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# Effect of diode laser and fluoride varnish on initial surface demineralization of primary dentition enamel: an *in vitro* study

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**Summary.** *Introduction.* Previous investigations have demonstrated improved enamel caries resistance after laser irradiation. The purpose of this *in vitro* study was to assess the caries-preventive potential of 809 nm diode laser treatment of the enamel of primary teeth compared to topical fluoride application.
*Methods.* Eighty samples of sound primary teeth were embedded in plastic and stored in saline solution. The enamel surface of 80 samples was polished in an area of 2 × 2 mm. These tooth specimens were randomly assigned to one control and three test groups: (1) no treatment/control; (2) application of 0·1 mg of fluoride varnish (Duraphat®) for 6 h; (3) diode laser application (809 nm, 140 mJ, 50 Hz, Ø 600 µm fibre, contact mode, absorber, 1 min; ORA-LASER01® I.S.T.); and (4) combined application – laser/fluoride varnish. Caries-like lesions were created by pH-cycling. After lesion formation, longitudinal sections were taken and examined by polarized light microscopy.
*Results.* In the control group, all samples showed lesions up to 30 µm in depth. After laser application, lesions could be identified in 15 out of 20 samples. Topical fluoride treatment in groups 2 (varnish) and 4 (laser/varnish) completely inhibited the development of caries-like lesions in all samples.
*Conclusion.* In this *in vitro* investigation, topical fluoride treatment enhances the resistance of sound enamel of primary teeth more effectively than diode laser application.

## Introduction

Although caries experience has declined in developed countries at least, treatment requires many expenses. Improved prophylaxis is, therefore, the key to increase dental health. To prevent enamel caries formation, topical fluoride treatment is the most respected regimen to avoid demineralization of dental hard tissues [1,2]. As an alternative to fluoride applications, previous investigations have demonstrated the potential of laser pre-treatment of enamel to inhibit subsequent, acid-induced dissolution of enamel *in vitro* at least [3,4]. Continuous-wave or pulsed $CO_2$ lasers, Nd:YAG and diode lasers in conjunction with absorbers, and Er:YAG and Excimer lasers have been used to enhance caries resistance and support remineralization [5,6]. Combined applications of laser irradiation and topical fluoride have been reported to have synergistic effects [7,8]. In a number of primarily basic studies, the advantages and side-effects have been discussed in order to develop safe and effective laser-assisted treatment procedures. Most of these investigations have been focused on permanent teeth. Little is known about the effects in primary enamel. Compared to permanent teeth, topical fluorides do not seem to completely prevent lesion development in primary teeth [9]. The aim of this study, therefore, was to

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examine the *in vitro* caries-inhibiting potential of 809 nm diode laser irradiation and topical fluoride application in artificial caries systems in primary teeth.

## Methods

In the present study, 80 samples of freshly extracted anterior primary teeth from the mandibular and maxillary arches (Teeth Bank of the University of São Paulo, São Paulo, Brazil) [53–63,73–83] which were deemed caries free by visual inspection were cleaned, cut into blocks and embedded in plastic. Every specimen was cut from a separate tooth. The samples were stored in saline solution. The buccal enamel surfaces of the samples were polished using silicon carbide paper grit 600 in a 2 × 2 mm area to standardize the optical surface properties for laser irradiation. This window was not covered by the plastic embedding medium. In this way, a flat surface without any contamination was obtained. The 80 enamel specimens were randomly assigned to a control and three test groups. The control group remained untreated. In the fluoride test group, the enamel was treated with 0·1 mg of fluoride varnish (Duraphat®) over a period of 6 h (Table 1). In the laser test group, the enamel was covered by an absorber (Contactin®, Oralia, Konstanz, Germany) and immediately irradiated for one minute in contact mode using a 600-μm fibre with a 809 μm diode laser (ORA-LASER01® I.S.T./Oralia, Konstanz, Germany). The output power was 140 mJ at 50 Hz. In the third group, laser treatment was followed by fluoride varnish application.

