Emanuela Carla dos Santos
(Organizadora)

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APRESENTAÇÃO

A Odontologia vem ampliando cada vez mais sua área de atuação dentro do campo da saúde. Hoje aliamos o conhecimento teórico de base às novas tecnologias e técnicas desenvolvidas através de pesquisas para elevar a qualidade e atingir excelência na profissão.

Diante da necessidade de atualização frequente e acesso à informação de qualidade, este E-book, composto por dois volumes, traz conteúdo consistente favorecendo a Comunicação Científica e Técnica em Odontologia.

O compilado de artigos aqui apresentados são de alta relevância para a comunidade científica. Foram desenvolvidos por pesquisadores de várias instituições de peso de nosso país e contemplam as mais variadas áreas, como cirurgia, periodontia, estomatologia, odontologia hospitalar, bem como saúde do trabalhador da Odontologia e também da área da tecnologia e plataformas digitais.

Espero que possam extrair destas páginas conhecimento para reforçar a construção de suas carreiras.

Ótima leitura!

Profª. MSc. Emanuela Carla dos Santos

SUMÁRIO

CAPÍTULO 28

EFFECT OF ND:YAG LASER AND FLUORIDE TREATMENT ON THE PERMEABILITY OF PRIMARY TOOTH ENAMEL

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Running title: Laser/fluoride effects on enamel permeability

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RESUMO: O objetivo deste estudo foi avaliar os efeitos da combinação do tratamento com laser Nd:YAG e gel de fluoreto de fosfato acidulado tópico ou verniz fluoretado sobre a permeabilidade do esmalte dentario. Vinte e quatro dentes decíduos humanos foram seccionados no sentido buco-lingual, e as amostras foram isoladas com verniz/cera, deixando uma área exposta na superfície externa (9mm^2). Estas amostras foram divididas aleatoriamente em 6 grupos (n=8) de acordo com os tratamentos fornecidos, os quais incluíram: G1 (sem tratamento), G2 (1,23% de flúor gel acidificado (DFL®; 4min), G3 (flúor acidulado gel + Nd:YAG a 0,5 W/10 Hz modo de contato), G4 (5% de verniz fluoretado (Duraphat®), G5 (verniz fluoretado + Nd:YAG a 0,5 W/10 Hz) e G6 (Nd:YAG a 0,5 W). Após o tratamento, foi realizado um método de coloração histoquímica utilizando sulfato de cobre e uma solução de ditiooxamida em todas as amostras, sendo retiradas três imagens da área exposta por meio de um microscópio óptico. A permeabilidade foi analisada nas imagens digitalizadas por meio da porcentagem de penetração de cobre sobre a espessura do esmalte. Os dados foram analisados usando ANOVA e LSD de Fisher. O grupo controle teve a maior penetração de cobre (67,48%) e foi significativamente diferente (p <0,05) do G2 (27,71%), G3 (23,24%), G4 (37,81%), G5 (39,88%) e G6 (36,80%) que apresentaram um menor grau de penetração de cobre. O tratamento com laser Nd:YAG, com ou sem gel/verniz fluoretado, mostrou ser semelhante à aplicação tópica de flúor em relação a redução da permeabilidade do esmalte.

PALAVRAS-CHAVE: Esmalte dentário; fluoretos; permeabilidade; Dente decíduo; Lasers.

