



## THE COMBINED USE OF SODIUM FLUORIDE AND ER: YAG LASER TO CONTROL THE PROGRESSION OF ENAMEL CARIES

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### ABSTRACT

This study aimed to evaluate in vitro the influence of the 0.05% NaF combined with Er:YAG laser irradiation to control dental enamel demineralization. Slabs of bovine enamel (3x3mm) were submitted to cariogenic challenges by pH-cycling method (5 days). After cycles, slabs were assigned into four groups, according to treatments (n=15): Placebo; Er:YAG Laser+Placebo; 0.05% NaF and Er:YAG Laser + 0.05% NaF. After surface treatment, new cariogenic challenges (14 days) were performed following the same protocol. The microhardness analysis was performed before caries formation and after cariogenic challenges. Data were submitted to analysis of variance (ANOVA) followed by DUNCAN test, at significance level of 1%. After cariogenic challenges, the placebo group ( $128.19 \pm 6.68$ ) presented the lowest microhardness values. The simultaneous action of Er:YAG laser + NaF ( $231.92 \pm 22.81$ ) promoted the highest values of enamel microhardness, followed by the treatments with Er:YAG laser ( $196.45 \pm 33.76$ ) and NaF ( $153.59 \pm 14.80$ ). The use of NaF combined with the Er: YAG laser may be an alternative to control the demineralization of enamel exposed to high cariogenic challenge.

**KEYWORDS:** Sodium fluoride, Er:YAG laser, enamel & caries.

### INTRODUCTION

Dental enamel is the most mineralized and hardest tissue in the human body.<sup>[1]</sup> The oral plaque contents acidogenic bacteria that converts fermentable carbohydrates to organic acids (lactic, formic, acetic or propionic), which can diffuse into the enamel, resulting in a reduction of pH and caries formation.<sup>[2, 3]</sup> Caries progression is a dynamic process in which an imbalance between the factors that cause demineralization or the remineralization<sup>[4]</sup> will result in the developing of incipient caries lesions or “white spot”.<sup>[5]</sup> It begins with the organic acid production and its diffusion through the enamel causing initial demineralization; dissolving the mineral crystals with the consequent loss of calcium and phosphate ions. At this initial stage, if the process is not reversed, subsurface lesions leading to cavitation will occur.<sup>[6-8]</sup>

The control of enamel caries requires a suitable treatment that is able to remineralize enamel by the diffusion of minerals into the defective tooth structure avoiding the progression of cavitation.<sup>[9, 10]</sup> The use of fluorides has been widely used to remineralize the enamel subsurface, resulting in arrestment or even reversal of caries lesions.<sup>[7, 11, 12]</sup>

Different types and forms of fluoride agents have been proposed; among them are toothpaste, mouth rinse, drops, varnish, gel, as well as, the combination of products.<sup>[13]</sup> The methods for caries prevention recommended by The World Health Organization (WHO) include the use of sodium fluorides. The action mechanism of this element is by reacting with the enamel and precipitating calcium difluoride (CaF<sub>2</sub>).<sup>[14,15]</sup> This deposits acts like a mechanical protection of the adjacent enamel surface and like a reservoir of fluoride ions.<sup>[16,17]</sup> In addition, a wide range of fluoride concentration can be found, being the most popular form the 0.05% NaF mouthrinse, sold over the counter without a prescription necessity.<sup>[18]</sup> However, the single use of fluoride compounds are insufficient for a caries-preventive effect and control of high caries risk in some individuals (high bacterial challenge or lack of salivary components), to overcome this challenge another adjunct methods are needed.<sup>[6]</sup> Other preventive strategies such as laser irradiation have been evaluated against enamel demineralization due to its favorable effect in decreasing enamel solubility against acid attack.<sup>[19]</sup> The Er:YAG laser emits light at a wavelength of 2,940 μm and is mainly absorbed by water present in the dental tissue.<sup>[20]</sup> The presence of water inside the tissue leads to evaporation and micro explosions that blast away the particles of hard tissue, promoting partial

denaturalization of the matrix and the formation of a mineral block that blocks acid diffusion.<sup>[21,22]</sup> At appropriate energy levels, laser irradiation on enamel promotes an increase in acid resistance, and followed by the application of topical fluoride compounds, the fluoride levels increased promoting an anti-cariogenic effect by reducing the dissolution rate of the enamel surface through the formation of fluorapatite.<sup>[23]</sup>