After treatment, the absorber dye and the varnish were removed after exactly 6 h using ethanol. To investigate the caries resistance of the treated enamel surfaces, the method employed by ten Cate and Duijsters [10] to create caries-like lesions was used to assess the presence or absence of lesions using polarized light microscopy. Over a period of 10 days, the teeth underwent 6 h of demineralization at 37 °C in a buffered acetate solution at pH 4·3. After rinsing in de-ionized water, the teeth were immersed for 18 h in a remineralizing solution at pH 7·0. After pH cycling, the samples were sectioned longitudinally and examined by polarized light microscopy to analyse any surface lesions [2,11]. The presence or absence of lesions was recorded to evaluate whether the protective effects were caused by laser effects or fluorides. All treated sections were characterized in an aqueous medium to avoid morphological artefacts caused by drying. Additionally, collateral damage caused by the laser application was recorded using light microscopy. The chi-square test was used for statistical analysis. Values were considered as statistically significant at $P < 0{\cdot}05$.

**Table 1.** Lesion formation in the test and control groups. Fluoride application inhibited lesion formation completely ($n = 20$ samples/group).

| Treatment group | (+) | Lesion formation | (–) |
|---|---|---|---|
| (1) No treatment/control | 20 | | 0 |
| (2) Fluoride varnish | 0 | | 20 |
| (3) Laser | 15 | | 5 |
| (4) Laser/fluoride varnish | 0 | | 20 |

## Results

All samples in the control group showed lesions up to 30 μm in depth (Fig. 1). The lesions were similar to naturally occurring ones. After laser application, lesions could be identified in 15 out of 20 samples

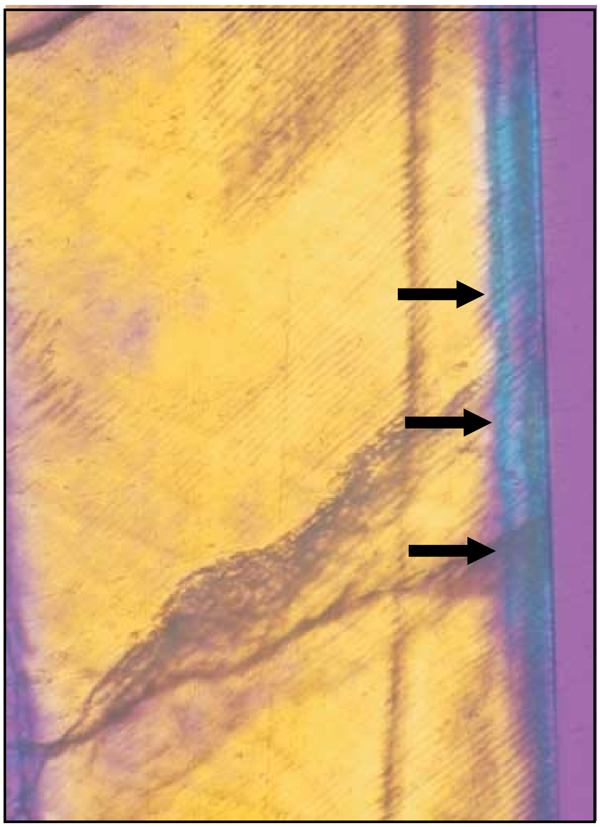

**Fig. 1.** Polarized light photomicrograph of a section of the control group. The arrows indicate the initial enamel lesions (positive birefringence lesion area, imbibition medium: water, × 250).

