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Crystalline structure of dental enamel after Ho:YLF laser irradiation

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Summary Irradiation of teeth with lasers using specific wavelengths and energy densities produces surface melting. This effect has been already applied to different procedures such as caries prevention and hypersensitivity reduction. The aim of this study is to characterize the crystalline structure of bovine enamel after holmium laser irradiation. A holmium laser (Ho:YLF) with emission wavelength of 2065 nm was used. Enamel tissues were irradiated in ablative regime and their structures before and after irradiation were analyzed using the powder X-ray diffraction technique. The X-ray diffraction patterns of non-irradiated enamel correspond to carbonated hydroxyapatite and those produced by irradiated samples indicate the existence of a mixture of two crystalline phases: hydroxyapatite and tetracalcium phosphate. The structural characteristics of holmium irradiated enamel were compared with those of the same tissue irradiated with other lasers.

Introduction

The enamel mineral matrix of teeth is usually named as enamel apatite (EAP) or carbonated hydroxyapatite because the major substitute is the carbonate radical, about 3.5 wt.% of the total tissue. Its general chemical composition is $Ca_{10}(PO_4)_6(OH)_2$. EAP can have different substitutes for Ca^{2+} such as Na^+, K^+, Mg^{2+} and for phosphate and hydroxyl such as CO_3^{2-}, F^-, HPO_4^{2-}, Cl^- and H_2O. The different substitutions occur because of the inhomogeneous environment under which enamel crystals grow during teeth mineralization. The crystallographic unit cell of EAP is hexagonal and its chemical composition and lattice parameters are very close to those of synthetic hydroxyapatites. In Table 1 these features for EAP are compared to those corresponding to synthetic calcium hydroxyapatites (HAP) prepared under non-aqueous and aqueous conditions.[1]

Heating enamel in an oven produces chemical changes in the mineral matrix such as those related to water and carbonate losses. Loosely bounded water losses occur when enamel is heated to temperatures up to 200 °C[2] while tightly bounded water is totally eliminated only at high temperatures (1300 °C).[3] Carbonate loss starts at 100 °C and the maximum loss rate occurs at about 800 °C. Finally, at 1100 °C, all carbonate is eliminated. Consequently, the total carbonate loss is a function of the whole thermal history associated to the heating and cooling process induced by laser irradiation.

Similar changes as those described above for conventional heating in an oven were also observed in enamel after irradiation with different lasers.[4]

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Table 1 Chemical properties and lattice parameters of non-aqueous, aqueous synthetic calcium hydroxyapatites (Ca-HAP) and enamel apatite (EAP).[1]

	$Ca_{10}(PO_4)_6(OH)_2$		
Apatite type	Ca-apatite (non-aqueous)	Ca-apatite (aqueous)	Enamel apatite
Substitution elements	—	HPO_4^{2-}; H_2O	Na^+; CO_3^{2-}; F^-; K^+; HPO_4^{2-}; Cl^-; Mg^{2+}; H_2O
Ca/P ratio	1.67	1.5—1.64	1.61—1.64
a-axis (nm)	0.9422	0.9433	0.9441
c-axis (nm)	0.6880	0.6882	0.6882

Reduction in water content promoted by Er:YAG (2.94 μm), CO_2 (10.6 μm) and Nd:YAG (1.064 μm) laser irradiation has been observed.[5,6] Carbonate losses were detected in irradiated enamel using a CO_2 laser with different wavelengths.[4,7]

Taking into account that the enamel tissue is mainly composed of hydroxyapatite and water, the light beam yielded by carbon dioxide laser has a wavelength for which the absorption coefficient (5,500 cm^{-1} at $\lambda = 9.3$ μm) is higher than that corresponding to holmium laser (<20 cm^{-1} at $\lambda = 2.1$ μm), this wavelength being also higher than that of neodymium laser (<1 cm^{-1} at $\lambda = 1.064$ μm). The holmium and neodymium pulsed lasers have pulse widths around 400 μs and 100 μs, respectively, while those of carbon dioxide[8] lasers have very short pulses, about 100—200 ns,[9] 2—200 μs,[7] or alternatively, a continuous emission.[10] The differences in absorption coefficient, pulse frequency and pulse width may induce very different local thermal effects. Consequently, differences in thermal history for tissues subjected to different types of laser irradiation are expected to modify the chemical composition of the irradiated samples.

