Photomedicine and Laser Surgery
Volume 24, Number 6, 2006
© Mary Ann Liebert, Inc.
Pp. 689–693
DOI: 10.1089/PHO.2006.1091

Effects of 960-nm Diode Laser Irradiation on Calcium Solubility of Dental Enamel: An *in Vitro* Study

ILKA T. KATO, M.S.,[1] EDUARDO K. KOHARA, M.S.,[1] JORGE E.S. SARKIS, Ph.D.,[2] and NIKLAUS U. WETTER, Ph.D.[1]

ABSTRACT

Objective: **The aim of this study was to investigate the effects of 960 nm diode laser and acidulated phosphate fluoride on calcium solubility of human dental enamel.** ***Background Data:*** **Interest in diode lasers has grown steadily since its invention due to its inherent advantages and its range of applications. Several other laser types have shown good results in caries prevention; however, there are few studies on dental tissue interactions using diode lasers.** ***Methods:*** **Acid resistance was evaluated using 65 enamel specimens, divided into five groups: control (C), fluoride (F), laser (L), laser + fluoride (LF), and fluoride + laser (FL). The laser was operated using the parameters of 6.5-W peak power, 5-msec pulse duration, 10-Hz repetition rate, and 33-mJ pulse energy. These parameters were previously tested regarding pulpal temperature rise and enamel morphology, and were determined to be safe. The amount of calcium lost during demineralization was measured.** ***Results:*** **The calcium solubility of the laser group was 12% higher than of the control group ($p > 0.05$). Group F showed a 33.6% increase of acid resistance ($p < 0.05$). When laser was associated with fluoride, the calcium solubility increased significantly ($p < 0.05$) compared to both the control group and the laser group. Groups treated with fluoride showed the same results ($p > 0.05$).** ***Conclusion:*** **The 960-nm diode laser promoted a slight increase in calcium solubility. A statistically significant reduction on calcium solubility was achieved with the three treatments that involve fluoride (F, FL, and LF). The additional application of laser irradiation did not cause any significant increase or decrease in calcium solubility.**

INTRODUCTION

THE REDUCED acid solubility of dental enamel after irradiation with high-intensity lasers is related to physical[1–3] and chemical[4–7] alterations caused by photothermal and photochemical effects. Depending on the temperature achieved by the laser irradiation, different effects occur that change the enamel's solubility. It was demonstrated[5] that the smallest level of acid dissolution of enamel is achieved after heating to 300–350°C. The author suggested that this effect is caused by denaturation and swelling of the organic matrix that leads to the obstruction of the diffusion pathways within the enamel. Above 200°C, a loss of carbonate occurs[4] that could contribute to increased acid resistance. Microspaces formed as a consequence of loss of water; carbonate and organic substances might prevent demineralization by trapping the dissolved ions.[2] An increase in pyrophosphates caused by heating up to 200–400°C strongly reduced hydroxyapatite dissolution rate.[6]

Laser wavelengths, from visible to infrared, applied to dental enamel promoted a significant inhibition of demineralization. After irradiation with different CO_2 lasers using 2.5–12.5 J/cm^2, a reduction in enamel mineral loss of 70–98% was achieved.[8–10] Several authors report significant increase ($p < 0.05$) in enamel decalcification resistance using the argon laser with fluences of 11.5–100 J/cm^2.[11–13] Also, the Nd:YAG laser demonstrated its potential in increasing acid resistance.[14–16]

Although high-intensity diode lasers are relatively new in dentistry, there are many interesting characteristics that make

---

[1]Centro de Lasers e Aplicações and [2]Grupo de Caracterização Química e Isotópica, Instituto de Pesquisas Energéticas e Nucleares (IPEN), Cidade Universitária, São Paulo, Brazil.

them quite popular amongst dentists. Their low cost, small dimension, and ease of application in the oral cavity due to fiber delivery are important characteristics that favor their use in clinical practice and encourage new studies. Successful studies employing these lasers are mainly focused on soft tissue interaction[17–20] and reduction of microorganisms.[21–23] Despite the large number of studies on enamel resistance employing lasers, there are only a few studies using diode lasers. It is therefore of interest to expand the range of applications of this type of high-power lasers to studies with hard dental tissue.

The objective of the present study is to investigate enamel decalcification resistance after application of a high-power 960-nm diode laser associated with topical fluoride.

