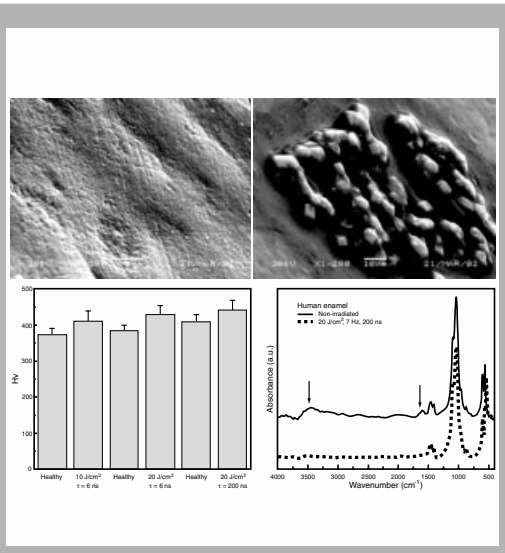
Abstract: Application and development of new methods in caries prevention is of paramount importance to reduce the incidence of chronic cases of the caries disease and to preserve dental structure. In this work, we tested nanosecond pulsed Nd:YAG laser on enamel surface examining the changes laser-induced by means SEM, EDS, and FTIR. SEM revealed, the existence of melted zone and bubble inclusions when 40 J/cm^2 (τ = 6 ns, 5 Hz) was applied on human sound enamel surface. The morphological alteration to 10 J/cm^2 (τ = 6 ns, 5 Hz) and to 20 J/cm^2 (τ = 200 ns, 7 Hz) no presented melting aspect, but a greater number of bubble inclusions. We have indicated that the Ca/P ratio increased to irradiation conditions employed here. In our previous study, the combination of the laser parameters not produce an excessive increase in temperature. The temperature variation was less than 2.5°C in the dental pulpal, following application of the energy density of 40 J/cm^2. Our results suggest that nanosecond pulsed Nd:YAG laser can be used to obtain minimal morphological alteration associated with a chemical reorganization enhancing the microhardness values and consequently inhibiting the acid dissolution by bacterial agents. In this work, the Vickers microhardness was quantified. Our results of FTIR analysis indicated that the laser effects occurring substantially in the organic compounds (such as water and proteins groups) and are reduced for mineral contend (such as phosphate and carbonate groups).



A representative SEM viewing of the healthy enamel surface (top-left); Laser treatment to 40 J/cm^2(τ = 6 ns) (top-right); Vickers hardness graphics to all energy densities (bottom-left); Infrared spectrum of healthy and irradiation human dental enamel to 20 J/cm^2(τ = 200 ns, 7 Hz) (bottom-right)

Surface morphology, elemental distribution, and spectroscopic changes subsequent the application of nanosecond pulsed Nd:YAG laser on dental enamel surface

A. Antunes, [1,*] *S.S. Vianna,* [2] *A.S.L. Gomes,* [2] *W. de Rossi,* [1] *and D.M. Zezell* [1]

[1] Instituto de Pesquisas Energéticas e Nucleares, Centro de Lasers e Aplicações, IPEN/CNEN-SP, São Paulo, Brazil
[2] Departamento de Física, Universidade Federal de Pernambuco, Recife, Brazil

Received: 12 October 2004, Accepted: 16 October 2004
Published online: 11 November 2004

Key words: teeth; enamel; laser irradiation; SEM; elemental distribution; infrared spectroscopy

PACS: 61.80.Ba, 68.37.Hk, 87.64.Je

1. Introduction

For decades, the evolution of the caries disease in the world has been associated to the social and public healthy problems.

Nowadays, the reduction of this disease has been observed in developed and undeveloped countries [1,2] in parts due to intensive programs to caries prevention. Investigations has been performed in Brazilian scholars evidencing this reality [3,4]. On the other hand, chronic clinical cases still occurs due to the high ingestion of cariogenic