Heating can also affect the structure of enamel inducing the formation of new phases. An X-ray diffraction study of enamel heated in an oven up to 800 °C detected the formation of a β-TCP phase dispersed in the natural HAP matrix. This minor phase remains at least up to 1200 °C. At 1200 °C the X-ray diffraction patterns exhibited two additional weak Bragg peaks corresponding to a *d*-spacing between 0.290 and 0.318 nm that were not identified.[11]

Thermal effects of laser irradiation are also expected to induce structural changes in irradiated enamel. Most of the previous studies were conducted on enamel irradiated with CO_2 lasers under different fluences and wavelengths. The corresponding X-ray diffraction patterns indicated the formation of traces of a α-TCP phase[9,12—15] while one of these studies[9] also detected an additional TetCP phase. More recently, a structural investigation of enamel irradiated with a neodymium laser[16] indicated the formation of traces of α-TCP and β-TCP.

The caries process is a series of demineralization and remineralization events involving enamel and dentin.[17] This process starts from a initial tissue dissolution. If this process go on, the caries lesion progresses and leads to cavity formation. Deposition of tooth minerals (remineralization) can repair or arrest caries lesions. New clinical procedures, such as laser irradiation, are expected to reduce the demineralization of the surface by changing the crystallographic features of the mineral matrix.

It was observed that laser irradiation with specific wavelengths and energy densities produces surface melting. Based on this effect and on the chemical changes mentioned above, the possibility of caries prevention by laser irradiation was extensively evaluated using different types of lasers: neodymium;[18—20]; holmium;[20—22] erbium[23—25] and CO_2.[26—28]

The aim of the study presented here is to characterize the changes in the crystalline structure of bovine enamel after holmium (Ho:YLF) laser irradiation and discuss the analogies and differences when comparing these effects with those obtained by irradiation using other classical lasers.

Materials and methods

The analyzed samples were permanent incisor teeth extracted from a single bovine animal. The tooth crown was separated from the root and sliced longitudinally. The slices of non-irradiated teeth and of those to be laser irradiated were 0.5 and 2 mm, respectively. The effect of laser irradiation was studied using bovine instead of human enamel because bovine teeth are easier to collect, have larger dimensions and are subjected to less formal health regulations. As we will see later, the crystallographic structure and the chemical composition of bovine and human enamel are similar,[29] so as the conclusion of this investigation regarding the effect

of laser irradiation is expected also apply to human enamel.

Enamel was separated from dentin tissue in the natural (non-irradiated) sample by cracking the 0.5 mm slice at the enamel-dentin junction. After the separation, the enamel pieces were ground manually in a mortar and pestle and the resulting powder sieved in order to select particle sizes between 25 μm and 38 μm. This powder sample was used as a reference for the studies of structural variations related to laser irradiation. Different grinding process can produce changes in the X-ray diffraction peak profiles but not in the angular peak positions.[30] Nevertheless, possible variations in peak profiles that may be produced by eventual differences in grinding procedure are not expected to affect phase identification that is the central issue of this paper.

Slices from samples treated with holmium laser were cut thicker than those from the non-irradiated sample because the purpose was to irradiate the surface and collect the melted and cooled tissue close to the border of the cavity produced by laser irradiation. The irradiation was performed on the cross-section surface and not on the buccal (external) surface of the teeth. This procedure was applied in order to avoid eventual contamination of the melted enamel with dentin from the deep part of the cavity, this effect being more probable if the irradiation is conducted on the external tooth surface.

A home-made holmium laser (Ho:YLF)[31] was used for the irradiation. The laser provided a Gaussian beam profile with an emission wavelength of 2065 nm and pulse duration of 400 μs.[32] The studied enamel tissue was irradiated in ablative regime. The energy per pulse ranged from 300 mJ up to 400 mJ, frequency was 0.5 Hz and 250 μm the diameter of the irradiated area. These irradiation conditions produce fluences between 600—800 J/cm^2. The laser beam was focused at the tissue surface with an optical lens ($f = 50.2$ mm), five pulses have being applied to the same area. The melted and quickly cooled tissue located at the cavity border was carefully collected with the sharp edge of a needle, under a stereoscopic vision; and introduced into a glass capillary for X-ray diffraction analysis. Three different samples were prepared using the same collection procedure. The remaining slice was observed by scanning electron microscopy in order to confirm the success of the selective removal of only the melted tissue.