ABSTRACT: The purpose of this study was to evaluate the effects of the combination of Nd:YAG laser treatment and a topical acidulated phosphate fluoride gel or a fluoride varnish on the permeability of primary enamel. Twenty-four human primary teeth were sectioned in the buccolingual direction, and the samples were isolated with nail varnish/wax, leaving an exposed area on the outer surface (9 mm^2). These samples were randomly divided into 6 groups (n=8) according to the provided treatments, which included the following: G1 (no treatment), G2 (1.23% acidulated fluoride gel (DFL ®; 4 min), G3 (acidulated fluoride gel + Nd:YAG at 0.5 W/10 Hz contact mode), G4 (5% fluoride varnish (Duraphat ®), G5 (fluoride varnish + Nd:YAG at 0.5 W/10 Hz) and G6 (Nd:YAG at 0.5 W/10 Hz). After the treatment, a histochemical coloring method using copper sulfate and a dithiooxamide solution was performed on all of the samples. Three sections taken from the exposed area of each sample were imaged using an optical microscope. The permeability was measured in the resulting digitized images as the percentage of copper penetration across the enamel thickness. The data were analyzed using ANOVA and Fisher's LSD. The control group had the deepest copper penetration (67.48%) and was significantly different ($p<0.05$) from G2(27.71%), G3(23.24%), G4(37.81%), G5(39.88%) and G6(36.80%), all of which were found to have a lesser degree of copper penetration. Nd:YAG laser treatment, either with/without fluoride gel/varnish, was found to be similar to topical fluoride application in terms of its effects in reducing enamel permeability.

KEYWORDS: Dental enamel; fluorides; permeability; Tooth, deciduous; Lasers.

1 I INTRODUCTION

The aim of high-intensity laser irradiation of dental enamel is to produce a surface with increased resistance to the acid dissolution that is produced during a cariogenic challenge (Hsu et al. 2000, Korytnicki et al. 2006, Colucci et al. 2015, Wen et al. 2014, Zancopé et al. 2016).

The Nd:YAG laser is one of the most-studied means of primary caries prevention (Korytnicki et al. 2006, Zezell et al., 2009, Shahabi et al. 2016). In the visible and near-infrared wavelengths (1.06 µm), this laser presents strong absorption by hemoglobin and other pigments, such as melanin (Cheong et al. 1990, Yue et al. 2016). On the other hand, it is not effectively absorbed by human enamel (Seka et al. 1995, Featherstone et al. 2000). In an attempt to improve Nd:YAG absorption by dental enamel while minimizing the possibility of pulpal injury, the use of a photosensitizer composed of blue ink (Jennett et al. 1847, Huang et al. 2001) or triturated coal (Zezell et al. 2009) was proposed. However, these photosensitizers are carbon-based, and their exposure to high temperatures may lead to chemical reactions with undesirable toxic byproducts (Gelskey et al. 1998). Accordingly, the present study avoided the use of photosensitizers because of their toxic effects and the low energy levels needed, thus minimizing the risk to the dental tissues.

Laser irradiation may modify the chemical-physical composition of the dental substrate, promoting an increase in resistance to demineralization and aiding in the prevention of caries development (Shahabi et al. 2016, Featherstone et al. 1997, Karandish 2014). It has recently been reported that laser irradiation produces an even greater increase in caries resistance when combined with a topical fluoride treatment (Zezell et al. 2009, Karandish 2014, Tagliaferro et al. 2007, Mathew et al. 2013, Stangler et al. 2013). The combination of fluoride and laser irradiation in the prevention of enamel demineralization has also been reported as a complementary procedure to prevent dental erosion (Ramalho et al. 2013, Braga et al. 2017). Several mechanisms might explain the improvement in caries resistance conferred by laser and fluoride treatment of the enamel (Zezell et al.2009). Laser irradiation might enhance the effects of fluoride by allowing a more effective interaction with the dental enamel or even by increasing the fluoride uptake and retention for longer periods (Delbem et al. 2003). However, these studies have mainly evaluated the use of lasers to promote acid resistance in the enamel of permanent teeth; few studies have addressed the same issue using primary human teeth.