The application of 1% sodium fluoride after Er: YAG surface irradiation leads to a significantly reduction of calcium loss.<sup>[24]</sup> In addition, Delbem et al showed that the acidulated phosphate fluoride gel associated to the Er:YAG laser promoted a synergistic effect by lowering the superficial mineral loss when compared to the APF group.<sup>[25]</sup>

Nevertheless, little is known about the association and effect of the Er:YAG laser and 0.05% NaF, over control of dental enamel caries. This study aimed to evaluate in vitro the influence of the 0.05% NaF combined with Er:YAG laser irradiation to control dental enamel demineralization.

## MATERIALS AND METHODS

### Experimental design

The factors under evaluation were Er:YAG laser irradiation associated with 0.05% sodium fluoride treatment to control the progression of carious enamel lesions. The control group received a placebo solution. Sixty bovine enamel fragments were randomly divided into four treatments (n=15): Placebo; Er:YAG Laser+Placebo; 0.05% NaF and Er:YAG Laser+0.05% NaF. The quantitative response variable was the subsurface Knoop microhardness (KHN) of the enamel subjected to cariogenic challenges. The design of the present study is presented in Figure 1.

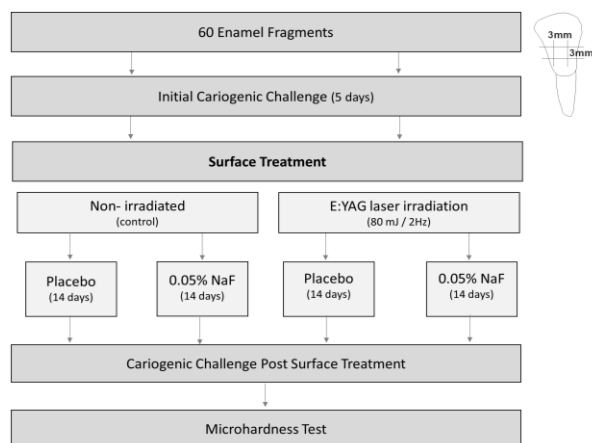


Fig. 1: Experimental design

### Selection and sample preparation

Bovine incisors freshly extracted were sectioned 2 mm below the cemento enamel junction in precision cutter water-cooled (Isomet 1000, Buehler, Lake Bluff, IL, USA). Subsequently, the crowns of the teeth were sectioned in mesio-distal direction and in the cervical-

incisal direction resulting in two middle third fragments (3x3mm).

The fragments were fixed in teflon matrix using melted wax with the enamel lateral surfaces (subsurfaces) facing the external environment. The subsurfaces were flattened and polished with #1200-grit silicon carbide paper in a water-cooled polishing machine (DP-9U2, Struers A/S, Copenhagen, Denmark) (Hermes Abrasives Ltd., VA, USA) and then, 0.3 um alumina paste by felt polisher (ATM, Altenkirchen, Germany).<sup>[26]</sup>

Three readings were performed on the subsurfaces 30 um from de edge and 100 um each other through a microhardness tester HMV-2000 (Shimadzu Corporation, Kyoto, Japan) using a diamond indenter for Knoop hardness under 25 g load for 20 seconds.<sup>[26-28]</sup> The repetitions were performed in order to obtain a sample of patterned fragments.

The average of the three measurements were averaged and used as the microhardness of the fragment. Specimens with microhardness values 20% above or below the mean were discarded.<sup>[29]</sup> Sixty dental fragments were selected based on Knoop harness values.