Powder X-ray diffraction measurements were conducted at the synchrotron XRD1 beamline (LNLS, Campinas, Brazil) using a monochromatic X-ray beam with a wavelength $\lambda = 0.148$ nm. A step scanning diffractometer (2θ step $= 0.02^\circ$) equipped with a scintillator photon counter was used for recording the diffraction spectra. Three samples subjected to the same irradiation conditions were studied and all of them yielded similar X-ray diffraction patterns, so indicating that the procedure of powder preparation leads to reproducible results. In order to reduce the effect of statistical errors, a single diffraction pattern obtained as the sum of the three individual patterns was analyzed.

Results

The experimental X-ray diffraction pattern of natural (non-irradiated) enamel is plotted in Fig. 1. In the same figure, the Bragg peaks of a reference hydroxyapatite (HAP) are also indicated (JCPDF 09-0432). The Bragg peaks obtained from JCPDF correspond to a crystalline HAP ($Ca_5(PO_4)_3OH$) phase with a hexagonal unit cell and lattice parameters $a = 0.942$ nm and $c = 0.687$ nm.

The equation that relates the lattice parameters, a and c, of the hexagonal unit cell with the lattice spacings, d_{hkl}, is given by

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

In order to calculate the d_{hkl} spacing, we have selected two intense and well defined peaks of the diffraction pattern of the natural (non-irradiated) bovine EAP, namely (3 0 0) and (1 1 2) and, by applying Eq. (1), we have obtained the unit cell dimensions $a = 0.946$ nm and $c = 0.687$ nm. These values are similar to those of the mentioned HAP ($a = 0.942$ nm and $c = 0.687$ nm) and also close

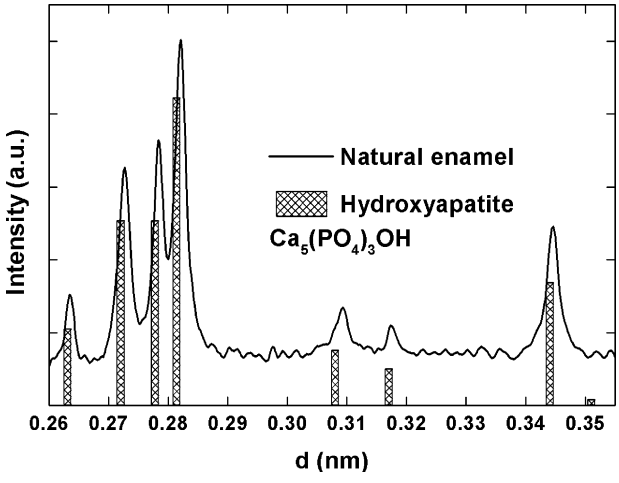

Figure 1 X-ray diffraction pattern of natural bovine enamel before laser irradiation and main hydroxyapatite (HAP) reflections.

