

Spectroscopic alterations on enamel and dentin after nanosecond Nd:YAG laser irradiation

A. Antunes^{a,*}, W. de Rossi^b, D.M. Zezell^b

^a Instituto de Física, Departamento de Física Aplicada, Rua do Matao, Travessa R, no. 187, Cidade Universitária, 05508-090 São Paulo, Brazil

^b Instituto de Pesquisas Energéticas e Nucleares, Centro de Lasers e Aplicações, IPEN/CNEN-SP, São Paulo, Brazil

Received 12 September 2005; received in revised form 8 November 2005; accepted 25 November 2005

Abstract

Laser irradiation on hard tissue has produced a resistant surface that is likely to prevent caries. In this study, human enamel and dentine were exposed to nanosecond pulsed Nd:YAG laser with energy densities of 20–40 J/cm² and pulse width of 6 ns inducing chemical changes in these tissues. Infrared analysis of human dental enamel and dentine was performed using the KBr method (2 mg sample/300 mg KBr). A correlation between non-lased and lased spectra was performed that gives an indication of the changes in organic and inorganic compounds after laser–tissue interaction. Spectra of teeth simultaneously show the inorganic and organic parts of the tissue. The principal bands: amide bands A, I, II, and III from the collagen-matrix, phosphate from the mineral content, and carbonate bands were identified. The normalized area of peak versus peak position was determined. Changes of the bands attributed to the collagen matrix were verified after Nd:YAG irradiation. The present results suggest a chemical modification of organic and mineral compounds by laser. The spectral results indicated an alteration in the absorption bands relative to, essentially, organic compounds.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Dental enamel; Dentine; Nanosecond laser; Caries prevention; Fourier transform infrared spectroscopy; Absorption bands

1. Introduction

The first experiments on teeth using laser were performed by Goldman et al. [1] and Stern and Sognnaes [2], with both groups using a pulsed Ruby laser. In dentistry, there is significant interest in the prospects of lasers for substituting conventional methods or to be an auxiliary apparatus for a wide range of oral and dental procedures [3]. Several studies have indicated that Nd:YAG laser can be used to modify the chemical composition and surface morphology of dental enamel and that it can serve as an adjunct in decay prevention [3–5]. Due to an intensive program to caries prevention investigations have appointed to a reduction of the disease in developed and undeveloped countries [6,7]. Many studies have revealed that laser irradiation of sound enamel increases its acid resistance [5]. Infrared spectroscopy has shown that the mineral in laser-irradiated sound enamel is another hydroxyapatite that has been changed to the modified apatite [8–10]. According to Fowler and Kuroda [11] the tem-

perature rise produced by laser irradiation on enamel surface was estimated at approximately 1400 °C. Based upon this temperature range, they predicted that several phase changes would arise with lasing, one of which was the conversion of HAP to β -TCP (β -Ca₁₀(PO₄)₆(OH)₂) [11,12]. These studies have provided information on chemical composition and shown chemical differences between non-lased and lased dental samples, through analysis by infrared spectroscopy.

Nowadays, studies focusing on IR spectroscopic features of human tissues have increased. Chemical characteristics of the sample following laser irradiation are important, and infrared spectroscopy gives information about the chemical structure. It is a common technique for examining both inorganic and organic materials, and has been used to establish quantitative measurements for mineralized tissue composition and physical properties to evaluate qualitative aspects. The FTIR has been employed successfully for analysis of hard dental tissue [12]. Current laser treatment of dental tissues involves diagnostic and prevention of formation of the lesions as well as mineral loss [13]. We have examined the changes through a comparison between the spectrum of each lased sample with the spectrum of the non-lased sample collected previously.

* Corresponding author.

E-mail address: antunes@if.usp.br (A. Antunes).

Many researchers have investigated infrared transmission spectroscopy in order to show the chemical changes induced in laser-irradiated dental enamel [8]. This mechanism is associated with the breaking of molecular and intermolecular bonds due to absorption of light laser whose frequency lies in the range of vibrational resonance of compounds of mineralized tissue [14]. The potential for modifying enamel and dentine by Nd:YAG laser with longer pulses is well recognized [15,16], but the action of shorter pulse laser Nd:YAG on dental structure requires more studies, because the chemical changes induced after short pulse Nd:YAG laser irradiation are not very well known. The aim of this paper is to contribute to the understanding of chemical changes due to laser interaction on human enamel and dentine.