METHODS

Human third molars and one mandibular incisor were obtained from the Faculty of Dentistry, University of São Paulo, Brazil. They were brushed with pumice, rinsed with water, and kept in de-ionized water until the experiment. Eighty-five caries-free enamel samples with area of 4 × 4 mm were cut from the crowns of the third molars.

Vegetable coal (Herbarium Laboratório Botânico Ltda., Paraná, Brazil) diluted in 0.9% physiological saline solution (NaCl) was used as absorber. The mixture was prepared with 0.060 g of vegetable coal and four drops of liquid. In all experiments, a thin layer of the absorber was applied over the enamel surface approximately 30 sec before irradiation with the diode laser started.

The 960-nm diode laser prototype used in this study (Centro de Lasers e Aplicações, IPEN, São Paulo, Brazil) was coupled to a 600-μm-diameter fiber with a numerical aperture (NA) of 0.22. During the experiment, the laser was operated in the quasi-continuous wave (qcw) mode. Before each laser application, the pulse energy was measured using a calibrated power meter (model 818T-150; Newport Corp., San Diego, CA). The fiber hand piece was held at a distance of approximately 1 mm from the enamel surface during irradiation.

Preliminary investigations were carried out to determine the pulp chamber temperature and enamel morphology changes caused by different irradiation parameters (16, 33, 49, 65, and 81 mJ of pulse energy). Peak power and repetition rate were fixed at 6.5 W and 10 Hz, respectively, in all five measurements. Only pulse duration was adjusted (2.5, 5, 7.5, 10, and 12.5 msec, respectively). Temperature was measured with a k-type thermocouple (0.005-inch-diameter; Omega Engineering Inc.) that was inserted into the incisor's pulp chamber, which had been filled with heat-conducting paste (KS-609; Shin-Etsu Chemical Co., Ltd., Japan). Prior to the temperature measurement, the incisor was kept at 36°C with the help of a thermoelectric heating plate and a programmable set-point controller (model 2416; Eurotherm, UK). The tooth was irradiated twice with each parameter, using a time interval of 1 min, and pulpal temperature increase was recorded with a digital storage oscilloscope (model TDS360; Tektronix Inc., Portland, OR). The morphology changes of 20 enamel samples, irradiated with the above parameters, were analyzed using scanning electron microscopy (SEM). Each specimen was divided into two halves, one of which was irradiated and the other was used as control. After the treatment, the specimens were dehydrated in crescent ethanol solutions (70%, 80%, 90%, and 100%) for 30 min, coated with a thin layer of gold and palladium (sputtering model SCD-040; Balzers Union, Germany), and observed with scanning electron microscopy (model XL30; Philips, Eindhover, Holland).

The other 65 caries-free enamel samples were used for the calcium solubility measurements. The specimens were coated with acid-resistant nail varnish leaving regions of 3 × 3 mm in the occlusal and middle portion of the enamel surfaces exposed for irradiation. The samples were divided into five groups of 13 specimens each: control (C), fluoride (F), laser (L), laser + fluoride (LF), fluoride + laser (FL).

Laser irradiation was applied before the absorber solution dried out (30 sec after dye application) to avoid excessive pulp heating. The samples were irradiated three times at an interval time of 1 min. Before each irradiation, a new absorber layer was applied, because the dye evaporates from the enamel surface during laser action.

The groups treated with topical fluoride (F, FL, and LF) received a 1.23% acidulated phosphate fluoride gel (Sultan Topex, DFL Indústria e Comércio Ltda., Rio de Janeiro, Brazil) that was applied over the enamel surface and removed after 4 min with a cotton swap. The group FL was irradiated 1 min after fluoride application. The LF group was cleaned after laser irradiation and then fluoride was applied. In all groups involving laser radiation, the same irradiation parameters were used.

After the treatment, the samples were demineralized in 10 mL of a 0.1 M lactic acid solution with pH 4.8.[24] The specimens within their solution were stored in a water bath (RTE-140; Neslab Instruments Inc., Newington, CT) at 37°C during 24 h. After this period, the enamel samples were removed from the acid solution and the calcium content of the solution was measured by inductively coupled plasma atomic emission spectrometry (Atomcomp series 800; Jarrell Ash Division).

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) with $p < 0.05$.

