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Effects of low power red laser on induced-dental caries in rats

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ABSTRACT

Objective: The purpose of this study was to investigate the effects of low power red laser associated with acidulated phosphate fluoride on the development of induced-dental caries in rats.

Design: Dental caries were induced in molars of 40 rats divided into five groups: control group (CG), the teeth were not submitted to any treatment; laser group (LG), teeth were irradiated with a low power red laser (LPRL), power of 30 mW and dose of 5 J/cm^2; fluoride group (FG), teeth were treated with topical acidulated phosphate fluoride (APF) 1.23% applied for 4 min; laser + fluoride group (LFG), teeth were irradiated with LPRL followed by APF; fluoride + laser group (FLG), teeth were treated with APF followed by LPRL. The animals were killed after 48 days, and the first and second molars were extracted to analyze the caries lesion area, microhardness, and calcium and phosphorus ratio.

Results: There were no statistical differences among FG, LFG, and FLG regarding to caries area and microhardness, although the caries area were smaller in LFG. Ca/P ratio did not show significant differences among all groups.

Conclusions: Although LPRL before APF application appeared to diminish the caries progression, LPRL did not present any additional benefit compared with acidulated phosphate fluoride on the prevention of induced-dental caries in rats.

1. Introduction

Despite the significant decline in caries incidence in the last few decades in developed countries, dental caries is still reported as the single most common chronic childhood disease.[1] In developing countries, more than 80% of caries prevalence has been reported in epidemiological studies.[2]

Because of this common problem, the constant search for cost-effective caries prevention techniques remains of paramount importance for global oral health.

Dental caries is a bacterial based disease. When it progresses, acids produced by bacteria diffuse into the tooth and dissolve the carbonated hydroxyapatite mineral. This process is called demineralization.[3]

The effects of fluoride in caries prevention are mainly due to the inhibition of the demineralization process, with minor role in retarding metabolism and acid production of cariogenic bacteria.[4,5] Fluoride can be taken up into the apatite lattice as ''firmly bound'' apatitic fluoride, which is less soluble than the original enamel apatite.[6]

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Abbreviations: APF, acidulated phosphate fluoride; BHI, brain heart infusion; CA, selected caries lesion area; CG, control group; CrA, crown total area; EDS, energy-dispersive X-ray spectrometer; FG, fluoride group; FLG, fluoride + laser group; KHN, Knoop hardness number; LFG, laser + fluoride group; LG, laser group; LPRL, low power red laser; MB, methylene blue; SCA, standardized caries lesion area

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The reasons for caries prevalence reduction during the last 20 years are difficult to identify. However, strong evidences suggest that the near universal use of fluoride containing products such as dentifrice, mouthrinses and topical gels applied in dental office have been major contributors. Earlier caries reductions of 40–70% (before the 1970s) had resulted from the fluoridation of public water supplies in many communities.[3] As fluoride seems to be the most impressive agent against dental caries, a cost-effective method of enhancing fluoride uptake would therefore be promising in caries prevention.

Since the 1960s, it has been consistently demonstrated that high power lasers can significantly increase the acid resistance of enamel by altering crystalinity, acid-solubility, permeability, and fluoride uptake.[7–10] It has been found that high power lasers combined with topical fluoride treatment can induce an even greater resistance of the dental enamel against demineralization than laser treatment alone.[11–13] Recently, studies with low fluence argon laser beam combined with topical fluoride treatment aiming an increased retention of fluoride can be found.[14–18]

Due to inherent cost of instrumentation, surgical lasers are still not widely employed in private practice, mainly in developing countries. Low power red and near-infrared lasers appear as an alternative approach, since reports in literature suggest that their association with topical fluoride or not, lead to an increase on teeth's resistance against dental caries.[19–21] Some authors suggest that the effect of this treatment is related to the time of the fluoride uptake.[22] The reported effect could also be attributed to alterations on the pulp microcirculation, having as result the reduction in the enamel solubility rate.[20] Until now, the possible mechanisms of action of this kind of laser in association or not with fluoride remain unclear.