2. Materials and methods

2.1. Sample preparation and FTIR spectra acquisition

Extracted non-carious, human molar teeth were selected and cleaned, after removing the tooth crown horizontally. Following washing and removal of soft tissues remains, the samples were stored in 0.9% sodium chloride solution, which was changed weekly. The Ethical Committee on Human and Animal Research of the Energy and Nuclear Research Institute approved this research (private communication). Enamel is a protective layer of the tooth preserving the internal region of the tooth [17]. Dentine is a portion of the tooth between the enamel and the pulp. Mature enamel contains approximately 96% mineral by tissue weight, the remainder is organic material and water. The principal inorganic constituent of enamel is hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Dentine is softer than enamel. The inorganic salt composition is the same as the enamel, but its organic fiber content is approximately 30% compared to 3% for the enamel [17].

2.2. Enamel pellets prepared with spectroscopically pure KBr

Fourier transformed infrared spectroscopy (FTIR) of human enamel and dentine powder (2 mg), dispersed in a KBr matrix (300 mg), was conducted on spectra recorded using FTIR spectrometer (BOMMEM-MB-100). Each spectrum was collected over the range from 4000 to 400 cm^{-1} with an average of 100 scans, each one taking approximately 2 min to be collected. Each spectrum was acquired subsequent to preparation of the KBr-pellet (see Fig. 1).

3. Nd:YAG laser parameters

The laser beam was provided by means of a commercial Sure-lite laser (Continuum), flash lamp pumped Q-switched Nd:YAG laser that delivers 6 ns pulses at 1.064 nm with a 5 Hz repetition rate for energy density of 20–40 J/cm^2 . In laser mode, the output energy was set at around 25 mJ to produce an estimated energy density of 40 J/cm^2 on the dental surface. All energy density values used were lower than the threshold for ablation of the enamel.



Fig. 1. Enamel pellets prepared with spectroscopically pure KBr.

4. Results and discussion

FTIR spectra were recorded as described previously. Peak intensities were calculated using two approaches: (1) peak fitting and (2) Fourier self de-convolution followed by band integration. Figs. 2 and 3 show an averaged infrared spectrum in the region from 4000 cm^{-1} to 800 cm^{-1} for non-lased and lased human dentine (20 J/cm^2 , 6 ns, 5 Hz). The peaks in these spectra have

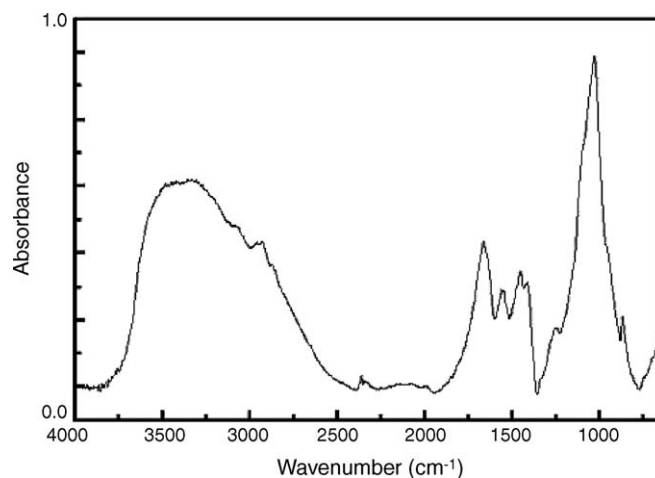


Fig. 2. Average absorption spectrum for healthy human dentine.

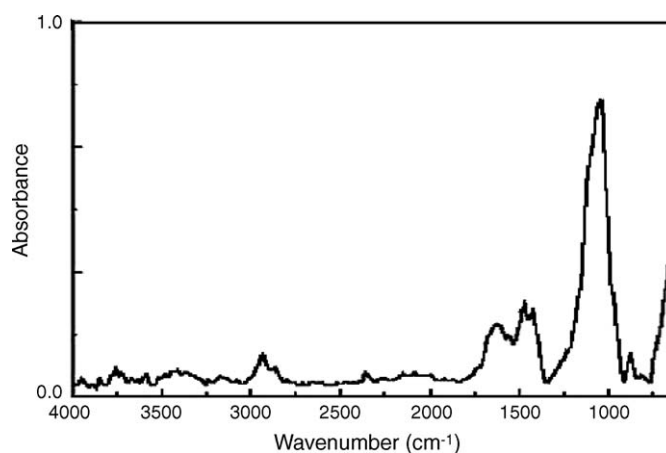


Fig. 3. Average absorption spectrum for irradiated human dentine.