* Corresponding author: e-mail: antunes@if.usp.br

products validating the constant analysis of successful preventive methods. Inspired on the pioneers works of Goodman et al. (1964) and Stern and Sognnaes (1964) [5,6], using pulsed Ruby laser, innovator studies have been performed in the world demonstrating the potential of laser inhibiting the caries action. The laser has been appointed as an efficient auxiliary method in distinct dental procedures and its use to combat the proliferation of caries has been observed in many investigations [7–9]. It is well know that intrinsic factors of the laser source (wavelength, emission mode, pulse energy, beam dimension, pulse duration, and application method on the tissue) and external parameters (energy density, and time exposition) are relevant to understand the interaction mechanism between laser and biological tissue [9,10]. The absorption coefficient to tissue components determine the degree of interaction between laser and tissue. Specifically, the Nd:YAG laser (near infrared) presents weak absorption for dental enamel components as water and hydroxyapatite. This fact justify the restrict use in some odontological procedures due to thermal damage induced. It is well establish the role of longer pulsed Nd:YAG laser on the enamel surface inducing morphological and chemical changes, but the application of nanosecond pulsed Nd:YAG laser on dental enamel surface still needs to be improved. However, the FDA (Food Drug Administration) has approved its use to some dental procedures on hard tissue, among them: laser assisted tooth whitening, caries removal and tooth preparation, etching and curing of preventive and restorative resins [11]. Commercially, the lasers have been included for use in dental practice in Australia since 1990 [11]. Thus, systematic investigations has included each day a new laser system and respective irradiation conditions contributing for discussions about the theme [12–16]. At this moment, the main question is related to what is the most adequate laser and/or most flexible for use in distinct odontological procedures, thus the role of the laser in the modern dentistry has been discussed by the scientific community. This fact was the main impulse for this investigation. Based on this viewpoint and understanding that laser irradiation on enamel leads a series of changes to tissue our purpose aim to characterize the morphological, chemical and spectroscopic changes on enamel surface subsequent application of nanosecond pulsed Nd:YAG laser. Complementary technique to evaluate the mechanical aspect, Vickers Microhardness, was carried out. The relation between microhardness values and compositional variation has also been discussed in this study.

2. Material and methods

2.1. Sample preparation

About enamel, it is established that the mineral content and ultrastructure of its contribute mainly to the hardness of the tissue. The chemical composition is 96 wt% inorganic content and 4 wt% organic material and water

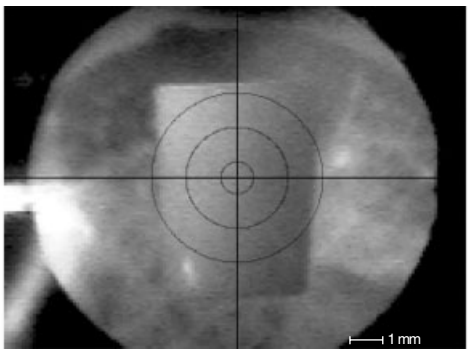


Figure 1 Optical microscope photograph of one of the enamel blocks positioned for analysis

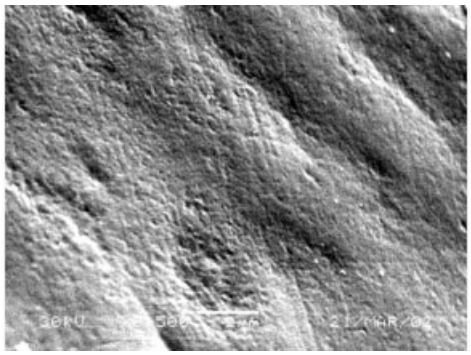


Figure 2 A representative SEM viewing of the enamel healthy surface

and the inorganic material corresponds the hydroxyapatite whose chemical formula is $Ca_{10}(PO_4)_6(OH)_2$. Extracted non-carious, third molars were selected and cleaned, after removing the tooth crown horizontally. This research was approved by the Ethical Committee on Human and Animal Research of the Institute of Research Energetic and Nuclear (Private Communication). Optical microscope photograph of one of the human enamel blocks positioned for analysis is shown in Fig. 1.

2.1.1. Morphological analysis

Scanning electron microscopy (SEM) and X-Ray analysis by energy dispersive spectrometry were carried out with JEOL-5900 instrument attaches to an analytical system for chemical analysis. The human dental enamel were prepared using a diamond disc in order to provide a plain area of approximately 6 mm^2 for the posterior morphological and chemical analysis (SEM-EDS). The samples were dehydrated in a graded sequence of aqueous ethanol (50, 70, 90, and 100% ethanol) by 20 min at each concentration.