There are notable differences between the microstructure and mineral composition of permanent and deciduous dental enamel (Wilson et al. 1989, Wang et al. 2006, De Menezes et al. 2010). It has been reported that the primary enamel has lower levels of Ca and P, a lesser thickness, and a higher rod density (De Menezes et al. 2010). The thinner layer of deciduous enamel combined with its lesser degree of mineralization might be responsible for the faster development of dental erosion and caries in primary enamel (Wang et al. 2006, Amaechi et al. 1999, Johansson et al. 2001) as well as its greater fluoride uptake (Percinoto et al. 1990). Thus, the remarkable differences between primary and permanent enamel substrates must be considered. To provide effective treatments for these different tissues (De Menezes et al. 2010), it is necessary to use specific and biological preventive protocols. Taking these differences into account, the ability of the Nd:YAG laser, either alone or in combination with fluoride treatment, to inhibit the development of dental caries in primary teeth must be further assessed as a viable treatment.

The aim of the present in vitro study was therefore to evaluate the effectiveness of Nd:YAG laser irradiation, with or without fluoride, in reducing the enamel permeability of primary teeth.

2 I MATERIALS AND METHODS

2.1 Tooth selection and sample preparation

Twenty-four human primary teeth were selected for use in this study. The teeth were sectioned 1 mm below the cement-enamel junction using a low-speed water-cooled diamond saw (IsoMet 1000^{Ò}, Buehler, Lake Bluff, IL, USA) to separate the root from the coronary portion. The crowns were then cut into 2 parts in the mesio-distal direction. Next, the samples were isolated with nail varnish/wax, leaving an exposed area on the outer surface (9 mm^{2}).

These samples were then randomly divided into 6 groups (n=8) according to the received treatment: G1 (control group; no treatment), G2 (1.23% acidulated fluoride gel (DFL ®) (4 min)), G3 (acidulated fluoride gel + Nd:YAG at 0.5 W/10 Hz contact mode), G4 (5% fluoride varnish (Duraphat ®)), G5 (fluoride varnish + Nd:YAG at 0.5 W/10 Hz) and G6 (Nd:YAG at 0.5 W/10 Hz).

2.2 Laser equipment, parameters and application

The Nd:YAG laser (SmartFile, DEKA, Italy) emits pulses at 1.064 *μ*m, with a 300 *μ*m quartz fiberoptic delivery system operating in contact mode (with a spot size of 300 *μ*m). It has a temporal width of 100 microseconds, and it operates at a repetition rate of 10-100. The Nd:YAG laser was used to irradiate the exposed surfaces, with the fiber positioned perpendicular to the sample with a scanning movement of 15 seconds. The parameters used were as follows: energy 50 mJ, power density 0.5 W and energy density 70.77 J/cm^{2}, with an irradiation time of 15 seconds. The FieldMaxII-TOP (Coherent, Inc., Santa Clara, CA, USA) device was used to measure the equipment's power output prior to each application.

2.3 Preventive treatments

Acidulated fluoride gel at a concentration of 1.23% (Sultan Topex, DFL Indústria e ComércioLtda, Rio de Janeiro, RJ, Brasil) was applied to the enamel surface with a microbrush for a period of 4 minutes and was later removed with gauze. The fluoride varnish Duraphat 5% (Colgate-Palmolive Company, Germany) was applied to the enamel surface with a microbrush and removed with gauze after 24 hours. Groups G3 and G5 were irradiated with the Nd:YAG laser for 1 minute after the application of the acidulated fluoride gel and fluoride varnish, respectively, which were applied simultaneously and removed at the determined time.

2.3.1 Histochemical coloring methods

After treatment, the specimens were subjected to a histochemical coloring method using copper sulfate and dithiooxamide solutions.[31] The histochemical coloring method was used to demonstrate the extent of diffusion of copper ions within the enamel. The specimens were immersed in 8 ml of a 10% copper sulfate aqueous solution and were kept in a vacuum for 5 minutes. Each specimen was then individually stored in 5 ml of this solution for seven days, then dried with absorbent paper and immersed in a 0.5% dithiooxamide alcoholic solution (revealer agent), following the same protocol described for the copper sulfate solution (five minutes in the vacuum followed by seven days in the solution). The specimens were rinsed with deionized water for 15 seconds, dried and kept in individual vials with ammonia steam (a fixative agent) for 7 days. The reaction of the copper sulfate with the dithiooxamide alcoholic solution produces a strong staining in the enamel structure, which ranges from dark blue to black depending on the concentration of the copper ions. It was thus possible to measure the thickness of the enamel and the penetration depth of the copper.