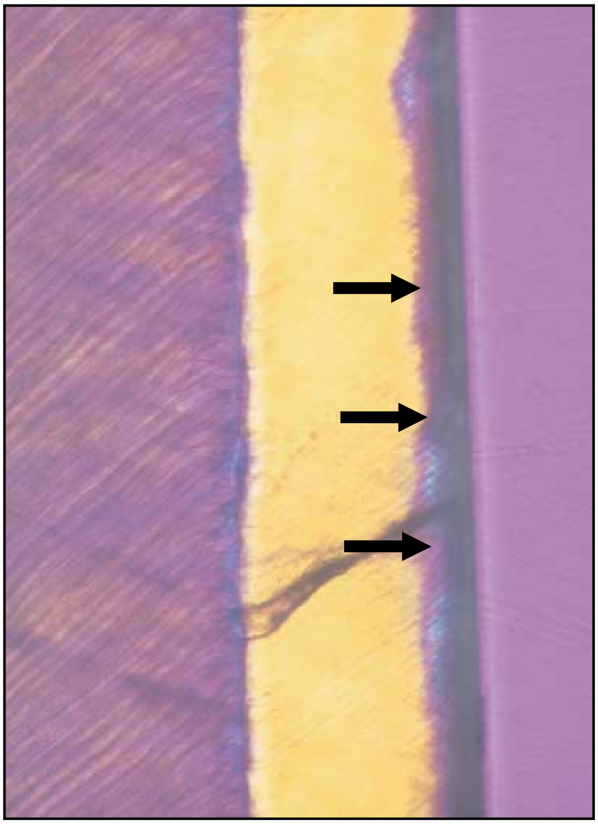

**Fig. 2.** Polarized light photomicrograph of a section of group 3 after laser applications. The arrows indicate the initial enamel lesion (imbibition medium: water, × 250).

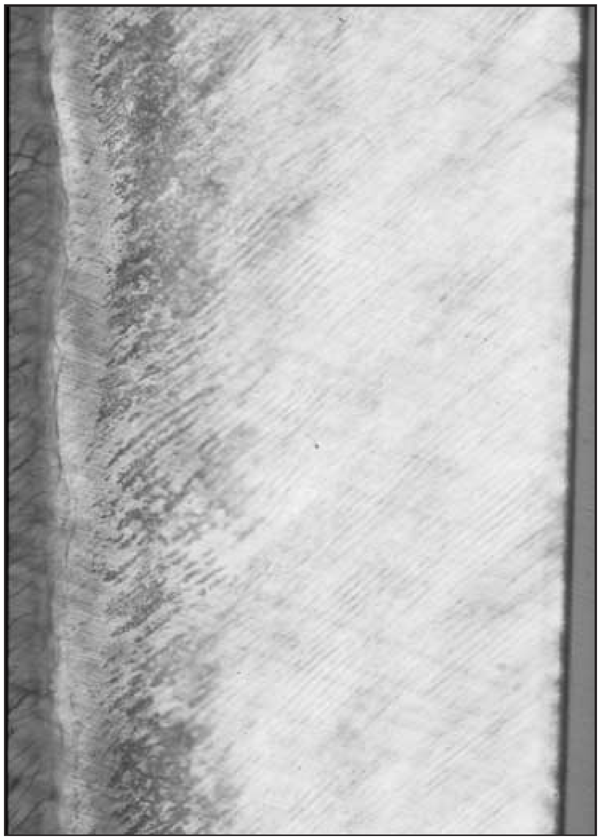

**Fig. 3.** Polarized light photomicrograpah of a section of group 2 (fluoride varnish). There is no lesion formation in the enamel (imbibition medium: water, × 250).

(Fig. 2). No collateral damage caused by the laser application was detectable. Topical fluoride application, and the combined treatment using the fluoride varnish and the laser inhibited the development of caries lesions completely in all samples (Figs 3 & 4). Topical fluoride treatment enhanced the resistance of the sound enamel of primary teeth more effectively than diode laser application (Table 1).

## Discussion

The complete inhibition of *in vitro* caries-like lesion progression achieved by topical fluoride application supports the potential of fluorides not only in permanent teeth, but also in primary ones. Fluoride effects in primary teeth may be different from those in permanent ones [10]. However, the results achieved by the *in vitro* model of pH cycling correspond to clinical practice and epidemiological data which describe the caries-protecting effect of fluorides in primary teeth.

In this study, laser application had some inhibiting potential. The treated areas showed no morphological changes. The parameters used, however, did not show the method to have the same effectiveness as the fluoride varnish that was tested.