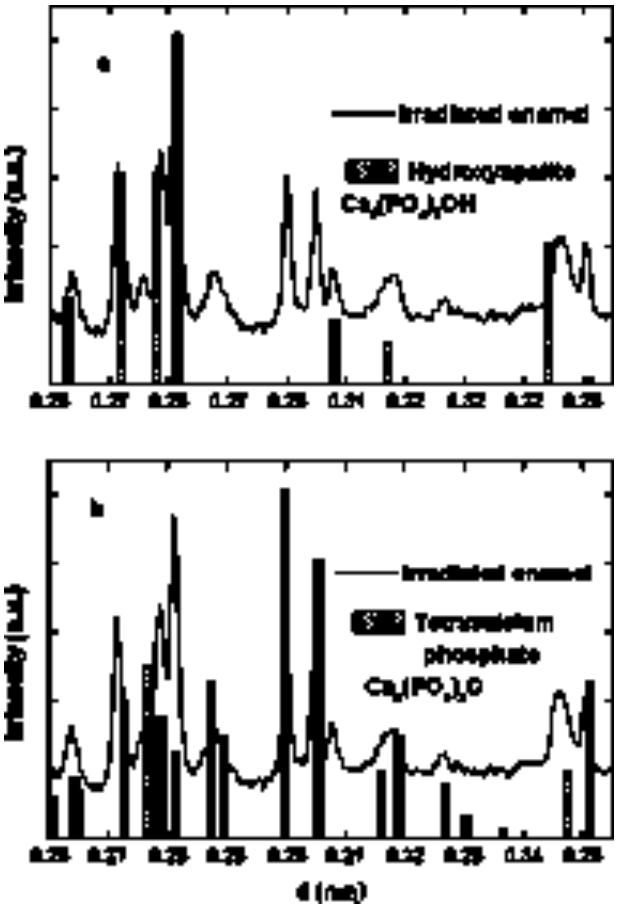

Figure 2 (a) X-ray diffraction pattern of irradiated bovine enamel and main hydroxyapatite (HAP) Bragg peaks. (b) The same experimental X-ray diffraction pattern including the main tetracalcium phosphate (TetCP) Bragg reflection peaks.

to those of human EAP ($a = 0.944$ nm and $c = 0.688$ nm).[33,34]

The experimental X-ray diffraction pattern corresponding to enamel irradiated with a holmium (Ho:YLF) laser is plotted in Fig. 2a and b. In Fig. 2a the Bragg peaks corresponding to HAP are also indicated. In Fig. 2b, the experimental pattern was plotted together with the expected peaks of tetracalcium phosphate—$Ca_4(PO_4)_2O$ (JCPDF 25-1137). The results indicate that, in the irradiated samples, there is a mixture of two crystalline phases: HAP and tetracalcium phosphate (TetCP). TetCP crystals exhibit a monoclinic lattice with unit cell parameters $a = 0.9473(2)$ nm, $b = 1.1986(4)$ nm, $c = 0.7023(1)$ nm and $\beta = 90,\ 90(1)^\circ$.[35]

The experimental d-spacing and the corresponding Miller indexes associated to all the observed Bragg reflections of natural and irradiated enamel are reported in Table 2.

We note in the X-ray diffraction patterns corresponding to the irradiated sample (Fig. 2a and b) that most of Bragg peaks contain contributions from reflections associated to both HAP and TetCP phases. However, in the region corresponding to d_{hkl} between 0.285 and 0.310 nm (Fig. 2a and b), a double peak, (2 2 1)/($\bar{1}$ 0 3), and two other isolated ones, (0 4 0) and (0 3 2), exclusively associated to TetCP, are observed, these four peaks being absent in the spectrum of natural enamel (Fig. 1). The rather large area of the Bragg peaks corresponding to TetCP suggests that the volume of the TetCP phase is a significant fraction of the total analyzed volume.

Discussion

Our X-ray diffraction study demonstrated that irradiation of bovine enamel with a holmium laser under a fluence of 600—800 J/cm^2 promotes the formation of a TetCP phase embedded in the initial HAP matrix. This finding differs from what occurs in enamel when they are irradiated with a neodymium laser, currently applied clinically to caries prevention.[18,36] In this case α- and β-TCP phases are formed.[16] Thus, the differences between the published results concerning neodymium, applied clinically, and those presented here are related to the nature of the phases. The different actions of both types of lasers are related to differences in the thermal effects in the irradiated volume.

While our results indicate the presence of an appreciable amount of TetCP in the analyzed samples, previous investigations reported only small volume fractions of α- and β-TCP.[13,15,16] This difference in the fraction of transformed volume is probably due to differences in the material collection procedure. Notice that we have carefully collected the enamel exclusively selecting the melted part, so as the volume fraction of non-irradiated enamel remaining in our samples was probably much smaller than those utilized in previous investigations. This difference occurs because the volume fraction of the tooth in which the temperature reaches a value above the melting point of enamel is confined into a thin surface layer.

We have noticed that the d-spacings derived from our X-ray diffraction patterns are close to those associated to the two strongest reflections observed for enamel heated in an oven, namely 0.290 nm and 0.318 nm.[11] Our results also indicate that the unidentified Bragg reflections previously observed in natural enamel heated in an oven actually correspond to TetCP. This implies that the new phase developed in holmium irradiated enamel is the same as that observed in enamel at 1200 °C.