RESULTS

The temperature of the pulp chamber during irradiation with each parameter is shown in Figure 1. Depending on the pulse energy used, the maximum temperature increase varied from 1°C to 6°C. SEM evaluation of lased enamel samples showed no morphological alterations on all samples irradiated with pulse energy of 16 mJ. Shallow and superficial exfoliation was observed in some areas (Fig. 2), on two of the four specimens irradiated with 33 mJ. Higher pulse energies caused increasingly larger areas of ablation (Fig. 3) and also fissures (Fig. 4).

The calcium content of the lactic acid solution of each sample is shown in (Table 1).

The mean calcium content in the acid solution was 12.4, 8.23, 13.9, 9.18, and 8.18 ppm for the groups C, F, L, LF, and FL, respectively. The standard error (SE) and the reduction of

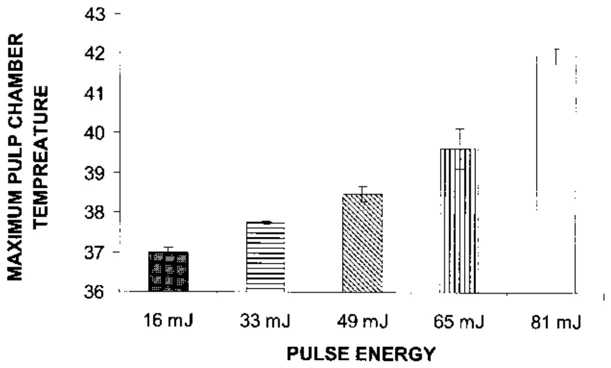

FIG. 1. Maximum pulp chamber temperature as a function of pulse energy and standard error.

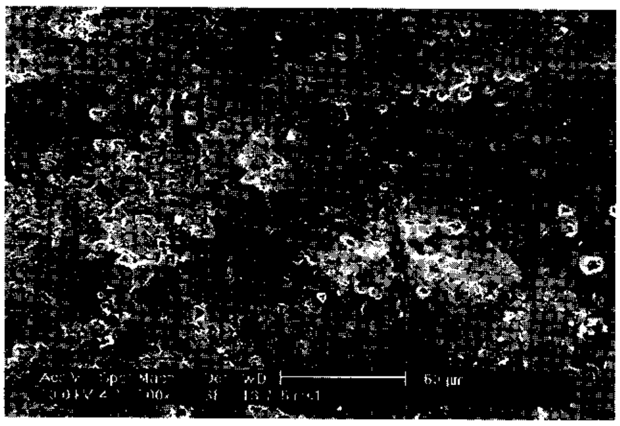

FIG. 2. SEM micrograph (original magnification, ×500) of the enamel surface after irradiation with 33 mJ. Scale bar = 50 µm. Several small areas of superficial enamel exfoliation can be seen (arrows).

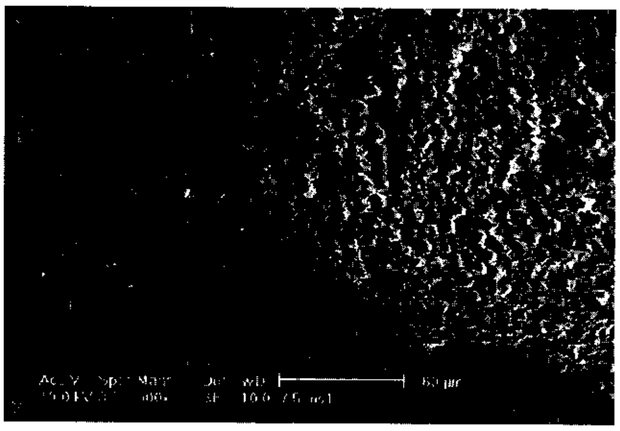

FIG. 3. SEM micrograph (original magnification, ×500) of the enamel surface after irradiation with 49 mJ showing a large area of ablation on the right half of the picture. Scale bar = 50 µm.

FIG. 4. SEM micrograph (original magnification, ×500) of the enamel surface after irradiation with 81 mJ showing cracks. Scale bar = 50 µm.

calcium solubility of each group are shown in Figure 5 and Table 2. The ANOVA test ($p < 0.05$) was applied to all groups and led to the conclusion that two or more means are significantly different. Levene's test for equal variance showed that the population variations are not significantly different ($p <$ 0.05). We therefore applied the independent t-test ($p < 0.05$) to establish what groups are significantly different from another.