On the other hand, acidulated phosphate fluoride (APF) has been shown to be an efficient cariostatic instrument.[23,24] The concentration of F in most APF solutions and gels is 1.23% (12,300 ppm) and the pH is typically in the range of 3–4, which is close to the pK_i of hydrofluoric acid (HF). Thus, approximately one-half of the F is ionic and the remainder is HF.[25]

Obviously, the application of laser technology and more specifically low power red laser (LPRL) in caries prevention requires a greater number of in vivo studies, with stronger scientific evidences. Thus, in this study, it was examined the effect of LPRL in association or not with APF on the prevention of induced-dental caries in rats. To accomplish these analysis three different techniques were employed. The caries lesion extension was determine via computer software coupled to an optical microscopy, the enamel resistance was verified through cross-sectional microhardness, and the calcium and phosphorus ratio in enamel was analyzed by energy-dispersive X-ray spectrometer.

2. Materials and methods

2.1. Caries in rats

Forty female Wistar rats were weaned at 18 days of age, fed with a cariogenic diet (Diet 2000)[26,27] and double deionized water ad libitum. The animals were maintained under standard laboratory conditions. Inoculation of *Streptococcus mutans* ATCC 1910 into rat's mouth started at the age of 19 days. *S. mutans* was used to enhance the cariogenic challenge as described by Seppä et al.[28] The microorganism was cultured on BHI (brain heart infusion) suspension (approximately 10^8 cells/mL), collected and injected directly (0.2 mL/rat) into the oral cavity of the rats once a day for three subsequent days. The experimental schedule is shown in Table 1.

Table 1 – Experimental schedule

Event	Day
Animal birth	0
Animal weaned and cariogenic diet beginning	18
Inoculation of *Streptococcus mutans*	19
Treatment	22
Animal sacrifice	66

Twenty-four hours after the last inoculation the rats were anesthetized with a sodium pentobarbital solution administered intraperitoneally and randomly divided into five groups: CG (control group), eight animals were not submitted to any treatment; LG (laser group), both maxillary and mandibular molars of eight rats were irradiated with a low power continuous GaAlAs laser at $\lambda = 660$ nm (Kondortech, São Carlos, SP, Brazil), 30 mW of output power, beam diameter of 0.2 cm, and an area of 0.03 cm^2. The irradiated area on the occlusal surface was equal to the beam diameter; thus, an energy density of 5 J/cm^2, corresponding to an exposure time of $\Delta t = 5$ s, was directly delivered on the teeth; FG (fluoride group), both maxillary and mandibular molars of eight rats were treated with topical application of acidulated phosphate fluoride (1.23% APF, fluoride gel Odahcam, Dentsply, tutti-fruti flavor, Rio de Janeiro, Brazil) for 4 min; LFG (laser + fluoride group), both maxillary and mandibular molars of eight rats were irradiated with LPRL as previously described, followed by topical application of APF; FLG (fluoride + laser group), both maxillary and mandibular molars of eight rats were treated with topical application of APF followed by LPRL irradiation.

After 48 days, the animals were sacrificed in a CO_2 gas chamber. The maxillaries were removed, dissected and washed with destilled water; the first and second molars were extracted and preserved in physiological solution. During the experimental period, all animals received humane care in compliance with the Ethical Principles of Animal Experimentation formulated by the Brazilian College for Animal Experimentation, and in accordance with guidelines approved by the Council of the American Psychological Society for the use of animal in experiments.

2.2. Caries lesion area

Seventy-five molars ($n = 15$ per group) had the roots coated with two layers of nail polish. The occlusal surface was left uncoated and the specimens dried for 24 h. The teeth were immersed in a 2% methylene blue (MB) solution for 2 h to stain the intrasulcular area affected by the caries lesion. After this procedure, the samples were abundantly washed in tap water.[29] Thereafter, the teeth were embedded in acrylic resin and sectioned along the sagittal medial–distal plane with a

diamond disk at a standard distance from the buccal and lingual surfaces. MB stained areas were examined and photographed with a digital camera coupled to an optical microscope (Leica DMLP, Germany). The pictures were then analyzed by a calibrated blind examiner with the Quantikov software[30,31] to determine the extent of the lesion. The software calculates the ratio between the total area of the crown and the area with caries. Since the crowns presented different sizes, this method allowed a standardization of the values. The calculation was performed according to the following equation:

$$\mathrm{SCA} = \frac{\mathrm{CA}}{\mathrm{CrA}}$$

where SCA is standardized caries lesion area, CA the selected caries lesion area, and CrA is the crown total area.