Table 1
Infrared characteristic bands and peaks associated

Characteristic bands	Absorption peaks (cm ⁻¹)
Amide I	1660
Amide II	1550
Amide III	1240
CO ₃ ⁻² (ν ₂)	870–960
CO ₃ ⁻²	1415
CO ₃ ⁻² (ν ₃)	1450–1550
PO ₄ ⁻³ (ν ₁)	960
PO ₄ ⁻³ (ν ₃)	1035
CO ₃ ⁻²	872
PO ₄ ⁻³ (ν ₁)	604
PO ₄ ⁻³ (ν ₁)	564

been assigned according to the literature that has been listed in Table 1 [18].

Features associated with the mineral component of dentine include the orthophosphate bands at 1030–1160 cm⁻¹, and 600–560 cm⁻¹, and the carbonate band at 870 cm⁻¹. The carbonate band at 1450–1425 cm⁻¹ is overlapped with the amide II band (1550 cm⁻¹, N–H, and C–N) and OH⁻ (1630 cm⁻¹) [19,20]. The amide peaks were observed between 1680 and 1200 cm⁻¹. The amide peaks were divided into the amide I peak at 1660 cm⁻¹, the amide II peak at 1550 cm⁻¹ and the amide III peak at 1240 cm⁻¹. The hydroxyl (OH) group absorption peak was recorded between 3600 and 2400 cm⁻¹. The orthophosphate group was recorded in the absorption peak, 1035 cm⁻¹. The peak at 2340 cm⁻¹ corresponds to molecular CO₂ and has been reported to be difficult to measure due to its volatility [20].

For human dentine, the area associated with organic compounds increased, such as amides I, II, and III. For human enamel, the change in the area of the 1030 and 1550 cm⁻¹ peaks can be observed. The mean peak areas versus peak position are shown in Figs. 4 and 5 for human dentine and Figs. 6 and 7 for human enamel. Studies of natural tooth samples, although

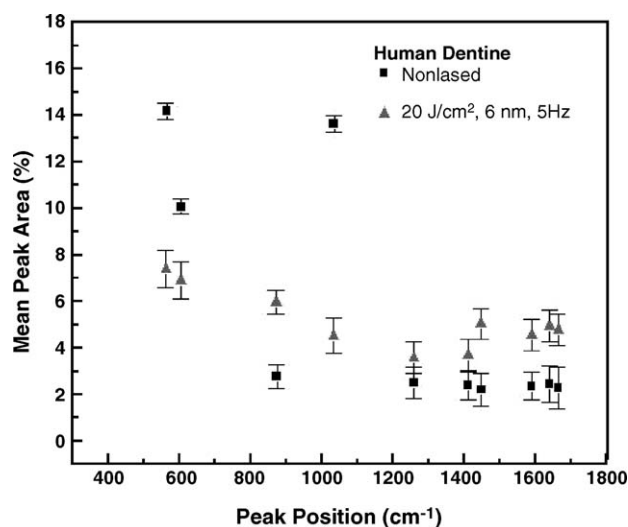


Fig. 4. Comparison of spectral analysis parameters for non-lased and lased human dentine for 20 J/cm². We observed an alteration in the peak area of the organic compounds and carbonate bands. These alterations are less pronounced than that for 40 J/cm².

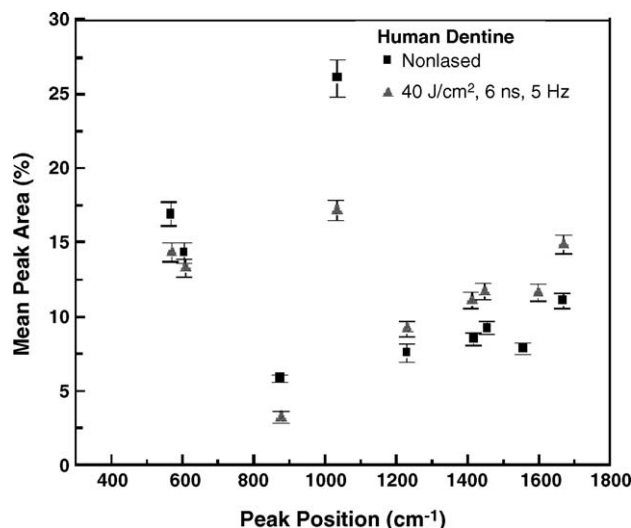


Fig. 5. Comparison of spectral analysis parameters for non-lased and lased human dentine for 40 J/cm². The spectral areas with the most pronounced difference occur in the organic matrix, for which the area increases (amides I, II, III and carbonate 1415 and 1450 cm⁻¹).

they have a very different history, contain mineral particles of disordered carbonated hydroxyapatite of the same morphology and size.