Although the difference between the groups C and L was not statistically significant ($p > 0.05$), there was a 12% increase in calcium solubility after diode laser irradiation. Fluoride treatment alone (group F) and fluoride associated with laser (groups LF and FL) resulted in significant calcium solubility reduction when compared to the control group ($p < 0.05$). However, no significant difference was found between the groups treated with fluoride alone and fluoride associated with laser ($p > 0.05$).

TABLE 1. CALCIUM CONTENT OF EACH SAMPLE

Sample	*C*	*F*	*L*	*LF*	*FL*
1	12.128	11.429	16.353	6.4661	5.9984
2	9.1341	8.5235	15.557	4.1266	7.554
3	5.0565	11.962	11.126	11.159	9.0386
4	13.011	5.2388	14.976	4.6226	6.722
5	6.9915	13.717	17.091	10.761	5.2958
6	19.765	12.064	14.06	14.674	13.181
7	13.81	4.6205	15.848	5.5057	16.7
8	18.304	6.0081	18.154	10.084	8.1933
9	10.923	5.5851	11.504	14.109	3.7831
10	14.058	10.364	10.405	8.9464	5.4343
11	14.739	6.5489	9.7178	9.712	9.3146
12	7.4769	5.2764	17.77	8.1782	5.426
13	15.462	5.6929	7.6096	10.996	9.7203

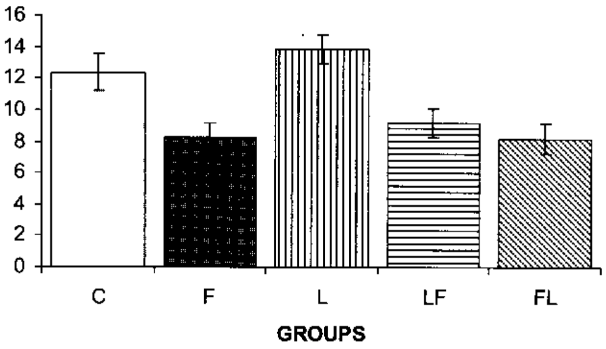

FIG. 5. Mean calcium content in the demineralization solution and standard error of each group.

DISCUSSION

Laser energy should be strongly absorbed and efficiently converted into heat to promote chemical or physical alterations in dental enamel. The degree of absorption inside the dental enamel depends on wavelength. In the case of laser wavelengths that are poorly absorbed by enamel, as is the case with high-power diode lasers, a coat of an appropriate dye absorbs the light, thus generating heat, and then transfers the heat to the target tissue.[15] In this study, a vegetable coal diluted in physiological solution was used. Besides its absorption at the 960-nm wavelength, this dye can also be indicated for use on dental tissue in virtue of its ease of removal from the surface. The 960-nm diode laser has the additional benefit of a ten times higher water absorption when compared to other commercial diode laser such as 808 and 830 nm. It was expected, therefore, that this physical property could contributed to promote better conversion of laser power into thermal energy, not only in the dental enamel but also in the water containing dye.

To our knowledge only two prior studies investigated the effects of diode lasers on acid resistance, using the 809-nm diode laser[25] (associated to fluoride varnish) and the 830-nm diode laser[24] (associated to $Ag(NH_3)_2F$). Similar to our results, no difference was observed between groups treated with fluoride only and groups that associated fluoride and laser. Only one study evaluated the effects of the laser alone, which is of importance in order to determine if the laser is necessary: primary teeth samples treated with 809 nm diode laser showed inhibition of the progression of caries lesion in five out of 20 specimens,[25] whereas complete inhibition in all samples was achieved with application of fluoride. Also, their pulse energy (140 mJ) is well above the energy we consider safe.

TABLE 2. CALCIUM CONTENT IN THE DEMINERALIZED SOLUTION IN EACH GROUP

Group	Mean (ppm)	SE	Percent reduction
C[a]	12.4	1.2	0%
F[b]	8.23	0.90	(33 ± 13)%
L[a]	13.86	0.95	(−12 ± 12)%
LF[b]	9.18	0.92	(26 ± 12)%
FL[b]	8.18	0.99	(34 ± 13)%

[a,b]Statistical difference exists between groups followed by different symbols.