### Initial cariogenic challenge

Artificial cariogenic challenges were performed in all enamel fragments simulating patients with high caries activity (microscopic enamel white spot lesions).

The specimens were fixed in teflon matrices with the buccal surface facing the external environment. The other surfaces were protected with wax. These matrices were stored in individual plastic containers and specimens were cycled for 5 days according to the protocol proposed by Featherstone et al and modified by Serra & Cury.<sup>[30,31]</sup> In this model, artificial caries lesions were produced by immersion of fragments in demineralizing solution (pH 5.0 for 6 hours) and remineralizing solution (pH 7.0 for 18 hours) at 37°C.

### Surface treatment

After the formation of caries lesions, the specimens were divided into 4 groups (n=15): non irradiated or Er:YAG laser irradiated (Fidelis Er III, Fotona, Ljubljana, Slovenia). Treatment was performed by Er:YAG laser in the MSP mode, using a pen (R02), at noncontact mode, pulse energy of 80 mJ and frequency of 2Hz, an output beam diameter of 0.9 mm and under water spray (6 mL/min).

During irradiation, the operator maintained the laser beam perpendicular to the substrate, and the laser beam was positioned 4mm from the substrate. After the irradiation, the specimens were kept in artificial saliva at 37°C.

The 0.05% sodium fluoride manipulated solution (pH 5.5) (Farmácia de Manipulação Flor Amarela. J.Biasioli

– ME.8 Jaú-SP) was composed as follows 0.05% Sodium Fluoride; 0.05% Propiliparabeno; 0.1% Methylparaben and distilled water. The specimens were immersed in 10 ml of 0.05% of sodium fluoride solution for 1 minute, one time per day, during 14 consecutive days.<sup>[32]</sup> Then, specimens were washed in deionized water for 10 seconds and kept in artificial saliva at 37°C.

The placebo solution was manipulated in the same way, except by the presence of sodium fluoride. The protocol used was the same mentioned above.

#### Cariogenic challenge post superficial treatment

Specimens were replaced in plastic containers and pH cycling was performed in the same way as described above, however this time, specimens were cycled for 14 days in order to simulate cariogenic severe challenge.

#### Microhardness test

After cariogenic challenge period, three readings were performed in the middle of the fragment 30 um from de edge and 100 um each other. The average of the three values hardness of each specimen was employed in the statistical analysis.

**Table 1: Microhardness values (mean and standard error) according to the superficial treatments in different experimental groups.**

Treatments	Baseline	Post-cariogenic challenges
Placebo	242.56 ± 7.32 <sup>aA</sup>	128.19 ± 6.68 <sup>aB</sup>
NaF 0.05%	251.90 ± 15.25 <sup>aA</sup>	153.59 ± 14.80 <sup>abB</sup>
Er:YAG	235.73 ± 11.41 <sup>aA</sup>	196.45 ± 33.76 <sup>bcA</sup>
Er:YAG + NaF 0.05%	259.91 ± 14.36 <sup>aA</sup>	231.92 ± 22.81 <sup>cA</sup>

*Different capital letters indicate comparison between baseline and post-cariogenic challenges Different lowercase letters indicate comparison between treatments*

Contemporary incipient caries management includes different approaches, minimal invasive ones such as fluoride therapy. However, on cariogenic challenge, the protocols for its application are not totally effective for controlling dental caries.<sup>[33]</sup> Therefore, additional methods for caries control should be applied.<sup>[34-36]</sup>

In the present study, the microhardness was decreased for those specimens that were not treated or treated only with 0.05% NaF. Incipient carious lesions are formed due the prolonged exposure to acidic environment which promote a substantially drop on the mineral content occur, leading to a mechanically weak structure that is vulnerable to permanent damage.<sup>[3,5]</sup>

Comparing groups after cariogenic challenges, the placebo group showed the lowest values, followed by 0.05% NaF-treated group. A high-risk cariogenic challenge was reproduced in this study; therefore, the time of exposure of the sodium fluoride to the enamel structure is directly related to better surface resistance to demineralization by deposition of fluorohydroxyapatite.<sup>[37]</sup> It is well known that the CaF<sub>2</sub> formation is responsible for the anticariogenic effect of

#### Statistical Analysis

Data were subjected to the two -way analysis of variance (ANOVA) and Duncan test using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA). The significance level adopted was 1%.