2.3. Microhardness assessment

Seventy-five molars ($n = 15$ per group) were embedded in acrylic resin and cut into sagittal medial–distal direction, with a diamond disk at a standard distance from the buccal and lingual surfaces. Subsequently, the samples were flattened and polished with 600, 1200, 1500, 2000 and 4000 grades of Al_2O_3 sandpapers, for 10 s with each paper, and thereafter, polished with 1/4 μm diamond slurry. The samples were ultrasonic cleaned for 10 min to remove debris.

Microhardness analysis was performed by Shimadzu HMV-2000 microhardness tester with a Knoop diamond with 25 g load for 15 s. Three indentations were made in the enamel located at the end of the occlusal fissure with 100 μm of distance between indentations (Fig. 1). The Knoop hardness number (KHN), expressed in $\mathrm{kgf/mm^2} \times 10^{-3}$, was obtained through the measure of the length of the major diagonal left by the penetration of the diamond, and calculated with the standard formula for Knoop microhardness.[32] Microhardness was used to evaluate the resistance of the enamel to demineralization considering that there is a good correlation between enamel microhardness and % of mineral in caries lesion.[33] For this reason, the microhardness of sound enamel was also measured.

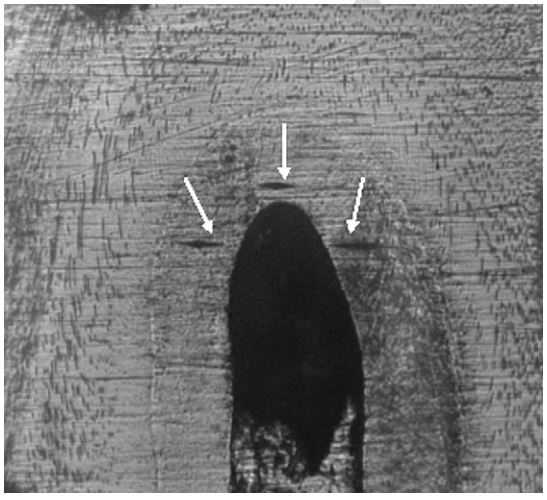

Fig. 1 – Photograph of indentations on the enamel located in the end of the fissure of the rat molar. The arrows point to indentations.

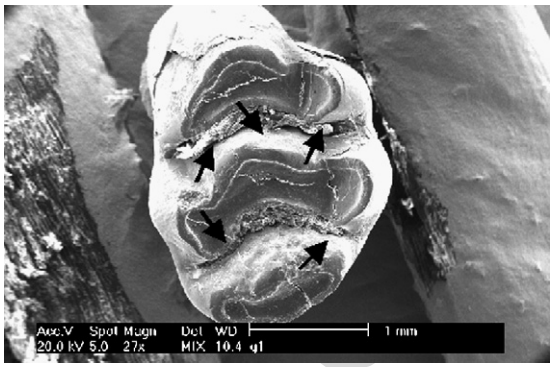

Fig. 2 – Electron-micrograph of the molar occlusal surface. The arrows point to random positions measured by EDS around sulcular area.

2.4. Energy-dispersive X-ray spectrometry

This technique was applied to detect alterations on the distribution of calcium and phosphorus on the teeth enamel in the occlusal surface.

Thirty molars ($n = 6$ per group) were mounted in aluminium stops and coated with carbon. Five points in each occlusal surface were randomly selected around the sulcular area (Fig. 2). The calcium and phosphorous ratio at these five points were then calculated by an energy-dispersive X-ray spectrometer (EDS) attached to a scanning electron microscope (Philips XL, Series 30, Eindhover, Holland). Sound enamel in the occlusal face was also analyzed.

2.5. Fluoride spectroscopy

The interaction between the APF and the red radiation was also investigated. The absorbance in the visible region of the electromagnetic spectrum of the APF ($V = 1$ mL) was determined in a Varian Cary 17 spectrophotometer using a quartz cell with optical path length of 1 cm. The room temperature was kept at 21 °C.

All mean values and standard deviation (S.D.) obtained through each employed method were computed and submitted to statistical analysis by one-way ANOVA and Tukey method. The results were considered significant when $p < 0.05$.