The *in vitro* pH-cycling technique was developed to study the effects of caries-preventive regimens and treatments [10]. Limited periods of alternating demineralization and remineralization enabled us to study a shifting in the mineral balance towards less cariogenic conditions caused either by an enhancement of remineralization or an inhibition of demineralization. This model seems to be very sensitive and realistic in simulating the reduction of lesion progression [10]. In primary teeth, the resulting lesion depths (~30 μm) represent the initial phase of surface demineralization. To avoid non-physiological parameters, the demineralizing effects of this pH cycling were not enhanced further to create deeper lesions. To evaluate whether protective effects were caused by the tested experimental procedures, the

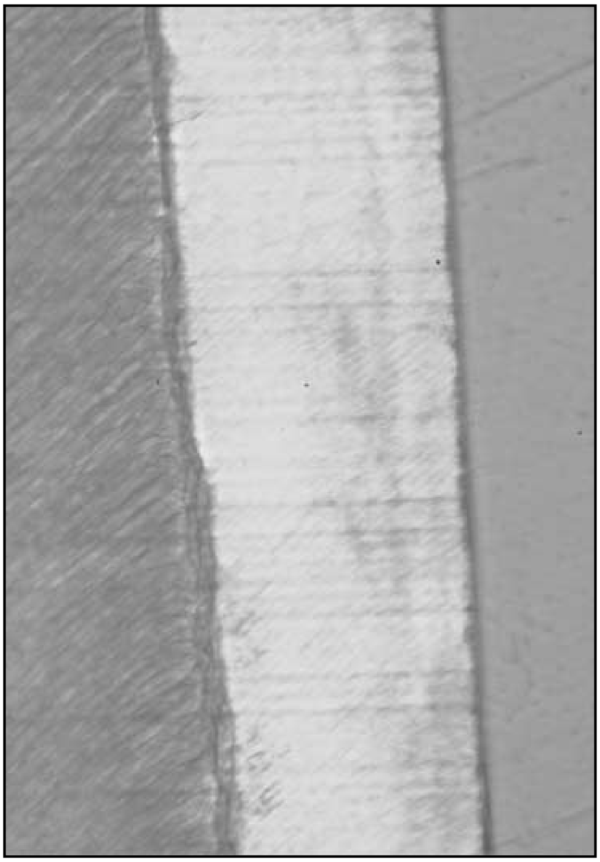

**Fig. 4.** Polarized light photomicrograph of a section of group 4 (laser/fluoride varnish). There are no signs of demineralization (imbibition medium: water, × 250).

presence or absence of lesions was selected as a distinct marker for caries resistance in this pilot study.

The laser parameters used were limited to a range in which collateral damage to the sound enamel was not expected. There are several theories regarding the mechanisms by which laser radiation enhances enamel resistance. These theories range from a surface melting through partial fusion and recrystallization of enamel prisms to changes in the organic matrix of the enamel [12–14]. In contrast to $CO_2$ laser radiation, which has been shown to have cariostatic effects in permanent teeth at least [4], 809 nm diode laser radiation is not directly absorbed by the enamel. An absorber dye at the enamel surface converts the diode laser energy into heat. The profiles of temperature rise may, therefore, be different from those of $CO_2$ lasers. It may be, for example, that the melting point of hydroxyapatite (> 1200 °C) is not reached in the subsurface layer.

Because of practicability and expenses, the diode laser would be preferable to other laser technologies in dental application. The results of our pilot study indicate that, up to now, little has been known about the cariostatic effects of this laser type. Further studies are needed to gain more detailed information. Under the limitations of *in vitro* conditions, however, this investigation has shown that topical fluoride treatment enhances the resistance of sound enamel of primary teeth more effectively than diode laser irradiation.