2.3.2 Enamel permeability evaluation

Each specimen was embedded in polyester resin (Milflex Indústria Química Ltda., São Bernardo do Campo, SP, Brazil), and three sections of 400 µm each were cut longitudinally in the center of the exposed area, using a low-speed diamond saw. These sections were hand polished, using Al_2O_3 abrasive papers (600- and 1200-grit) that were cooled with water, to a thickness of approximately 200 µm. The prepared samples were imaged using an optical microscope (Axiostar Plus, Carl Zeiss) at x100 magnification, and the permeability was measured using the digitized images (Axion Vision 3.1, Carl Zeiss Vision) to assess the percentage of copper ion penetration in terms of the total enamel thickness. Five measurements (in millimeters) were performed for each of the three sections obtained per specimen. The average of these five measurements was converted to a percentage as the outcome value for each specimen.

The application and evaluation of the test agents were performed by different operators.

2.4 Statistical analysis

The average of three sections from the same specimen was considered to be the outcome value for each specimen. The data were analyzed using ANOVA and Fisher’s LSD (a = 5%).

3 I RESULTS

ANOVA showed significant differences in terms of enamel permeability ($p<0.05$). Fisher's LSD revealed the deepest copper penetration in control group (67.48%), which differed from groups G2 (27.71%), G3 (23.24%), G4 (37.81%), G5 (39.88%) and G6 (36.80%) (Table 1). Figure 1 shows the penetration of copper ions for all of the assessed groups. In the control group (Fig 1A), the penetration was deeper and more significant than in groups G2 (Fig 1B), G3 (Fig 1C), G4 (Fig 1D), G5 (Fig 1E) or G6 (Fig 1F), all of which had lower copper ion penetration levels.

Group	Enamel thickness	Enamel diffusion	Permeability
G1	0.34	0.23	67.48 ± 12.82 (b)
G2	0.23	0.08	27.71 ± 20.88 (a)
G3	0.23	0.05	23.24 ± 17.83 (a)
G4	0.43	0.16	37.81 ± 2187 (a)
G5	0.30	0.12	39.88 ± 13.34 (a)
G6	0.33	0.12	36.80 ± 14.23 (a)

* The same letter indicates statistical similarity

Table 1. Enamel thickness (mm), diffusion (mm), mean (%) and standard deviation of the permeability for each of the different treatments.