3. Results

3.1. Caries lesion area

The averages and the S.D. of the SCA were 0.13 ± 0.06 for CG group; 0.07 ± 0.04 for FG group; 0.14 ± 0.05 for LG group; 0.08 ± 0.05 for FLG group and 0.038 ± 0.025 for LFG group. Fig. 3 displays these findings. It can be observed that the effect of LPRL associated with APF was more pronounced when compared to others groups. Caries lesion decreased about 70% for LFG group, while FG and FLG groups showed a

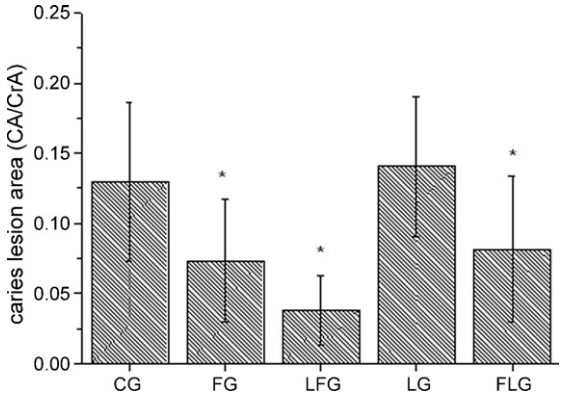

Fig. 3 – Mean values and ± standard deviation (S.D.) of the standardized caries lesion area assay ($n = 15$). No statistically significant differences were observed between FG, LFG, and FLG ($p > 0.05$), although there was a statistically significant difference between those groups and CG and LG. CG, control group; FG, fluoride group; LFG, laser before fluoride group; FLG, fluoride before laser group; LG, laser group.

reduction of approximately 40% compared to control. ANOVA statistical test at 95% level, however, showed that animals from FG, FLG and LFG groups presented the same percentage of caries lesion without statistical significant differences, although statistical differences were observed among those groups and GC and LG groups. There was no statistical difference between CG and LG groups.

3.2. *Microhardness*

Mean values and S.D. of the KHN were: 62.8 ± 15.6 for CG group; 116.7 ± 33.7 for FG group; 57.5 ± 12.4 for LG group; 103.8 ± 49.2 for FLG group; 105.7 ± 29.7 for LFG group (Fig. 4). Sound enamel showed a mean microhardness of 221.2 ± 58.2. ANOVA statistical test at 95% level showed no significant differences between FG, FLG, and LFG groups, however, there was significant difference comparing those groups with LG and CG. All experimental groups showed mean values of microhardness significantly different from sound enamel ($p < 0.05$).

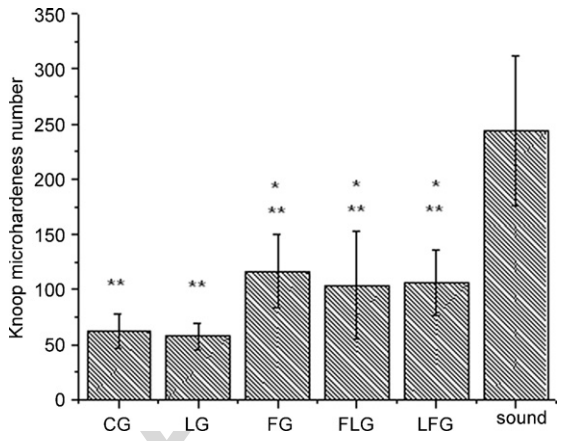

Fig. 4 – Mean values and ± standard deviation (S.D.) of the microhardness assay ($n = 15$). No statistically significant differences were observed between FG, LFG, and FLG ($p > 0.05$), although there was a statistically significant difference between those groups and CG and LG. All experimental groups showed microhardness values significantly different from sound enamel. CG, control group; FG, fluoride group; LFG, laser before fluoride group; FLG, fluoride before laser group; LG, laser group.

Table 2 – EDS data

Group	Ca	P	Ca/P	S.D.
CG	63.14	36.86	1.71	0.09
LG	61.67	38.33	1.61	0.07
LFG	62.85	37.15	1.69	0.1
FG	62.55	37.45	1.67	0.05
FLG	62.66	37.34	1.68	0.07

Values are means of $n = 6$ molars per group. In each group, five random sites around sulcular area were measured on occlusal surface. No statistically significant differences were observed among the groups ($p > 0.05$).