Several studies conducted with different lasers have demonstrated significant inhibition of enamel demineralization. The argon laser has shown effective inhibition of demineralization for low fluences of 11.5–13.5 J/cm^2 without causing heating or fusion of the enamel.[11] When using CO_2 laser wavelengths (9.6 and 10.6 μm), enamel fusion is not necessary in order to increase acid resistance.[9,10] The Nd:YAG laser radiation promoted less calcium solubility in fused enamel.[15]

For safe laser irradiation, the pulp chamber temperature should not increase beyond 5.5°C.[27] Also, the enamel surface should not show cracks and ablation craters, because these morphological changes increase surface area and therefore exposure to acid attack. Using both criteria it may be concluded (Figs. 1–4) that energies per pulse of 16 or 33 mJ are safe. The next higher energy parameters (49 and 65 mJ) cause already large ablation areas and higher parameters cause excessive pulpal temperature rise. Therefore the highest temperature rise on the enamel surface caused by the diode laser that may still be considered safe occurs at pulse energy of 33 mJ.

Enamel irradiated with energy per pulse of 33 mJ showed superficial enamel exfoliation (Fig. 2) in a few irradiated areas, which could explain the mean increase in calcium solubility for group L. Similar changes in morphology were observed in enamel irradiated with CO_2.[28] The authors state that this enamel exfoliation caused by the explosive ablation is probably due to the rapid expansion of water vapor,[28,29] indicating a minimum temperature increase of at least 100°C. Although a reduction in the enamel's solubility was demonstrated for temperatures of the order of 100°C[4,5] and 150°C,[30] these changes were not observed in our study. The difference may be due to the fact that the diode laser heats the enamel only during a few microseconds whereas in these studies, the samples were heated for 24 h.

The results clearly show that, if safe parameters are considered, irradiation with the 960-nm diode laser alone does alter the enamel surface, but does not increase acid resistance, as observed with CO_2, Nd:YAG and argon lasers.

CONCLUSION

Calcium solubility was investigated in dental enamel irradiated with a 960-nm diode laser. Treatment with the laser by its own resulted in a small increase of calcium solubility. A statistically significant reduction on calcium solubility was achieved with the three treatments that involve fluoride (F, FL, and LF). Amongst these three treatments no statistically significant decrease or enhancement in acid resistance was achieved due to the additional application of the laser.

ACKNOWLEDGMENTS

We would like to thank FAPESP (State of São Paulo Research Foundation) for research grant 02/05535-5 and CNPq (National Council for Scientific and Technological Development) for grant 472640/03.