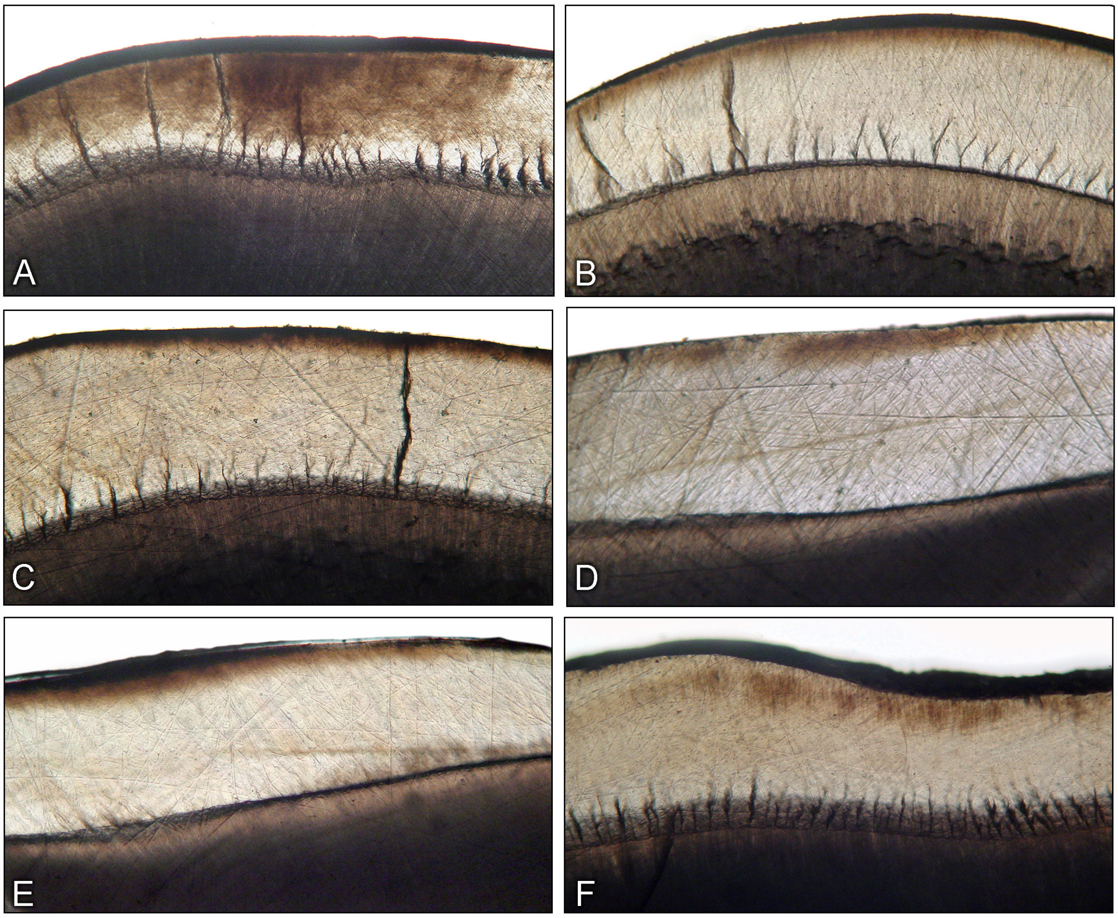

Figure 1. Digital images obtained by Optical Microscopy, representing the different depths of penetration of copper ions in relation to the thickness of the enamel. A) Group C. B) GroupFA. C) Group FAL. D) Group VF. E) Group VFL. F) Group L.

4 I DISCUSSION

Although the worldwide incidence of dental caries has been declining over the last several decades, this condition is still the most prevalent disease in childhood and adolescence. Modern sealing materials are widely accepted in the scientific community despite their disadvantages: they can contaminate the operation field, they can be accompanied by non-uniform and ineffective etching, and they are known to contract during polymerization (Borsatto et al. 2010, Durmus et al. 2017). These issues have led researchers to investigate alternative solutions that might overcome these limitations (Cochrane et al. 2010).

Several investigations have demonstrated that different types of lasers with different operating modes and energy outputs, such as Nd:YAG, CO_2 and Er:YAG lasers, can reduce the rate of enamel surface demineralization and thereby prevent dental caries development (Colucci et al. 2015, Wen et al. 2014, Shahabi et al. 2016, Featherstone et al. 1997, Karandish 2014, Tepper et al. 2004, Castallan et al. 2007, Schmidlin et al. 2007). Different explanations for the increased acid resistance of laser-treated enamel have been suggested, which include decreased enamel permeability and alterations in the chemical composition and/or surface morphology (Tepper et al.

2004).