3.3. *EDS*

The results of the Ca/P weight percentages detected at the borders of the lesion zones and intact enamel are summarized in Table 2. The results of the statistical analyses showed that CG group presented a Ca/P mean value and S.D. of 1.71 ± 0.09; FG group 1.67 ± 0.05; LG group 1.61 ± 0.07; FLG group 1.68 ± 0.07; LGF group 1.69 ± 0.10. The calculated Ca/P weight percentage did not confirm a statistical significant difference among groups ($p > 0.05$).

3.4. *Fluoride absorbance spectrum*

Fig. 5 shows the optical absorption of the 1.23% APF used in this study. It is possible to observe a spectral band between 500 and 700 nm with peak centered at λ = 630 nm.

4. Discussion

In this study, the effectiveness of the LPRL on dental caries prevention was investigated comparing the use of LPRL alone, topical application of fluoride alone or the combination of the topical fluoride with LPRL. Under the applied cariogenic challenge none of the tested settings avoided the incidence of the dental caries, although the progression of caries lesion had been diminished with the use of fluoride alone or in combination with LPRL.

The applied model focused on the sulcular area of the occlusal face, which is the most vulnerable site of the molars teeth that are, in turn, the most susceptible teeth in terms of caries prevalence. Most of the study models use facial surfaces to test the efficacy of a preventive method, but in fact, the sulcular areas are the most vulnerable ones due to its winding anatomy that facilitates adherence and growth of bacteria.

Although the frequent use of fluoride in low concentration by the patient has been the best way to control dental caries development, the professional application of fluoride in high

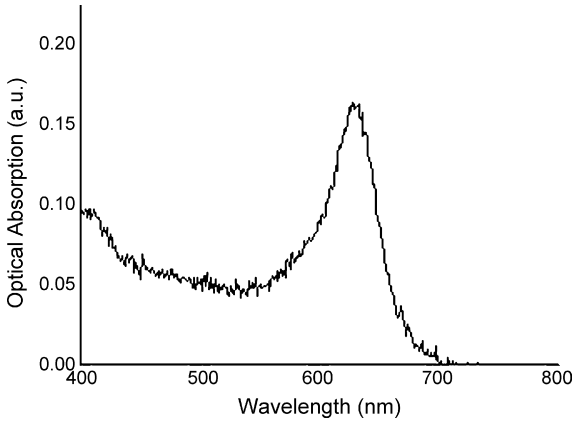

Fig. 5 – Visible optical absorption of 1.23% APF centered at λ = 630 nm.

concentration is still needed in some clinical situations.[34] Since the 1940s when fluoride started to be applied to the teeth, there is a constant search for a cost-effective method to enhance the fluoride uptake, and consequently, to prevent dental caries. Some researches suggest that the clinical efficacy of fluoride could be enhanced through improved delivery or use in combination with supplementary agents.[35] Indeed, CO_2, Nd:YAG, argon and others high power lasers exhibit efficacy in prevention of induced-dental caries in vitro and in vivo.[7–13] The mechanisms of action of those lasers are mostly through thermal effect leading to structural changes on the crystalline matrix of the dental enamel.[9,12,13]

Some reports had showed that LPRL improves caries prevention.[19,20,21] LPRL as the one used in this research, do not promote heat, therefore the mechanisms of action would be completely different.

The combination of LPRL and an exogenous photosensitizer as MB or toluidine blue results in a drop off of the bacterial load over the target area.[36,37] This therapy, commonly nominated photodynamic therapy, is under investigation and could be useful in caries prevention due to its bactericidal effect.[38,39] In this approach the photosensitizer absorbs light with the appropriated wavelength, selected according to the dye absorption spectra, and the excited photosensitizer reacts with the substrate. The result of these interactions is highly reactive oxygen species, which induce injury and death of microorganisms. In our study, an exogenous photosensitizer was not used, therefore, a photodynamic effect is not expected, and furthermore, if a photodynamic effect would be possible due to bacterial production of endogenous photosensitizer as protoporphyrin IX, the group that received LPRL alone would have presented better results due to the photodynamic effect. MB was applied in this work only as a dye employed for staining procedures, and the use of this dye as a photosensitizer was not tested in this study.

The results from optical microscopy indicated that the SCA, directly proportional to the dye (MB) penetration, was reduced for LFG group, in which LPRL treatment was performed before APF, although no statistical significant differences were observed between LFG, FG and FLG groups. LFG group presented a percentage of lesion reduction of 70%, while FG and FLG groups presented a percentage of caries reduction of 40% when compared with CG group.