Table 2 Measured interplanar spacing (d), Miller indexes (hkl) and phase (HAP and TetCP) association corresponding to natural and irradiated enamel.

d (nm)	Phase	(hkl)	d (nm)	Phase	(hkl)
Natural enamel					
0.263	HAP	(2 0 2)	0.309	HAP	(2 1 0)
0.273	HAP	(3 0 0)	0.317	HAP	(1 0 2)
0.278	HAP	(1 1 2)	0.345	HAP	(0 0 2)
0.282	HAP	(2 1 1)			
Irradiated enamel					
0.264	TetCP	(1 4 1)	0.305	TetCP	(0 3 2)
	TetCP	($\bar{1}$ 4 1)			
	HAP	(2 0 0)			
0.271	TetCP	(2 1 2)	0.308	HAP	(2 1 0)
	HAP	(3 0 0)			
0.275	TetCP	($\bar{2}$ 1 2)	0.318	TetCP	(2 1 1)
				TetCP	($\bar{2}$ 1 1)
				HAP	(1 0 2)
0.278	TetCP	(1 3 2)	0.327	TetCP	($\bar{1}$ 3 1)
	TetCP	(1 1 3)			
	HAP	(1 1 2)			
0.281	TetCP	($\bar{1}$ 3 2)	0.346	TetCP	(1 3 0)
	HAP	(2 1 1)		HAP	(0 0 2)
0.287	TetCP	(2 2 1)	0.350	TetCP	(2 0 0)
	TetCP	($\bar{1}$ 0 3)		HAP	(2 0 1)
0.300	TetCP	(0 4 0)			

Unit cells of HAP and TetCP used for pattern indexation are hexagonal and monoclinic, respectively.

The TetCP phase that we have detected in holmium irradiated enamel was also observed in one of the previous structural investigation of CO_2 laser irradiated enamel.[9] This phase was not observed when using other lasers.[12–16] This difference is due to the variety of thermal histories that can be expected for samples with non-equivalent irradiation conditions. The fact that the TetCP phase was detected either in enamel irradiated with holmium laser and also in a CO_2 laser under specific conditions,[9] suggests that the thermal history of the samples is in both cases similar.

Beside the appearance of a new phase in holmium irradiated enamel, changes in overall chemical composition are also expected.[4] It is known that CO_2 (9.6 μm) laser irradiation induces carbonate loss in the enamel surface,[7] its elimination promotes an increase in acid resistance,[1] according to surface dissolution experiments.[37] Moreover, this effect is expected to occur in the holmium irradiated enamel studied here. This effect may contribute to an improved overall resistance of holmium irradiated enamel in spite of the presence of the detected, more soluble, TetCP. Even though pure TetCP is known to be less resistant to acids than natural EAP,[4] this effect was not yet quantitatively evaluated for holmium irradiated enamel in which TetCP phase is embedded in the EAP. Anyway, in order to clearly establish the relative contributions of the different factors that can affect the acid resistance, a systematic chemical and crystallographic study is required.

Previous in vitro studies demonstrated that the irradiation of Ho:YLF ($\lambda = 2.065$ μm) and Ho:YAG ($\lambda = 2.1$ μm) lasers melt enamel and dentin tissues, suggesting that they can eventually be applied to caries prevention.[21] The interaction of a holmium laser ($\lambda = 2.065$ and 2.1 μm) with dental tissues exhibits some features that differ from those associated to other lasers that are currently used for caries prevention(Nd:YAG) or evaluated for that procedure (CO_2—9.6 μm). For example, the interaction with water molecules of holmium lasers ($\lambda \sim 2$ μm) is stronger than for neodymium ($\lambda \sim 1$ μm) lasers. This feature of holmium laser is probably advantageous because the increase in temperature is expected to be confined in a more superficial volume layer. On the other hand, photons from CO_2 ($\lambda \sim 9.3$–9.6 μm) lasers interact mainly with the phosphate radicals and not with water like

holmium and neodymium lasers. The differences in interaction mechanisms probably affect the thermal process and differences in the maximum temperature in the teeth pulp. For this reason, prior to any attempt of evaluating the application of holmium lasers to caries prevention in living systems, eventual excessive thermal effects should be properly evaluated.

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