2.1.2. Compositional analysis

Twenty dental enamel blocks were analyzed for quantitative elemental analysis using an energy-dispersive spec-

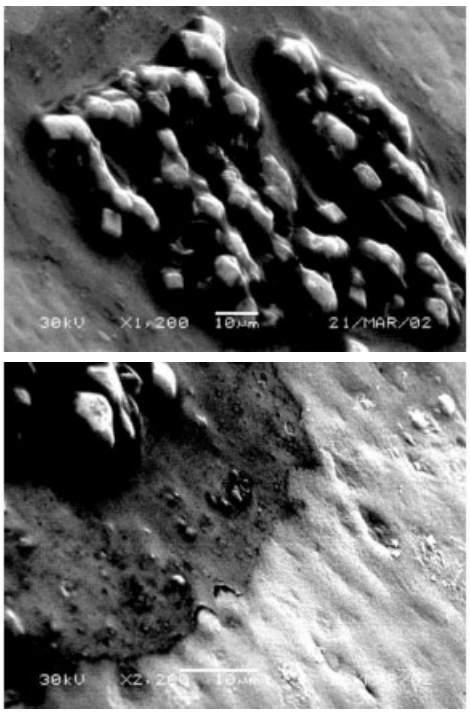


Figure 3 A representative SEM viewing of the enamel surface after laser treatment to 40 J/cm^2 ($\tau = 6$ ns, repetition rate 5 Hz) (top). Effects as surface fusion, melting in the borders can be visualized (bottom)

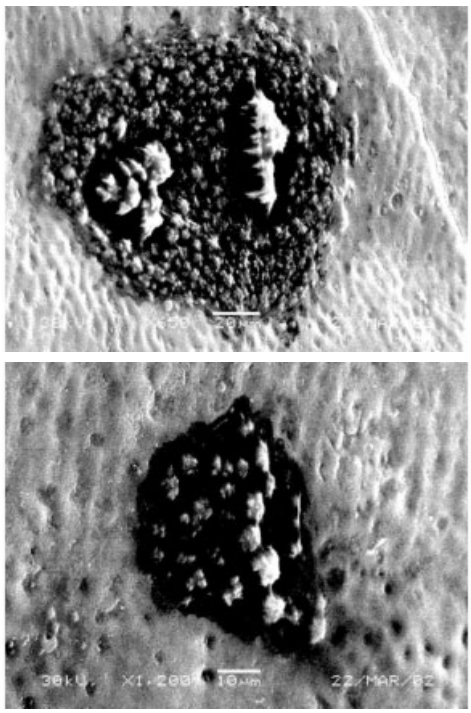


Figure 4 SEM micrographs showing the enamel surface morphologies to laser treatment to 10 J/cm^2($\tau = 6$ ns, repetition rate 5 Hz) (top) and laser treatment to 20 J/cm^2 ($\tau = 200$ ns, repetition rate 7 Hz) (bottom)

trometry (EDS) connected to JEOL-5900 scanning electron microscope (SEM). The operating conditions for both healthy and irradiated enamel blocks were 15 KV and counting times of 100 seconds. The detector used was a Si(Li). In order to obtain representative compositions were selected five areas in each healthy and irradiated enamel blocks. The analysis are presented as normalized to 100%.

2.1.3. Microhardness test

The operation principle for microhardness measurements uses a diamond indentator of standard geometry, typically 136°C square based pyramidal (Vickers) that was indented under enamel blocks with known load for a fixed period. Microhardness was determined using a Vickers diamond (model MHT-1, Matsuzawa, Japan) at a load of 100 grams applied for 10 seconds. Five indentations were performed on each enamel block and respective average determined, the points were randomly selected.

2.1.4. Spectroscopic analysis

Fourier Transformed Infrared Spectroscopy (FTIR) of human enamel was recorded using FTIR spectrometer (BOMMEM-MB -100). Each spectrum was collected over the range from 4000 to 400 cm^{-1} with resolution of 4 cm^{-1} and an average of 100 scans, each one taking approximately 2 min to be collected. Spectra were acquired from different areas of healthy and laser-irradiated enamel blocks.