Even though statistically significant differences were not detected in this work, to some extent, reduction in the caries lesion depth were detected with the association of LPRL and APF. According to Garcia-Godoy et al., 4-min APF treatment prior to caries-like lesion formation diminished lesion depth by 37–40%.[40] These values are in accordance with our data for FG and FLG (about 40%). The values obtained for LFG (70%) were higher than FG and FLG.

In process of caries induction in rats, high sucrose ingestion causes a deficiency on dentinogenesis.[41] According to Pekkala et al.[41] sucrose in newborn rats causes a reduced dentine apposition and an enhanced occurrence and progression of dental caries. This effect could be due to changes on odontoblastic response, originated by the sucrose diet, resulting in a reduction of dentine apposition during primary dentinogenesis, which in turn, contributes to the progression of dental caries thereafter.

Since the results showed that, to some extent, a reduction in the caries lesion depth may be due to the association of LPRL and APF, the effect of this combination on primary dentinogenesis cannot be completely discarded.

Besides, another important factor is that there is no evidence that all individuals would respond in the same way to radiation and either to fluoride uptake, thus a significant standard deviation is expected. Although all rats have been managed in the same way, caries initiation and progression is determined by the overall condition of each animal, i.e. frequency of food ingestion, water consume, etc.

Interestingly, for FLG and FG caries-like lesion formation diminished lesion depth in about 40%. This result could be due to the fluoride gel absorption at the red wavelength range (see Fig. 5). Therefore, the teeth covered with fluoride could not receive the same fluence at deeper layers. The smaller penetration depth of the red radiation in FLG could be the responsible for the different results obtained from LFG.

The fluoride, as it is well established, plays the most important role on the inhibition of the caries progression. The use of laser radiation alone did not promote any additional benefit on the control of dental caries since LG did not show any differences comparing to CG.

Furthermore, in the hypothesis of laser plus fluoride actually represents a beneficial outcome, this effect is essentially concentrated on the dentin level. The topical fluoride operates in enamel with its well-known mechanisms of action. Neither the microhardness surface assay performed in this study nor the EDS analysis showed any advantageous effect of the laser irradiation on enamel. Studies suggest that LPRL associated, or not, with topical fluoride may increase the enamel resistance to an acidic attack, although the mechanisms involved would be unclear.[20,21] Microhardness measurements have been accepted to evaluate mineral lost or gain by enamel because there is a correlation between the mineral content of the enamel surface and the length of the indentations.[42,43] Thus, measures of enamel cross-sectional microhardness show a correspondence between percentage of mineral and KHN in caries lesion.[33]

The cross-sectional microhardness analysis in this study did not show significant differences between FG, LFG and FLG

groups, although significant differences among those groups and LG and CG groups were observed, showing that fluoride plays a pivotal role in caries prevention. It was used a 25 g load for 15 s. This load was selected in a pilot study and it shows to be the best load allowing a correct indentation in the rat's enamel. A noteworthy remark is that measurements were not carried out on dentine to check caries progression due to an even smaller load necessary to perform the analyses on dentin, which would not provide useful data.

The results of this work suggest that the combination of low power laser and topical application of APF gel did not have a significant effect on the prevention of induced-dental caries in rats, although LPRL before APF application appeared to diminish the caries progression.

The constant exposure of the teeth to low concentrations of fluoride seems to be an effective method to control dental caries. In this study the animals did not received any source of fluoride besides the topical application performed in a single day. Even the supplied water was double deionized, thus it did not provide an external source of fluoride. This method was preferred because of the increased concern about dental fluorosis. The prevalence of fluorosis has increased in the past 50 years in optimally fluoridated communities as well as in fluoride-deficient areas, probably due to dietary fluoride supplements.[44] The ingestion of fluoride-containing toothpastes used by toddlers and young children without supervision can provide a source of fluoride exposure,[39] thus a method of preventing dental caries without repeatedly exposing young children to fluoride content products would be highly desired.

Besides the fluoride exposition, the laser parameters as well might play an important role in the outcomes. Different wavelengths, fluences and irradiances should be tested in order to verify the efficiency of low power laser treatment on dental caries prevention.