**Résumé.** *Objectifs.* De précédentes études ont montré une résistance de l'émail améliorée face à la carie après irradiation au laser. Le but de cette étude in vitro a été de comparer le potentiel cario-préventif d'un laser à diode de 809 nm sur l'émail de dents temporaires à celui de l'application de fluor topique. *Echantillons et méthodes.* Quatre-vingt échantillons de dents temporaires saines ont été inclus dans du plastique et conservés dans du sérum physiologique. La surface amélaire des 80 échantillons a été polie sur une surface de 2 × 2 mm. Ces dents ont été assignées au hasard au groupe témoin ou aux trois groupes test: 1. pas de traitement/témoin; 2. application de vernis à 0,1 mg de fluor (Duraphat®) pendant 6 h; 3. Application de laser à diode (809 nm, 140 mJ, 50 Hz, fibre de Ø 600 µm, mode contact, absorbeur, 1 min (ORA-LASER01® I.S.T.); 4. Application combinée _ Laser/vernis fluoré. Des lésions pseudo-carieuses ont été créées par pH-cycling. Après formation de la lésion, des sections longitudinales ont été prises et examinées au microscope sous lumière polarisée. *Résultats.* Tous les échantillons du groupe témoin ont montré des lésions allant jusqu'à 30 µm de profondeur. Des lésions ont pu être identifiées chez 15 dents sur 20 après application laser. Le traitement par application de topique fluoré dans le groupe 2 (vernis) et le groupe 4 (laser/vernis) a inhibé le développement de pseudo-lésions dans tous les échantillons.
*Conclusion.* Dans cette étude in vitro, le fluor topique augmente mieux la résistance de l'émail sain des dents temporaires que l'application de laser à diode.

**Zusammenfassung.** *Ziele.* Vorangegangene Untersuchungen hatten gezeigt, dass nach Laseranwendung die Kariesresistenz von Schmelz gesteigert wurde. Das Ziel dieser Studie war es, das kariespräventive Potential eines 809 nm Diodenlasers an Milchzahnschmelz zu untersuchen im Vergleich zu Fluoridapplikation.
*Stichprobe und Methode*: Achzig Proben aus kariesfreien Milchzähnen wurden in Kunststoff eingebettet

und in Kochsalzlösung aufbewahrt. Die Schmelzoberfläche wurde auf einer Fläche von 2 × 2 mm poliert. Diese Zahnproben wurden zufällig einer Kontrollgruppe sowie drei Testgruppen zugeteilt: Gruppe 1 keine Behandlung, Gruppe 2 Applikation von 0.1 mg Fluoridlack (Duraphat) für 6 h; 3. Diodenlaserapplikation (ORA-LASER01® I.S.T., 809 nm, 140 mJ, 50 Hz, ∅ 600 µm Faser, Kontaktmodus, Absorber, 1 min) 4. Kombinierte Laser/ Fluoridlackanwendung. Durch pH-Cycling wurden kariesartige Läsionen erzeugt. Nach Ausbildung der Läsionen wurden Längsschnitte angefertigt und polarisationslichtmikroskopisch ausgewertet.
*Ergebnisse*. In der Kontrollgruppe zeigten alle Läsionen eine Tiefe von 30 µm. Nach Laserapplikation konnten bei 15 von 20 Proben Läsionen nachgewiesen werden. Die Lokalfluoridierung in Gruppe 2 (nur Lack) und Gruppe 4 (Laser plus Lack) verhinderten vollständig die Entstehung kariesartiger Läsionen bei allen Proben.
*Schlussfolgerung*. In dieser in-vitro-Untersuchung erhöhte Lokalfluoridierung die Kariesresistenz von Milchzahnschmelz effektiver als die Anwendung eines Diodenlasers.

**Resumen.** *Objetivos*. Investigaciones previas han demostrado una mejora en la resistencia a la caries después de la irradiación con láser. El propósito de este estudio in vitro fue valorar el potencial preventivo a la caries de un diode láser de 809 nm en el esmalte de dientes deciduos comparado con la aplicación tópica de flúor.
*Muestra y método*. Se incluyeron en plástico y se almacenaron en solución salina, ochenta muestras de dientes deciduos sanos. Se pulió un área de 2 × 2 mm de la superficie del esmalte de las 80 muestras. Estas muestras dentarias se asignaron aleatoriamente a un grupo control y a otros tres de prueba: 1. sin tratamiento/control; 2. aplicación de 0.1 mg de barniz de flúor (Duraphat®) durante 6 h; 3. aplicación de láser diodo (809 nm, 140 mJ, 50 Hz, Ø 600 µm de fibra, modo contacto, absorbente, 1 min (ORA-LASER01® I.S.T.); 4. aplicación combinada – láser/barniz de flúor. Se crearon por ciclos de pH, lesiones de caries artificiales. Después de la formación de la lesión se tomaron secciones longitudinales y se examinaron por microscopio de luz polarizada.
*Resultados*. En el grupo control todas las muestras mostraron lesiones de hasta 30 µm en profundidad. Después de la aplicación del láser en 15 de 20 muestras podían identificarse las lesiones. El tratamiento con flúor tópico en el grupo 2 (barniz) y en el grupo 4 (láser/barniz) inhibió completamente el desarrollo de las lesiones artificiales de caries en todas las muestras.
*Conclusión*. En esta investigación in vitro el tratamiento con flúor tópico mejora la resistencia del esmalte sano de dientes deciduos de forma más efectiva que la aplicación de láser diodo.

