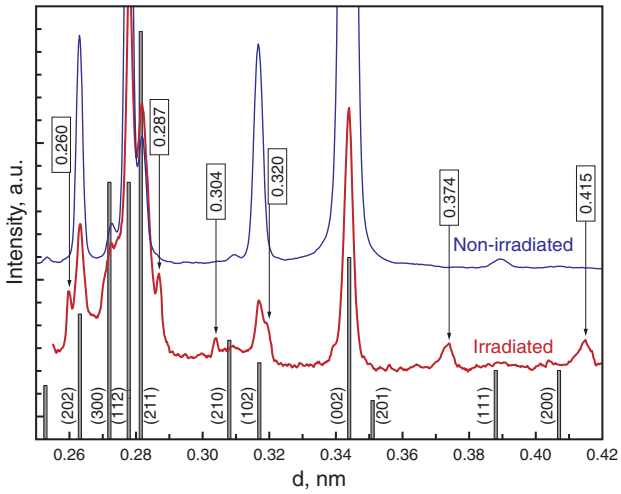

Figure 1 (online color at www.lphys.org) X-ray diffraction patterns of non-irradiated and Er,Cr:YSGG laser-irradiated human enamel. The main Bragg peaks expected for hydroxyapatite (HAP, JCPDF 09-0432) and the six additional peaks corresponding to the enamel sample after irradiation are indicated

as tetracalcium phosphate (TetCP) $\{Ca_4(PO_4)_2O\}$ [1,8–10]. The formation of new crystalline phases was observed in enamel irradiated with different types of lasers, namely neodymium (Nd:YAG – 1.064 μm), holmium (Ho:YLF – 2.065 μm), and carbon dioxide lasers (CO_2 – 9 – 11 μm). The detection of new crystallographic phases after irradiation is one of a possible explanation for the results observed in the literature for caries resistance [11–13] and changes in adhesion after laser irradiation [14–16].

The aim of this work is to characterize the crystalline structure of melted/resolidificated layers that develop in enamel after irradiation with Er,Cr:YSGG (2.79μm) lasers. In the literature, the formation of new crystalline phases in enamels after irradiation with this laser system had not yet been reported.

The Er,Cr:YSGG laser system is commonly applied to tissue removal. In addition, it has recently been clinically evaluated for caries prevention [17–19] including your thermal analysis [20] evaluating the safety for clinical application. The present work will characterize structural changes that this laser system promotes in enamel and try to explain the previous clinical and pre-clinical observations related to caries prevention promoted by Er,Cr:YSGG laser irradiation.

2. Materials and methods

The samples to be analyzed were obtained from three sound human third molar teeth. A $3\times3\times2$ mm^3 slab of enamel was cut from the smoothed surfaces of these teeth, and from it an area of 9 mm^2 was selected for laser irradiation and X-ray analysis. Three samples were prepared, one of them being not irradiated in order to characterize by X-ray diffraction the structure of the initial enamel and the other two were submitted to same Er,Cr:YSGG laser irradiation with a power of 12.5 mJ/pulse, 0.25 W, and 2.8 J/cm^2.

The Er,Cr:YSGG laser device (Millennium, Biolase Inc., San Clemente, CA, USA) operates at a wavelength of 2.79 μm and the pulse width duration is 140 μs, with a repetition rate of 20 Hz of spot size of 750 μm. During irradiation, the laser handpiece was positioned on the optical support that is coupled to a computer that controls the lateral displacements of the sample (Newport, Irvine, CA) with a speed of 4 mm/s. After preparation, the samples were stored in a moist environment until the X-ray analysis was carried out.

The crystalline structures of the enamel samples were evaluated by X-ray diffraction using the XRD1 synchrotron beamline (LNLS, Campinas, Brazil), which provides a monochromatic X-ray beam with a wavelength $\lambda = 0.0954$ nm. The sample surface was oriented in order to have the direct X-ray beam at grazing incidence. This allowed for the maximization of the diffraction signal coming from surface layer of enamel and, thus, for a better characterization of new crystallographic phases that may eventually be induced by laser irradiation. A step scanning diffractometer ($2\theta_{step} = 0.01°$) equipped with a scintillator photon counter was employed in order to record the diffraction patterns.

In order to reduce the effect of statistical errors, a single diffraction pattern was determined as the sum of the two individual scans corresponding to two enamel samples irradiated with the laser under equivalent conditions.

3. Results

The experimental X-ray diffraction patterns produced by our sample of non-irradiated human enamel and another corresponding to enamel irradiated with the Er,Cr:YSGG laser are plotted in Fig. 1. The positions of Bragg peaks expected for hydroxyapatite (JCPDF 09-0432) are also indicated in the diffraction patterns. The unit cell of crystalline hydroxyapatite $[Ca_5(PO_4)_3OH]$ phase is hexagonal and its lattice parameters are a = 0.942 nm and c = 0.687 nm (JCPDF 09-0432).