#### RESULTS AND DISCUSSION

**Table 1** shows the microhardness values according to the superficial treatments in different experimental groups. There were evidence of difference among the treatments (p=0.01). The effect of treatments post-cariogenic challenges were compared to initial condition (baseline). There was a decrease of microhardness for placebo and 0.05% NaF-treated group (p>0.01). Meanwhile, for the groups treated with Er:YAG laser or Er:YAG Laser+0.05% NaF, no evidence of difference was found in relation to the initial values (p>0.01).

After cariogenic challenges, comparing the experimental groups, the placebo group presented the lowest microhardness values (p>0.01). The simultaneous action of Er:YAG Laser+0.05% NaF presented the highest values of microhardness, being different of the groups placebo and 0.05% NaF (p<0.01), but similar to the Er:YAG laser-treated group (p>0.01).

topical fluoride and its formation is directly related to the concentration of fluoride applied to enamel.<sup>[15,38]</sup> These factors make 0.05% NaF, when used alone, not appropriate for an anticariogenic and remineralizing role. The simultaneous action of Er:YAG Laser+0.05% NaF showed the highest microhardness values between all treatments, however, no evidence of difference was found in between the Er:YAG Laser+0.05% NaF and Er:YAG laser-treated group. The interaction of laser irradiation with teeth causes impact on biological constituents of the dental hard tissue.<sup>[39]</sup> In that sense, in order to achieve satisfactory clinical results, it is important the determination of the appropriate laser parameters, before tooth irradiation.<sup>[21,40]</sup> The most plausible explanation for the effect of Er:YAG laser on enamel describes several proposals of mechanisms that includes structural and chemical changes to explain laser-induced prevention on enamel demineralization.

When light from the laser is absorbed by specific components of the dental enamel, is converted directly into heat. Then, carbonate is released from the heated enamel and water evaporation occurs, with the consequent formation of pyrophosphate, which has been

described as a component more stable and less soluble.<sup>[23,25,39]</sup> Another theory widely accepted attributes the effect of acid resistance of the organic matrix and the interaction with the porous complex, present on enamel.<sup>[41]</sup> Before the acid promotes dissolution of the apatite crystal within the enamel, the acid has to diffuse into the crystal surfaces through the interprismatic and intercrystalline spaces. During laser irradiation, the organic matters may block diffusion pathways and seal the enamel micropores due to partial denaturation.<sup>[22,42]</sup> On the other hand, the little variance of microhardness values between baseline and after cariogenic challenges may indicate the presence of a synergical effect of fluoride and laser, suggesting that during laser irradiation the uptake of fluoride is enhanced into the tooth crystalline structure in the form of firmly bound fluoride.<sup>[43]</sup> This findings are supported by other studies, which demonstrated high fluoride incorporation after samples irradiation, promoting beneficial effect on enamel's acid resistance.<sup>[1]</sup> It has been also observed lower surface mineral loss<sup>[25]</sup> and fewer changes on enamel microhardness<sup>[23]</sup> when the Er:YAG laser combined with another type of fluoride were evaluated.

## CONCLUSION

It can be concluded that the use of 0.05% NaF combined with the Er: YAG laser may be an alternative to control the demineralization of enamel exposed to high cariogenic challenge.