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REFERENCES

1. U.S. Department of Health Human Services. National Institute of Dental and Craniofacial Research, National Institute of Health, Oral Health in America: a report of the Surgeon General. *J Calif Dental Assoc* 2000;**28**:685–95.
2. Stephen KW. Caries in young populations-worldwide. In: Bowen WH, Tabak LA, editors. *Cariology for the nineties*. Rochester, New York, USA: University of Rochester Press; 1993. p. 37–50.
3. Featherstone JD. The science and practice of caries prevention. *J Am Dental Assoc* 2000;**131**:887–99.
4. Wefel JS. Effects of fluoride on caries development and progression using intra-oral models. *J Dental Res* 1990;**69**:626–33.
5. Featherstone JD. Prevention and reversal of dental caries: role of low-level fluoride. *Comm Dent Oral Epidemiol* 1990;**27**:31–40.
6. DePaola PF. Reaction paper: the use of topical and systemic fluorides in the present era. *J Pub Health Dent* 1991;**51**:48–52.
7. Stern RH, Sognnaes RF, Goodman F. Laser effect on in vitro enamel permeability and solubility. *J Am Dental Assoc* 1966;**73**:838–43.
8. Yamamoto H, Ooya K. Potential of yttrium–aluminium–garnet laser in caries prevention. *J Oral Pathol Med* 1974;**3**: 7–15.
9. Cecchini RCM, Zezell DM, Oliveira E, Freitas PM, Eduardo CP. Effect of Er:YAG laser on enamel acid resistance; morphological and atomic spectrometry analysis. *Lasers Surg Med* 2005;**37**:373–80.
10. Chin-Ying SH, Gao XL, Pan JS, Wefel JS. Effects of CO_2 laser on fluoride uptake in enamel. *J Dent* 2004;**32**:161–7.
11. Morioka T, Tagomori S. Combined effects of laser and fluoride on acid resistance of human dental enamel. *Caries Res* 1989;**23**:225–31.
12. Nelson DGA, Jongebloed W, Featherstone JDB. Laser irradiation of human dental enamel and dentin. *NZ Dental J* 1986;**82**:74–7.
13. Delbem ACB, Cury JA, Nakassima CK, Gouveia VG, Theodoro LH. Effect of Er:YAG laser on CaF_2 formation and its anti-cariogenic action on human enamel: an in vitro study. *J Clin Laser Med Surg* 2003;**21**:197–201.
14. Hicks MJ, Flaitz CM, Westerman GH, Blankenau RJ, Powel GL, Berg JH. Enamel caries initiation and progression following low fluence (energy) argon laser and fluoride treatment. *J Clin Pediatr Dent* 1995;**20**:9–13.
15. Haider S, White GE, Rich A. Combined effects of argon laser irradiation and fluoride tretments in prevention of caries-like lesion formation in enamel: an in vitro study. *J Clin Pediatr Dent* 1999;**23**:247–57.
16. Anderson JR, Ellis RW, Blankenau RJ, Beiraghi SM, Westerman GH. Caries resistance in enamel by laser irradiation and topical fluoride treatment. *J Clin Laser Med Surg* 2000;**18**:33–6.
17. Nammour S, Demortier G, Florio P, Delhaye Y, Pireaux J-J, Morciaux Y, et al. Increase of enamel fluoride retention by low fluence argon laser in vivo. *Lasers Surg Med* 2003;**33**: 260–3.
18. Nammour S, Rocca J-P, Pireaux J-J, Powel GL, Morciaux Y, Demortier G. Increase of enamel fluoride retention by low fluence argon laser beam: a 6-month follow-up study in vivo. *Lasers Surg Med* 2005;**36**:220–4.
19. Slujáiev IF, Pak AN. He–Ne laser effect on dental enamel solubility in health and caries. *Stomatol* 1990;**5**:6–9.
20. Andreu MIG, Zaldivar CV, Dben AG. Influencia de la radiación láser de baja potencia em molares permanente inmaduros. *Rev Cub Estomatol* 1996;**33**:1–4.
21. Fagnoni V, Sapino S, Zulian P, Iemma D. Ionofluor + laser = prevenzione. *Min Stomatol* 1989;**38**: 769–72.
22. Janse van Rensburg SD, Wiltshire WA. The effect of soft laser irradiation on fluoride release of two fluoride containing orthodontic bonding materials. *J Dental Assoc S Afr* 1994;**49**:127–31.
23. Diaz-Arnold AM, Holmes DC, Wistrom DW, Swift Jr EJ. Short-term fluoride release/uptake of glass ionomer restoratives. *Dent Mater* 1995;**11**:96–101.
24. Jiang H, Tai BJ, Du MQ, Peng B. Effect of professional application of APF foam on caries reduction in permanent first molars in 6–7-year-old children: 24-month clinical trial. *J Dent* 2005;**33**:469–73.
25. Whitford GM, Adair SM, Hanes CMMcK, Perdue EC. Enamel uptake and patient exposure to fluoride comparison of APF gel and foam. *Pediatr Dent* 1995;**17**:199–203.