## References

1 Marinelli CB, Donly KJ, Wefel JS, Jakobsen JR, Denehy GE. An *in vitro* comparison of three fluoride regimens on enamel remineralization. *Caries Research* 1997; **31**: 418–422.
2 Itthagarun A, Wei SH, Wefel JS. The effect of different commercial dentifrices on enamel lesion progression: an *in vitro* pH-cycling study. *International Dental Journal* 2000; **50**: 21–28.
3 Featherstone JDB, Barrett-Vespone NA, Fried D, Kantorowitz Z, Seka W. $CO_2$ laser inhibition of artificial caries-like lesion progression in dental enamel. *Journal of Dental Research* 1998; **77**: 1397–1403.
4 Kantorowitz Z, Featherstone JD, Fried D. Caries prevention by $CO_2$ laser treatment: dependency on the number of pulses used. *Journal of the American Dental Association* 1998; **129**: 585–591.
5 Kimura Y, Wilder-Smith P, Arrastia-Jitosho AM, Liaw LH, Matsumoto K, Berns MW. Effects of nanosecond pulsed Nd: YAG laser irradiation on dentin resistance to artificial caries-like lesions. *Lasers in Surgery and Medicine* 1997; **20**: 15–21.
6 Hossain M, Nakamura Y, Kimura Y, Yamada Y, Kawanaka T, Matsumoto K. Effect of pulsed Nd: YAG laser irradiation on acid demineralization of enamel and dentin. *Journal of Clinical Laser in Medical Surgery* 2001; **19**: 105–108.
7 Hicks MJ, Flaitz CM, Westerman GH, Blankenau RJ, Powell GL, Berg JH. Enamel caries initiation and progression following low fluence (energy) argon laser and fluoride treatment. *Journal of Clinical and Pediatric Dentistry* 1995; **20**: 9–13.
8 Anderson JR, Ellis RW, Blankenau RJ, Beiraghi SM, Westerman GH. Caries resistance in enamel by laser irradiation and topical fluoride treatment. *Journal of Clinical Laser and Medical Surgery* 2000; **18**: 33–36.
9 Sonju Clasen AB, Ogaard B, Duschner H, Ruben J, Arends J, Sonju T. Caries development in fluoridated and non-fluoridated primary and permanent enamel *in situ* examined by microradiography and confocal laser scanning microscopy. *Advances in Dental Research* 1997; **11**: 442–447.
10 ten Cate JM, Duijsters PPE. Alternating demineralization and remineralization of artificial enamel lesions. *Caries Research* 1982; **16**: 201–210.
11 Hicks MJ, Parkins FM, Flaitz CM. Kinetic cavity preparation effects on secondary caries formation around resin restorations: a polarized light microscopic *in vitro* evaluation. *Journal of Dentistry for Children* 2001; **68**: 115–121.
12 Stern RH, Sognnaes RF, Goodman F. Laser effect on *in vitro* enamel permeability and solubility. *The Journal of the American Dental Association* 1966; **73**: 838–843.
13 Featherstone JDB, Nelson DGA. Laser effects on dental hard tissues. *Advances in Dental Research* 1998; **71**: 21–26.
14 Featherstone JDB, Fried D, Bitten ER. Mechanism of laser-induced solubility reduction of dental enamel. In: Wigdor HA, Featherstone JDB, Rechmann P (eds). *Lasers in Dentistry III*. [Proceedings.] Bellingham, WA: SPIE – The International Society for Optical Engineering, 1997: 112–116.