The lattice parameters (a and c) of hexagonal unit cells are related to the interplanar spacing, d_{hkl}, by

$$\frac{1}{d_{hkl}^2} = \left[\frac{4}{3}\left(\frac{h^2+hk+k^2}{a^2}\right) + \frac{l^2}{c^2}\right] . \quad (1)$$

We have determined a set of four d_{hkl} spacings for the studied human enamel, prior to irradiation, from the Bragg angles associated to four strong and well-defined diffraction peaks, namely (112), (002), (213), and (004), as displayed in Fig. 1. We have determined, by applying Eq. (1),

the lattice parameters of the hexagonal unit cell of non irradiated enamel as being a = 0.944 nm and c = 0.687 nm. These values are close to those expected for hydroxyapatite as reported in JCPDF 09-0432 [13] (a= 0.942 nm and c = 0.687 nm and also close to the values reported in the literature for non-irradiated human enamel (a = 0.944 nm and c = 0.688 nm) [22,23].

The X-ray diffraction pattern corresponding to irradiated enamel also plotted in Fig. 1 displays a series of diffraction peaks similar to those observed for non-irradiated enamel (corresponding to hydroxyapatite), and also six additional peaks corresponding to a different crystallographic phase. This finding indicates that the Er,Cr:YSGG (2.79 μm) laser irradiated enamel sample contains a mixture of at least two crystalline phases. The procedure for indexing the six additional peaks that are seen in Fig. 1 was started by using the database PDF2-Release (International Centre for Diffraction Data – ICDD, 2005). Our search did not directly lead to a known crystallographic phase for the observed set of additional Bragg peaks.

We have then decided to carefully compare our experimental diffraction pattern corresponding to irradiated enamel to those expected for three crystalline phases previously observed after different types of laser irradiation or thermal treatments, namely α-tricalcium phosphate (α-TCP), β-tricalcium phosphate (β-TCP), and tetracalcium phosphate (TetCP). In Fig. 2a, our experimental pattern was plotted together with a set of strong peaks expected for α-TCP (JCPDF 29-0359). The diffraction peaks expected for α-TCP match well to only two of our experimental peaks (0.260 nm and 0.287 nm), while other four peaks, expected for α-TCP, do not exactly coincide with them and several others are not apparent in our experimental diffraction pattern.

The X-ray diffraction pattern expected for the same tricalcium phosphate but in beta phase (β-TCP, JCPDF 09-0169) is displayed in Fig. 2b together with the experimental pattern for irradiated enamel. In this case only three diffraction peaks for β-TCP match to experimentally observed peaks (0.260 nm, 0.287 nm, and 0.320 nm).

The third pattern corresponding to a known crystallographic phase that we have compared to our experimental data was that of tetracalcium phosphate (TetCP, JCPDF 25-1137). The results plotted in Fig. 2c indicates that most of the diffraction peaks of the expected pattern for TetCP match well to the Bragg peaks observed for irradiated enamel that not apparent in the pattern produced by non-irradiated samples.