The chemical composition of the teeth [2,11,18] is variable with respect to the total mineral content and organic matrix, which is similar to the characteristic spectra obtained in our study for different constituents of teeth. During the curve-fitting process, peak parameters such as exact position, peak width, and peak height were allowed to vary. By plotting these parameters, the area of the fitted peaks obtained as a function of peak position was determined, and a parameter for comparison between the non-lased and lased samples was established.

Figs. 6 and 7 show the comparison of average spectra for non-lased enamel and for two different energy densities, respectively.

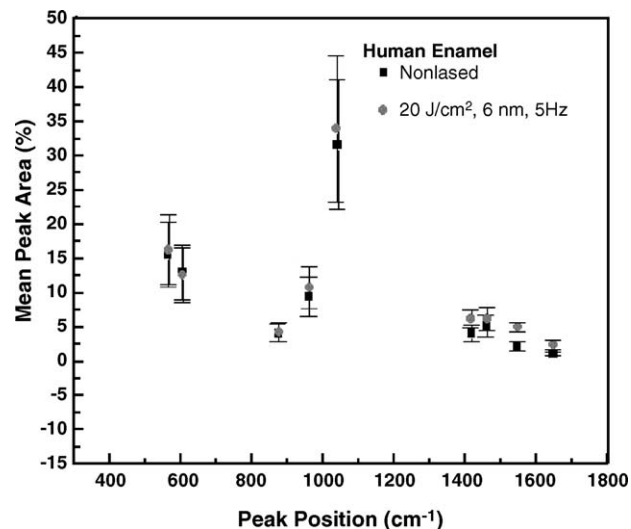


Fig. 6. Comparison of spectral analysis parameters for non-lased and lased (20 J/cm²) has shown an alteration in organic matter (amides I and II, and carbonate peaks) and a statistically insignificant change in the peak positions associated with inorganic compounds.

The relative percent areas of the majority of the peak components are similar. The absorption spectrum of lased human dentine for different energy density is shown in Fig. 3 for an energy density of 20 J/cm^2 . The main features of the 1700 and 1100 cm^{-1} (regions where the organic matrix absorbs) contains valuable information on the amides I (1650 cm^{-1}), II (1540 cm^{-1}), and III (1240 cm^{-1}) modes. A strong band was reported in the 1650 cm^{-1} region that corresponds to the human dentine samples and was attributed to the C–O stretching of amide I band. This position was modified after laser interaction.

Other important changes occur in the organic matrix when the amide I overlaps with water bands, appearing as a band at 1645 cm^{-1} . All samples of human dentine studied showed bands in 1240 cm^{-1} , which can indicate the presence of the amide III band [20] and changes in the mineral compound associated with carbonate bands. The FTIR spectrum of enamel differed from the dentine with less intense bands from organic compounds functional groups, which agrees with the chemical composition. Carbonate bands in the positions shown in Table 1 also overlap with organic matrix bands. After a nanosecond pulse Nd:YAG laser exposure, human dentine samples for the two energy densities displayed an increase in the areas relative to the organic compounds (Figs. 4 and 5), indicating an alteration in the associated band. The linear increase in the area of 1030 cm^{-1} peak indicates that the normalized area of the peak is directly related to the total amount of calcium and phosphorus present in the inorganic matter and to the modification of this compound after interaction between laser and dental hard tissue. Characteristic absorption bands observed routinely in dental tissues include features attributable to the mineral as well as organic components. The most intense bands in Figs. 6 and 7 are associated with the mineral component and their contribution to the spectra. Vibration bands due to organic components will interfere with the CO_3 vibration bands (associated with mineral component) in the region of $1500\text{--}1400 \text{ cm}^{-1}$.

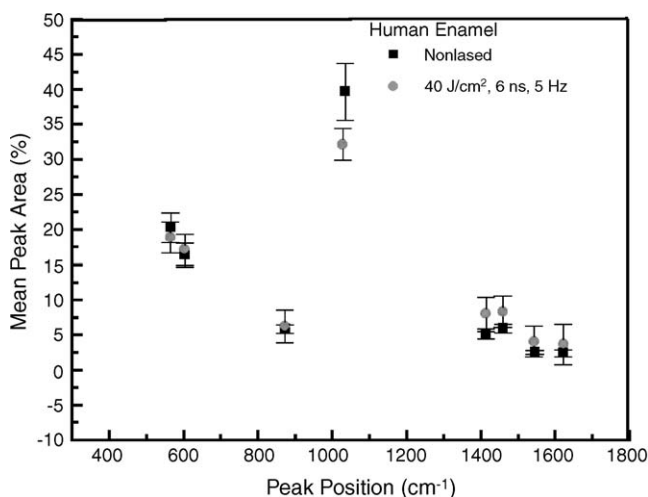


Fig. 7. Comparison of spectral analysis parameters for sound and irradiated human enamel for 40 J/cm^2 . The spectral areas altered are organic matter (amide I, II and carbonate in the peak position 1415 and 1461 cm^{-1} (associated the carbonate type B and type A, respectively).