2.2. Laser parameters

The laser beam is provided from a commercial laser Surelite (Continuum), flash lamp pumped Q-switched Nd:YAG laser ($\lambda = 1064$ nm). Energy densities from 10 J/cm^2 to 40 J/cm^2 ($\tau = 6$ ns, repetition rate 5 Hz) was applied in the first experiment. The second experiment was composed by energy density from 10 J/cm^2 to 20 J/cm^2 ($\tau = 200$ ns, repetition rate 7 Hz).

The dental enamel blocks were then exposed to nanosecond pulsed Nd:YAG laser using distinct laser conditions. An x-y translation system with adjustable speed controller was used to move each block during the laser irradiation procedure.

3. Results and discussion

3.1. Morphological changes

SEM observations of healthy enamel surface is shown in Fig. 2. The Fig. 3 presents morphological changes on

Groups	Ca (%)	P (%)	Na (%)	Cl (%)	K (%)	Ca/P ratio
Healthy	65	34	<1	<1	<1	1.91
40 J/cm^2($\tau = 6$ ns)	46	22	11	19	2	2.09
Healthy	66	32	1	<1	<1	2.06
10 J/cm^2($\tau = 6$ ns)	48	22	10	16	4	2.18
Healthy	64	33	1	1	<1	1.93
20 J/cm^2($\tau = 200$ ns)	54	24	6	14	2	2.25

Five measuring points were selected on three blocks of each tooth resulting in a total number of 15 measuring points per tooth

Table 1 Average results for dental enamel contend by EDS analysis (wt%) for healthy and irradiated enamel

healthy enamel surface induced by nanosecond laser for distinct energy densities. Laser parameters used were energy densities from 10 J/cm^2 and 40 J/cm^2 ($\tau = 6$ ns, repetition rate 5 Hz). To maximum energy density applied the area of the affected zone was 3 times bigger than expected. The morphological changes observed on enamel surface were fusion, recrystallization, and the structure with various pores and bubble inclusions shown in the Fig. 3 (top). The Fig. 3 (bottom) shows details of melting zone to maximum energy density applied on enamel surface.

Similarly, the SEM observations to 10 J/cm^2 ($\tau = 6$ ns, repetition rate 5 Hz) presented fusion and recrystallization aspect with a reduced melting zone visualized in the Fig. 4 (top). Fig. 4 (bottom) shows the modified region enamel to 20 J/cm^2 ($\tau = 200$ ns, repetition rate 7 Hz), again the fusion aspect is observed with few pores and bubble inclusions.

No others significant morphological alternations such as: craters, or laser induced fractures were found. The SEM analysis of the surface of each enamel block have shown the evidence of melting surface, morphological changes and fusion aspect. Significative difference in the SEM examination was found to enamel blocks irradiated laser treatment to 40 J/cm^2 ($\tau = 6$ ns, repetition rate 5 Hz).

3.2. Elemental analysis

The irradiation conditions are sufficient to modify the enamel surface. The means for the Ca/P ratio are shown in Tab. 1. The Ca/P ratio was greater than in the irradiated areas. Typical X-Ray spectra obtained for irradiated dental enamel is shown in the Fig. 5. The results of EDS measurements show that after laser irradiation on enamel surface decrease in Ca and in P. Tab. 1 also shows the mineral contend percentage average for healthy and irradiated dental enamel for conditions of irradiation here applied and its respective Ca/P ratio. Fig. 5 shows a typical EDS spectrum collected for irradiated surface(irradiation condition: 40 J/cm^2, 6 ns, 5 Hz).

The present results showed that laser treatments modify more organic components of hard tissue than inorganic

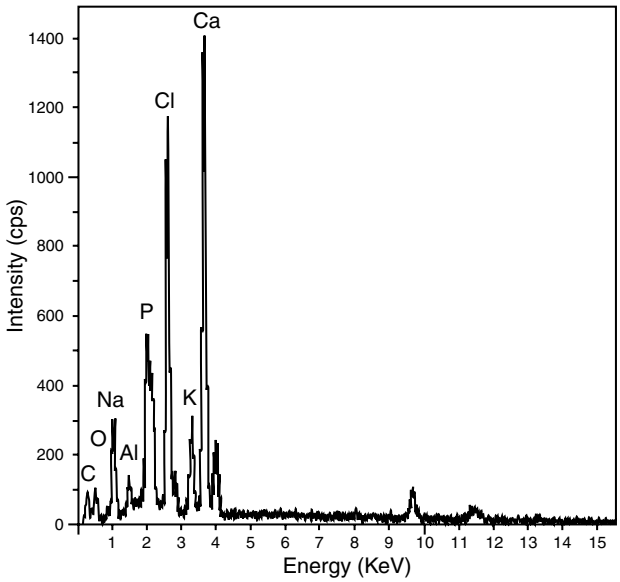


Figure 5 EDS analysis results of dental enamel surface to irradiation condition of 40 J/cm^2 ($\tau = 6$ ns, repetition rate 5 Hz)