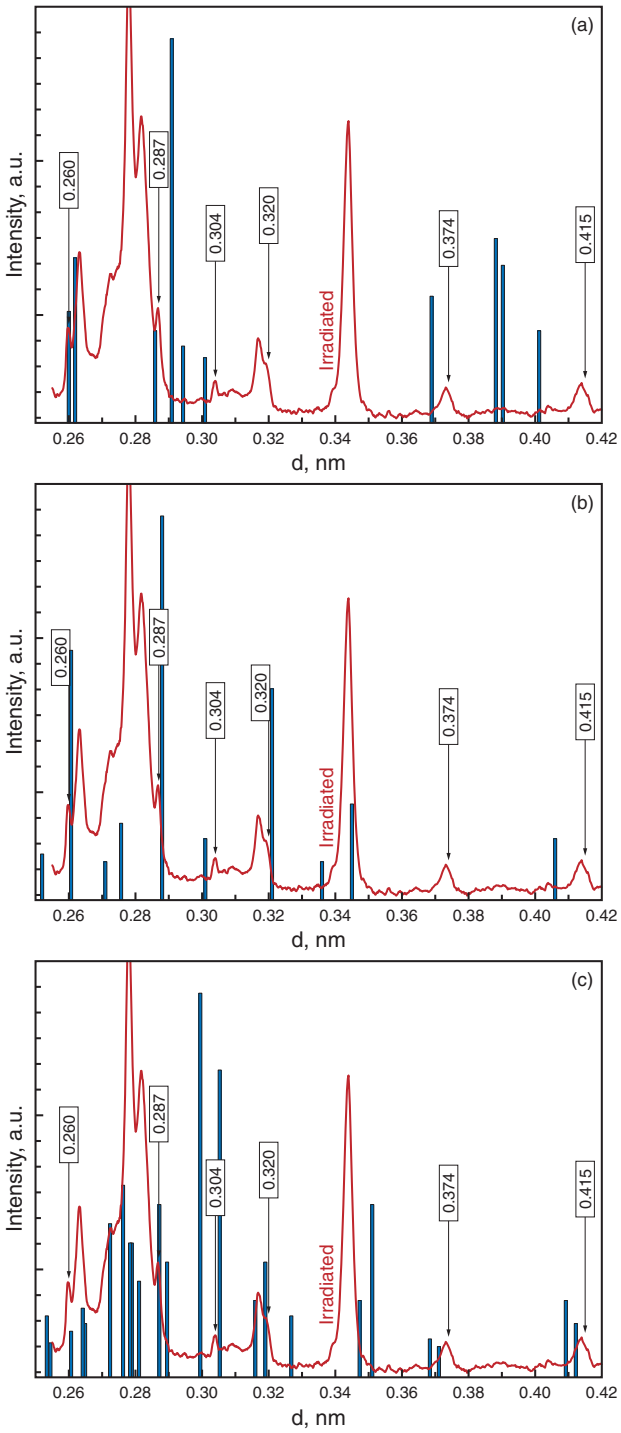

Figure 2 (online color at www.lphys.org) Experimental X-ray diffraction patterns of the irradiated enamel sample studied here and the expected patterns for other three phases which have been previously observed in enamels subjected to irradiation with other laser systems: (a) – tricalcium phosphate in alpha phase (TCP-α, JCPDF 29-0359); (b) – tricalcium phosphate in beta phase (TCP-β, JCPDF 09-0169); and (c) – tetracalcium phosphate (TetCP, JCPDF 25-1137)

4. Discussion

Our X-ray diffraction study demonstrates that irradiation with Er,Cr:YSGG (2.79 μm) of human dental enamel maintains a major phase with the same structure as the initial crystalline hydroxyapatite [$Ca_5(PO_4)_3OH$] phase but,

in addition, it induces the formation of one or more minor crystalline phases. We have concluded that, within the angular domain analysed by us, the additional crystalline phases produce six diffraction peaks corresponding to d-spacings ranging from 0.25 nm to 0.42 nm.

Only some of the diffraction peaks corresponding to alpha and beta phases of tricalcium phosphate match our experimental peaks, but not all of them. The presence of several unmatched peaks led us to disregard the possibility for the new phase formed after irradiation to be (α- or β-) TCP. However, we should point out that eventual and particular preferred crystal orientations in the new phases could also explain the lack of some expected diffraction peaks in the experimental patterns.

According to the general features of the patterns plotted in Fig. 2a, Fig. 2b, and Fig. 2c and described in the proceeding section, we have concluded that Er,Cr:YSGG (2.79 μm) laser irradiation promotes the formation of a single additional phase, which most probably is tetracalcium phosphate (TetCP). Overlappings of diffraction peaks of the minoritary phase (TetCP) with those of the major phase of hydroxyapatite did not allow us to precisely determine the lattice parameters corresponding to the hexagonal unit cell of the new minor phase.

The presence of the new phase suggested from our experimental results (TetCP) was also observed in enamels irradiated with CO_2 laser [9], holmium laser [10], and it was also detected after thermal treatment [24,25]. Thus the phase observed in our enamel irradiated with a Er,Cr:YSGG laser can also be explained as a consequence of the thermal effects that our laser irradiation produces on the enamel surface. As discussed in the literature [10], the three crystallographic phases observed in enamel samples irradiated with different laser systems are the same as those observed in enamel held at high temperatures: β-TCP, α-TCP, and TetCP. As a matter of fact, β-TCP is formed at $T > 800^{\circ}C$ [25], while α-TCP and TetCP are formed at $T > 1100^{\circ}C$ [24].

As it was mentioned in the introduction section, the Er,CR:YSGG laser system emitting at 2.79 μm is often applied to tissue removal and recently was also clinically evaluated for caries prevention [17–19]. The results presented here indicate that Er,Cr:YSGG-laser irradiation promotes the formation of a minor phase of tetracalcium phosphate embedded in a hydroxyapatite matrix. The appearance of the mentioned new phase and the induced changes in chemical composition of Er,Cr:YSGG-irradiated enamel is expected to improve the overall caries resistance as suggested in previous investigations [17–19].

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