26. Keyes PH, Jordan HV. Periodontal lesions in the Syrian hamster. Part III. Findings related to an infectious and transmissible component. *Arch Oral Biol* 1964;**9**:377–400.
27. Ikeno K, Ikeno T, Miyazawa C. Effects of propolis on dental caries in rats. *Caries Res* 1991;**25**:347–51.
28. Seppä L, Koskinen M, Luoma H. Caries prevention by two fluoride varnishes in Osborne–Mendel rats. *Caries Res* 1982;**16**:453–9.
29. Piva E, Meinhardt L, Demarco FF, Powers JM. Dyes for caries detection: influence on composite and compomer microleakage. *Clin Oral Invest* 2002;**6**:244–8.
30. Machado G, Denardin ELG, Kinast EJ, Goncalves MC, de Luca MA, Teixeira SR, et al. Crystalline properties and morphological changes in plastically deformed isotatic polypropylene evaluated by X-ray diffraction and transmission electron microscopy. *Eur Polym J* 2005;**41**: 129–38.
31. Coutinho ACS, Bressiani JC, Bressiani AHA. Influence of microstructural characteristics on the mechanical properties of silicon nitride with Al_2O_3, Y_2O_3 and Nd_2O_3 as sintering aids. *Adv Powder Technol III Mater Sci Forum* 2003;**416**:567–72.
32. Craig RG, Peyton FA. The microhardness of enamel and dentin. *J Dental Res* 1958;**37**:661–8.
33. Featherstone JD, ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. *Caries Res* 1983;**17**:385–91.
34. Cury JA, Delbem ACB. Effect of the application time of APF and NaF gels on microhardness and fluoride uptake of in vitro enamel caries. *Am J Dent* 2002;**15**:169–72.
35. Bowen WH. Do we need to be concerned about dental caries in the coming millennium? *Crit Rev Oral Biol Med* 2002;**13**:126–31.
36. Wainwright M. Photodynamic antimicrobial chemotherapy (PACT). *J Antimicrob Chemother* 1998;**42**:13–28.
37. Jori G, Fabris C, Soncin M, Ferro S, Coppellotti O, Dei D, et al. Photodynamic therapy in the treatment of microbial infections: basic principles and perspective applications. *Lasers Surg Med* 2006;**38**:468–81.
38. Williams JA, Pearson GJ, Colles MJ, Wilson M. The photo-activated antibacterial action of toluidine blue O in a collagen matrix and in carious dentine. *Caries Res* 2004;**38**:530–6.
39. Zanin ICJ, Gonçalves RB, Brugnera Jr A, Hope CK, Pratten J. Susceptibility of *Streptococcus mutans* biofilms to photodynamic therapy: an *in vitro* study. *J Antimicrob Chemother* 2005;**56**:324–30.
40. Garcia-Godoy F, Hicks MJ, Flaitz CM. Acidulated phosphate fluoride treatment and formation of caries-like lesions in enamel: effect of application time. *J Clin Pediatr Dent* 1995;**19**:105–10.
41. Pekkala E, Hietala E-L, Puukka M, Larmas M. The effect of sucrose diet of rat dams on the dentine apposition and dental caries of their pups. *Arch Oral Biol* 2000;**45**: 193–200.
42. Arends J, Schuthof J, Jongebloed WG. Lesion depth and microhardness indentations on artificial white spot lesions. *Caries Res* 1980;**14**:190–5.
43. Zero DT. In situ caries models. *Adv Dental Res* 1995;**9**:214–30.
44. Billings RJ, Berkowitz RJ, Watson G. Teeth. *Pediatrics* 2004;**113**:1120–7.