REFERENCES

1. Sognnaes, R.F., and Stern, R.H. (1965). Laser effect on resistance of human dental enamel to demineralization *in vitro*. J. South Calif. Dent. Assoc. 33, 328–329.
2. Oho, T., and Morioka, T. (1990). A possible mechanism of acquired acid resistance of human dental enamel by laser irradiation. Caries Res. 24, 86–92.
3. Tagomori, S., and Iwase, T. (1995). Ultrastructural change of enamel exposed to a normal pulsed Nd-YAG laser. Caries Res. 29, 513–520.
4. Holcomb, D.W., and Young, R.A. (1980). Thermal decomposition of human tooth enamel. Calcif. Tissue Int. 31, 189–201.
5. Sato, K. (1983). Relation between acid dissolution and histological alteration of heated tooth enamel. Caries Res. 17, 490–495.
6. Fowler, B.O., and Kuroda, S. (1986). Changes in heated and in laser-irradiated human tooth enamel and their probable effects on solubility. Calcif. Tissue Int. 38, 197–208.
7. Featherstone, J.D. (2000). Caries detection and prevention with laser energy. Dent. Clin. North Am. 44, 955–969,ix.
8. Featherstone, J.D., Barrett-Vespone, N.A., Fried, D., et al. (1998). CO_2 laser inhibitor of artificial caries-like lesion progression in dental enamel. J. Dent. Res. 77, 1397–1403.
9. Hsu, C.Y., Jordan, T.H., Dederich, D.N., et al. (2000). Effects of low-energy CO_2 laser irradiation and the organic matrix on inhibition of enamel demineralization. J. Dent. Res. 79, 1725–1730.
10. Kantorowitz, Z., Featherstone, J.D., and Fried, D. (1998). Caries prevention by CO_2 laser treatment: dependency on the number of pulses used. J. Am. Dent. Assoc. 129, 585–591.
11. Westerman, G.H., Flaitz, C.M., Powell, G.L., et al. (2002). Enamel caries initiation and progression after argon laser irradiation: *in vitro* argon laser systems comparison. J. Clin. Laser Med. Surg. 20, 257–262.
12. Hicks, J., Winn, D., 2nd, Flaitz, C., et al. (2004). *In vivo* caries formation in enamel following argon laser irradiation and combined fluoride and argon laser treatment: a clinical pilot study. Quintessence Int. 35, 15–20.
13. Flaitz, C.M., Hicks, M.J., Westerman, G.H., et al. (1995). Argon laser irradiation and acidulated phosphate fluoride treatment in caries-like lesion formation in enamel: an *in vitro* study. Pediatr. Dent. 17, 31–35.
14. Yamamoto, H., and Sato, K. (1980). Prevention of dental caries by acousto-optically Q-switched Nd: YAG laser irradiation. J. Dent. Res. 59, 137.
15. Tagomori, S., and Morioka, T. (1989). Combined effects of laser and fluoride on acid resistance of human dental enamel. Caries Res. 23, 225–231.
16. Huang, G.F., Lan, W.H., Guo, M.K., et al. (2001). Synergistic effect of Nd:YAG laser combined with fluoride varnish on inhibition of caries formation in dental pits and fissures *in vitro*. J. Formos. Med. Assoc. 100, 181–185.
17. Coluzzi, D.J. (2000). An overview of laser wavelengths used in dentistry. Dent. Clin. North Am. 44, 753–765.
18. Goharkhay, K., Moritz, A., Wilder-Smith, P., et al. (1999). Effects on oral soft tissue produced by a diode laser *in vitro*. Lasers Surg. Med. 25, 401–406.
19. Romanos, G., and Nentwig, G.H. (1999). Diode laser (980 nm) in oral and maxillofacial surgical procedures: clinical observations based on clinical applications. J. Clin. Laser Med. Surg. 17, 193–197.
20. Yousuf, A., Hossain, M., Nakamura, Y., et al. (2000). Removal of gingival melanin pigmentation with the semiconductor diode laser: a case report. J. Clin. Laser Med. Surg. 18, 263–266.
21. Moritz, A., Gutknecht, N., Goharkhay, K., et al. (1997). *In vitro* irradiation of infected root canals with a diode laser: results of microbiologic, infrared spectrometric, and stain penetration examinations. Quintessence Int. 28, 205–209.
22. Moritz, A., Schoop, U., Goharkhay, K., et al. (1998). Treatment of periodontal pockets with a diode laser. Lasers Surg. Med. 22, 302–311.
23. Yilmaz, S., Kuru, B., Kuru, L., et al. (2002). Effect of gallium arsenide diode laser on human periodontal disease: a microbiological and clinical study. Lasers Surg. Med. 30, 60–66.
24. Yu, D.G., Kimura, Y., Fujita, A., et al. (2001). Study on acid resistance of human dental enamel and dentin irradiated by semiconductor laser with $Ag(NH_3)2F$ solution. J. Clin. Laser Med. Surg. 19, 141–146.
25. Santaella, M.R., Braun, A., Matson, E., et al. (2004). Effect of diode laser and fluoride varnish on initial surface demineralization of primary dentition enamel: an *in vitro* study. Int. J. Paediatr. Dent. 14, 199–203.
26. Tsai, C.L., Lin, Y.T., Huang, S.T., et al. (2002). *In vitro* acid resistance of CO_2 and Nd-YAG laser-treated human tooth enamel. Caries Res. 36, 423–429.
27. Zach, L., and Cohen, G. (1965). Pulp response to externally applied heat. Oral Surg. Oral Med. Oral Pathol. 19, 515–530.
28. McCormack, S.M., Fried, D., Featherstone, J.D., et al. (1995). Scanning electron microscope observations of CO_2 laser effects on dental enamel. J. Dent. Res. 74, 1702–1708.
29. Apel, C., Schafer, C., and Gutknecht, N. (2003). Demineralization of Er:YAG and Er,Cr:YSGG laser-prepared enamel cavities *in vitro*. Caries Res. 37, 34–37.
30. Hsu, J., Fox, J.L., Higuchi, W.I., et al. (1994). Heat-treatment-induced reduction in the apparent solubility of human dental enamel. J. Dent. Res. 73, 1848–1853.

Address reprint requests to:
Dr. Niklaus U. Wetter
Centro de Lasers e Aplicações
Instituto de Pesquisas Energéticas e Nucleares (IPEN)
Av. Lineu Prestes, 2242
Cidade Universitária
São Paulo, SP, 05508-900, Brazil

E-mail: nuwetter@ipen.br