Permeability reduction through the melting and re-crystallization of the enamel surface was believed for many years to be an essential contributing factor in caries prevention (Stern et al. 1972). However, in evaluating enamel treated with different lasers, Borggreven et al. (Borggreven et al. 1980) observed a significant increase in the permeability of surfaces that were irradiated with high energy levels. Additional research has supported this finding, demonstrating that high energy levels may produce undesirable changes on and within the enamel surface, such as cracks, glazed surfaces and columns separated by voids (Chen et al. 2009, Rodriguez-Vilchis et al. 2011). These alterations derive from the melting and the subsequent re-crystallization of the enamel during cooling (Chen et al. 2009, Rodriguez-Vilchis et al. 2011). At present, morphological alterations in the enamel surface, such as melting and re-crystallization, are known to be unnecessary to reduce demineralization and thus are not necessary for the reduction and prevention of dental caries (Apel et al. 2005, Bedini et al. 2010).

Scientific literature also suggests that laser irradiation can promote modifications of the bands within the phosphate and collagen matrix (Castellan et al. 2007). According to Ying et al. (2004), the partial denaturation of the organic matrix by laser irradiation can cause a decrease in the enamel pore volume and surface area, which may help explain the laser-induced blocking of the diffusion pathway and the subsequent prevention of enamel demineralization. In a previous study (Castellan et al. 2007), the use of Nd:YAG energy density values that were lower than the threshold for ablation of the enamel affected (Castellan et al. 2007) a small quantity of inorganic compound, whereas most of the modified material was composed of organic matter. These authors have also indicated that the results of Nd:YAG laser–tissue interactions are not merely restricted to their heating effects; the laser heating of dental tissues induces the formation of $TCPCa_3(PO_4)_2$. Lower energies were also used by Bedini et al. (2010), who observed minimal alterations in an irradiated enamel surface that were generated with 0.6 W of power. Thus, the low energy levels used in this study were justified by the fact that they can reduce enamel demineralization (through chemical-physical changes) and thus preserve its integrity.

Several studies also suggest that an increased fluoride uptake occurs in laser-treated dental enamel, increasing the resistance of the enamel to acid (Kumar et al. 2016, Fornaini et al. 2014, Mohan et al. 2014). The major effect of topical fluoride treatment is the formation of CaF_2-like globules on the enamel surface or in the decalcified enamel lesions (Zancopé et al. 2016, Rošin-Grget et al. 2013) This globular surface material is often combined with phosphates or proteins, and it is thought to be generally insoluble (Rošin-Grget et al. 2013). However, this material is known to be lost from the enamel surface over a time period of days to weeks, due to daily brushing and chewing (Dijkman et al. 1983, Dijkman et al. 1988). Therefore, certain researchers have argued that these deposits provide only a limited protection (Jeng et al. 2008). Regarding fluoride association, we observed that the use of the Nd:YAG laser with gel and varnish reduced permeability. However, a similar reduction in permeability was noted with the use of topical fluoride alone. These results are not unexpected, given that fluoride itself is known to have a preventive effect on demineralization, reducing acid diffusion through the dental enamel (Chersoni et al. 2011).

Our findings also demonstrated that laser irradiation without fluoride treatment produces chemical and physical changes in the enamel surface that reduce its overall permeability, corroborating previous results obtained by Márquez et al. (1993). According to such previous studies, this reduction in permeability could be associated with the water loss that occurred between 80 and 120 °C, the decomposition of a small quantity of organic substance at 350°C or the initial loss of carbonate hydroxyapatite between 400 and 660°C (Bedini et al. 2010). It is believed that in this temperature range, certain enamel proteins and their products decompose, thereby promoting a reduction in enamel microporosity and ion penetration (Hsu et al. 2000).

The current literature contains conflicting reports on the effects of laser treatment on enamel structure, which is likely due to the high number of variables involved in the irradiation process, including power level, pulse frequency and duration. Therefore, additional in vitro and clinical studies will be necessary to determine the most efficient laser parameters for use in caries prevention.

5 I CONCLUSION

Despite the inherent limitations of this in vitro study, it can be concluded that the combination of fluoride treatment and Nd:YAG laser irradiation reduces enamel permeability by approximately the same level as topical fluoride treatment alone.

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