5. Conclusion

We have shown that FTIR is a powerful technique for measuring inorganic and organic compounds present in dental hard tissue by the KBr method, and that it is possible to obtain a good quality FTIR spectrum by this method. From the molecular assignments proposed, it is possible to identify bands, which are sensitive to tissue after laser irradiation. In fact, the laser radiation causes morphological changes to the dental surface and leads to an alteration in the band characteristics for mineral content as well as in band characteristics for organic content such as water, amides I, II, and III. The collagen matrix bands are suppressed after Nd:YAG laser irradiation of human enamel and dentin.

This study has demonstrated that surfaces irradiated by Nd:YAG laser with a pulse width of 6 ns or less displayed a small modification of the bands for phosphate and the collagen matrix. The application of this technique as complementary to other analysis is ideal due to the minimal amount of tissue used in KBr method. We also indicated that nanosecond Nd:YAG laser treatments change a small quantity of inorganic compound and the greater part of compound modified has been organic matter. The data of the present study are the first to establish the comparison of mean peak areas in non-lased and lased enamel and dentine. This research is innovative because the use of short pulse Nd:YAG on human enamel and dentine, under the conditions investigated, and the subsequent FTIR analysis have not previously been studied. Authors have indicated that the result of laser–tissue interaction is not merely a restricted heating effect: the heating of the dental tissues by laser induces the formation of $\text{TCP-}\text{Ca}_3(\text{PO}_4)_2$.

Acknowledgement

This work was supported by the State of Sao Paulo Research Foundation-FAPESP, Process 00/07378-9.

References

- [1] L. Goldman, P. Hornby, R. Meyer, B. Goldman, *Nature* 203 (1964) 417.
- [2] R.H. Stern, R.F. Sognnaes, *J. Dent. Res.* 43 (1964) 873.
- [3] A. Antunes, S.S. Vianna, A.S.L. Gomes, W. de Rossi, D.M. Zetzell, *Laser Phys. Lett.* 2 (2005) 141.
- [4] S. Tagomori, T. Morioka, *Caries Res.* 23 (1989) 225.
- [5] T. Oho, T. Morioka, *Caries Res.* 24 (1990) 86.
- [6] R.L. Zanata, M.F.L. Navarro, J.C. Pereira, et al., *Braz. Dent. J.* 14 (2003) 75.
- [7] L.K.A. Rodrigues, M.N. Santos, D. Pereira, A.V. Assaf, V. Pardi, *J. Dent.* 32 (2004) 531.
- [8] T. Hamakawa, P. Senawongse, M. Otsuki, J. Tagami, Y. Sato, T. Eguchi, K. Shihoyama, M. Kawachi, *Int. Congress Ser.* 1248 (2003) 121.
- [9] A. Bahar, S. Tagomori, *Caries Res.* 28 (1994) 460.
- [10] K. Sato, *Caries Res.* 17 (1983) 490.
- [11] B.O. Fowler, S. Kuroda, *Calcif. Tissue Int.* 38 (1986) 197.
- [12] A. Aminzadeh, S. Shahabi, L.J. Wash, *Spect. Acta A* 55 (1999) 1303.
- [13] M. Hossain, Y. Nakamura, Y. Kimura, Y. Yamada, T. Kawanaka, K. Matsumoto, *Laser Surg. Med.* 19 (2001) 105.
- [14] J.D.B. Featherstone, D.G.A. Nelson, *Adv. Dent. Res.* 1 (1987) 21.

- [15] M.H. Niemz, *Laser –Tissue Interactions: Fundamentals and Applications*, Springer, 1996.
- [16] A. McDonald, N. Claffey, G. Pearson, W. Blau, D. Setchell, *J. Dent.* 29 (1) (2001) 43.
- [17] Ten Cate, *Oral Histology Development, Structure, and Function*, third ed., St. Louis, 1989. pp. 213.
- [18] K. Kawasaki, J. Ruben, H. Tsuda, M.C.D.N.J.M. Huysmans, O. Takagi, *Caries Res.* 34 (2000) 395.
- [19] P. Griffiths, J. Chalmers (Eds.), *Handbook of Vibrational Spectroscopy*, Wiley, Chichester, 2001.
- [20] M.C.P. Lin, B.S. Lee, F.H. Lin, S.H. Kok, W.H. Lan, *J. Endod.* 27 (2001) 389.