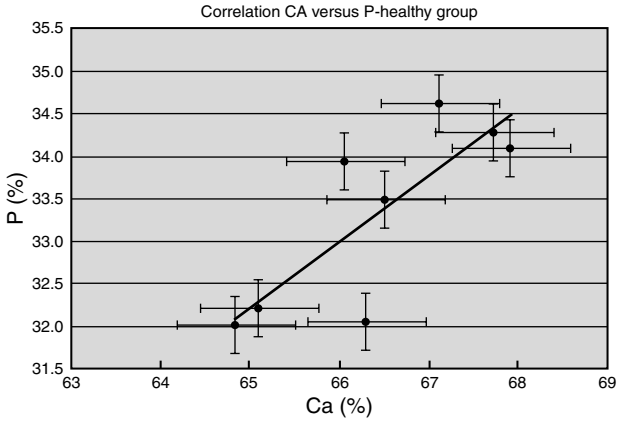


Figure 6 The Pearson coefficient was calculated to the healthy group 1 ($r = 0.803$) indicating a strong correlation between Ca and P contend

components. Table 1 lists percentage of the chemical composition and the Ca/P ratio. The Ca content of the ranges from 65% to 46% and P contend from 34% to 22% for irradiation condition 1 (40 J/cm^2, $\tau = 6$ ns, 5 Hz). To the condition 2 (10 J/cm^2, $\tau = 6$ ns, 5 Hz) the amount of Ca varied of 66% to 48% and P contend from 32% to 22%. The enamel blocks treated in the irradiation condition 3 (20 J/cm^2, $\tau = 200$ ns, 7 Hz) presented average percentage varies between 64% and 54% to Ca contend and between 33% and 24%, respectively. The Ca/P ratio determined to heathy groups is similar. The Pearson coefficient was determined to healthy and irradiated groups.

Irradiation Conditions	Hv Initial ± SD	Student t-test p<0.05	HvFinal ± SD
10 J/cm^2, 6 ns	374±17	sig	412±27
20 J/cm^2, 6 ns	385±15	sig	430±24
20 J/cm^2, 200 ns	410±18	sig	442±26

Statistical comparisons were performed using Student's Paired t-test

Table 2 Mean Vickers hardness values (±SD)

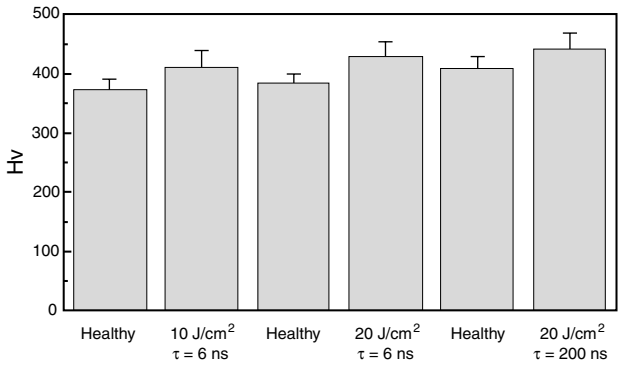


Figure 7 Vickers microhardness test results at distinct irradiation condition

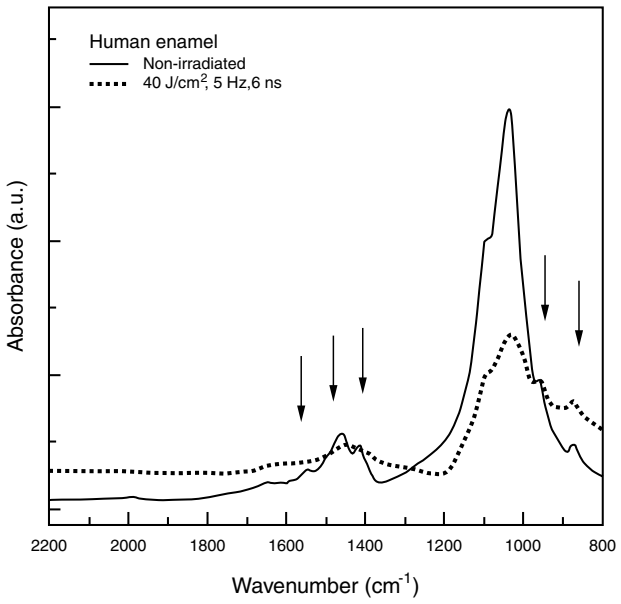


Figure 8 Averaged spectrum of human enamel for 40 J/cm^2(τ = 6 ns, repetition rate 5 Hz. The amide I and II have been enlarged in irradiated conditions

The results have indicating that the positive correlation for majority elements (Ca and P) is preserved after irradiation for all groups, however the healthy groups present strong correlation and to irradiation groups present moderate correlation. The Fig. 6 presents a correlation between the majority elements.

Still, in according to the Table 1, the results of EDS analysis to wt% Ca/P ratio indicated that the Ca/P content is similar in the health groups, but an increase is observed to all irradiation groups.

The dental enamel sub-structure consists of crystalline grains containing some impurities such as Cl, F, Na^+, K^+, and Mg^{2+} [17]. Some trace elements were observed in the EDS analysis. Averaged content to elements that are present in the enamel sub-structure vary significantly to irradiated groups indicating that the laser induces a chemical reorganization of elements inside of the thin layer modified.

Recent investigations about biochemical aspects revealed that the laser-interaction on organic contend in enamel has a relevant role in the inhibition of its demineralization [18–20].

These data confirm that the decrease in P after irradiation has been correlated to volatile at a temperature, at least, as high as 1125°C [21].

3.3. Microhardness test

The mechanical properties of the calcified tissues has been correlated to its mineral content [22]. Several investigations reported present Vickers Microhardness average values for healthy enamel ranging from 263 ± 26 to 431 ± 35 [23–27]. The Vickers microhardness (Hv) values for distinct irradiation conditions are presented as percentages in Tab. 2. Our results show that the hardness values increased for all irradiation conditions applied in this study (Tab. 2). Enhancing hardness values its resistance to acid dissolution increase too, in according to others investigations published [21,22]. The Fig. 7 shows the Vickers Microhardness average values obtained to healthy enamel and irradiated groups.

Paired Student's t-test was used to identify statistical significant differences in the enamel blocks analysis. The average Vickers microhardness obtained to irradiation groups in comparison to healthy groups, shown in the Tab. 2, presented statistically significant difference ($p < 0.05$).

3.4. Spectroscopic analysis

The laser radiation changes morphologically the dental enamel surface and occurs an alteration in bands characteristics for mineral content as well as in bands characteristics of organic content as water, Amide I and II.

The protein bands are reduced after Nd:YAG laser irradiation on the human enamel in according to Figs. 8, 9, and

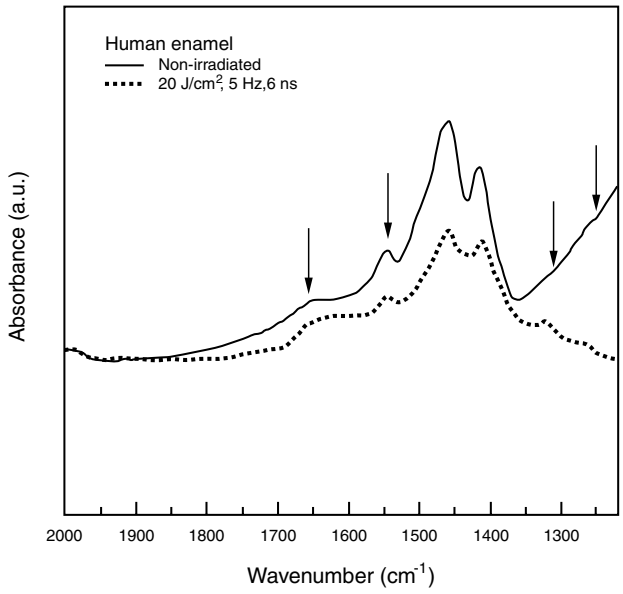


Figure 9 Averaged spectrum of human enamel for 10 J/cm^2($\tau =$ 6 ns, repetition rate 5 Hz). The figure shows the infrared spectra of human enamel before (black line) and after laser irradiation (dot line). There are spectral difference in each band, notably in the amide I, amide II, and carbonate peaks

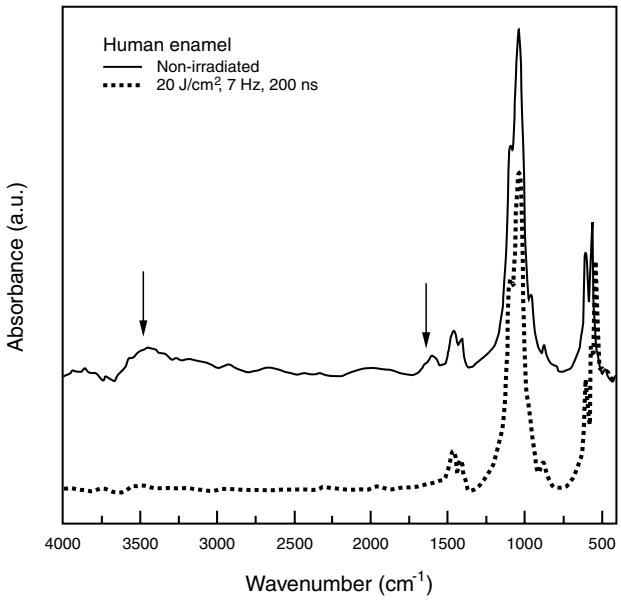


Figure 10 Averaged spectrum of human enamel for 20 J/cm^2 ($\tau =$ 200 ns, repetition rate 7 Hz). In this case, the principal alterations occurs to amide I and amide A + H_2O

Characteristic Bands	Absorption peaks (cm^{-1})
Amide I	1650
Amide II	1540
Amide III	1240
$CO_3{}^{-2}$ (ν2)	870-960
$CO_3{}^{-2}$	1415
$CO_3{}^{-2}$ (ν3)	1550-1450
$PO_4{}^{-3}$ (ν1)	960
$PO_4{}^{-3}$ (ν3)	1030
$CO_3{}^{-2}$	872
$PO_4{}^{-3}$ (ν1)	604
$PO_4{}^{-3}$ (ν1)	564

Table 3 Infrared characteristic bands and peaks associated [28]

10. Our study confirms that due to the superficial heating, quantitatively the organic matter and phosphate vibration bands are altered. In contrast to the spectral profile of the spectra of all the irradiated samples presents reduction or suppression of water band (group O-H) and group amide I and II.

The peaks in these spectra have been assigned according to the literature that has been showed in Tab. 3 [28].

The infrared absorption peaks before and subsequent Nd:YAG irradiation for distinct laser parameters conditions are shown in Figs. 8, 9, and 10. The absorption peaks of the stretching vibration mode of amide I, and amide II bands correspond respectively to 1550 cm^{-1} and 1650 cm^{-1}. Surfaces that were irradiated by Nd:YAG laser with pulse width of 6 ns or inferior presented a decrease on the OH and amide bands, which are mainly related to organic components. A reduction in the intensity of $PO_4{}^{-3}$ (ν_1 964 cm^{-1}, ν_3 1030 cm^{-1}, ν_4 585 cm^{-1}), and $CO_3{}^{-2}$ (ν_1 1070 cm^{-1}) was obtained proportionally to energy density and pulse width used.

4. Conclusion

The results of this work demonstrate that the application of nanosecond laser ($<$ 10 ns) on enamel surface produces a compositional alteration without thermal damage pulpal ($<$ 2.5°C). The morphological changes observed on enamel surface were fusion, recrystallization, and the structure with various pores and bubble inclusions. This observation are consistent with morphological alterations produced by laser. The compositional analysis by EDS has indicated that the P contend decrease subsequent irradiation justifying an increase in the Ca/P rate. This decrease in the P contend was observed to be more accentuated after the irradiation with pulse width 6 ns and moderate for pulse width 200 ns laser irradiation. We observed that the irradiation at the non-resonant wavelength caused loss in the element P, especially in the irradiated area. In this area, a decrease in P means an enlarged in the hardness of the dental enamel that was observed in the Vickers microhardness test. Thus, this would indicate recrystallization in enamel structure. The organic and mineral contend has been altered after nanosecond laser-tissue interaction.

The changes in water contend and protein groups (such as amide A, amide I, amide II) have been observed in the spectra obtained. In summary, we report distinct experimental techniques to characterize changes in dental enamel irradiated with nanosecond laser. New experiments still can be performed to determine optimal parameters of application in others dental tissues and dental procedures.

Acknowledgements This work was supported by the State of São Paulo Research Foundation-FAPESP, Process 00/07378-9 and CAPES/PROCAD 0156/01-9.

References

[1] G. Rolla, B. Oggard, and R.A. Cruz, Int. Dent. J. **41**, 171–174 (1991).
[2] P.C. Narvai, R.A. Castellanos, and P. Frazäo, Rev. Saúde Pública **34**, 196–200 (2000).
[3] R.L. Zanata, M.F.L. Navarro, J.C. Pereira et al., Braz. Dent. J. **14**, 75–81 (2003).
[4] K.G.A. Peres, J.R.M. Bastos, and M.R.D.O. Latorre, Rev. Saúde Pública **34**, 402–408 (2000).
[5] R.H. Stern and R.F. Sognnaes, J. Dent. Res. **43**, 873–873 (1964).
[6] R.H. Stern, R.F. Sognnaes, and F. Goodman, J. Am. Den. Association **73**, 838–843 (1966).
[7] S. Tagomori and T. Morioka, Caries Res. **23**, 225–231 (1989).
[8] T. Oho and T. Morioka, Caries Res. **24**, 86–92 (1990).
[9] L.K.A. Rodrigues, M.N. Santos, D. Pereira, et al., J. Dent. **32**, 531–540 (2004).
[10] L.S. Miseredino and R.M. Pick, Lasers in Dentistry Quintensence (Publishing Co. Inc., Chicago, 1995) p. 344.
[11] L.J. Wash, Australian Dent. J. **48**, 146–155 (2003).
[12] H.A. Wigdor, J.T. Walsh, J.D.B. Featherstone, et al., Lasers Surg. Med. **16**, 103–133 (1995).
[13] M.H. Niemz, Lasers Surg. Med. **16**, 103–133 (1995).
[14] J.D.B Featherstone and D.G.A. Nelson, Adv. Dent. Res. **1**, 21–26 (1987).
[15] H.E. Goodis, J.M. White, G.W. Marshall Jr, et al., Archs Oral Biol. **42**, 845–854 (1997).
[16] A. Antunes, W. de Rossi, V.L.R. Salvador, and D.M. Zezell, J. Oral Laser Appl. **1**, 45–45 (2001).
[17] M.H. Niemz, Laser - Tissue Interactions (Springer Verlag, 2002) p. 303.
[18] S.H. Chin-Ying, G. Xiaoli, P. Jisheng, and J.S. Wefel, J. Dent. **32**, 161–167 (2004).
[19] A. Antunes, Dr. Thesis (Institute of Research Energetic and Nuclear, Sao Paulo, Brazil, 2003).
[20] K. Sato, Caries Res. **17**, 490–495 (1983).
[21] M.C.P. Lin, B.S. Lee, F.H. Lin, et al., J. Endodontics **27**, 389–393 (2001).
[22] T. Kodaka, K. Debari, M. Yamada, and M. Kuroiwa, Caries Res. **26**, 139–141 (1992).
[23] R.G. Craig and F.A. Peyton, J. Dent. Res. **37**, 661–668 (1958).
[24] K. Collys, D. Slop, R. Cleymaet, et al., Dent. Mater. **8**, 332–335 (1992).
[25] T.G. Wilson and B. Love, Am. J. Orth. and Dent. Orthop. **107**, 379–381 (1995).
[26] M.P. Gutierrez-Salazar and J. Reyes-Gaza, Mat. Res. **6**, 367–373 (2003).
[27] L. Lupi-Pegurier, M. Muller, E. Leforestier, et al., Arch. Oral Biol. **48**, 141–145 (2003).
[28] A. Aminzadeh, S. Shahabi, and L.J. Walsh, Spect. Acta A **55**, 1303–1308 (1999).