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## REFERENCES

1. Bevilacqua FM, Zezell DM, Magnani R, da Ana PA, Eduardo Cde P. Fluoride uptake and acid resistance of enamel irradiated with Er: YAG laser. *Lasers Med Sci*, 2008; 23(2): 141-147.
2. Kudiyirickal MG, Ivancaková R. Early enamel lesion part II. Histo-morphology and prevention. *Acta Medica (Hradec Kralove)*, 2008; 51(3): 151-156.
3. Mohanty B, Dadlani D, Mahoney D, Mann AB. Characterizing and identifying incipient carious lesions in dental enamel using micro-Raman spectroscopy. *Caries Res*, 2013; 47(1): 27-33.
4. Kudiyiricka MG, Ivancaková R. Early enamel lesion part I. Classification and detection. *Acta Medica (Hradec Kralove)*, 2008; 51(3): 145-149.
5. Dickinson ME, Wolf KV, Mann AB. Nanomechanical and chemical characterization of incipient in vitro carious lesions in human dental enamel. *Arch Oral Biol*, 2007; 52(8): 753-760.
6. Featherstone JD. Remineralization, the natural caries repair process--the need for new approaches. *Adv Dent Res*, 2009; 21(1): 4-7.
7. Clarkson BH, Exterkate RAM. Noninvasive dentistry: a dream or reality?. *Caries Res*, 2015; 49(1): 11-17.
8. Featherstone JD. The science and practice of caries prevention. *J Am Dent Assoc*, 2000; 131: 887-899.
9. Heravi F, Ahrari F, Mahdavi M, Basafa S. Comparative evaluation of the effect of Er: YAG laser and low level laser irradiation combined with CPP-ACPF cream on treatment of enamel caries. *J Clin Exp Dent*, 2014; 6(2): e121-126.
10. Sathe N, Chakradhar Raju RV, Chandrasekhar V. Effect of three different remineralizing agents on enamel caries formation--an in vitro study. *Kathmandu Univ Med J*, 2014; 12(45): 16-20.
11. Yin W, Hu DY, Li X, Fan X, Zhang YP, Pretty IA, Mateo LR, Cummins D, Ellwood RP. The anti-caries efficacy of a dentifrice containing 1.5% arginine and 1450ppm fluoride as sodium monofluorophosphate assessed using Quantitative Light-induced Fluorescence (QLF). *J Dent*, 2013; 41(2): S22-28.
12. Featherstone JDB. The continuum of dental caries—evidence for a dynamic process. *J Dent Res*, 2004; 83: C39-C42.
13. Weyant RJ, Tracy SL, Anselmo TT, Beltrán-Aguilar ED, Donly KJ, Frese WA, Hujoel PP, Iafolla T, Kohn W, Kumar J, Levy SM, Tinanoff N, Wright JT, Zero D, Aravamudhan K, Frantsve-Hawley J, Meyer DM. Topical fluoride for caries prevention: executive summary of the updated clinical recommendations and supporting systematic review. *J Am Dent Assoc*, 2013; 144(11): 1279-1291.
14. Comar LP, Wiegand A, Moron BM, Rios D, Buzalaf MA, Buchalla W, Magalhães AC. In situ effect of sodium fluoride or titanium tetrafluoride varnish and solution on carious demineralization of enamel. *Eur J Oral Sci*, 2012; 120(4): 342-348.
15. Saxegaard E, Rölla G. Fluoride acquisition on and in human enamel during topical application in vitro. *Scand J Dent Res*, 1988; 96(6): 523-535.
16. Ogaard B, Seppa L, Rolla G. Professional topical fluoride applications-clinical efficacy and mechanism of action. *Advances in Dental Research*, 1994; 8: 190-201.
17. Marinho VC, Worthington HV, Walsh T, Chong LY. Fluoride gels for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev*, 2015; 15(6): CD002280.
18. Rattanawiboon C, Chaweewannakorn C, Saisakphong T, Kasevayuth K, Trairatvorakul C. Effective fluoride mouthwash delivery methods as an alternative to rinsing. *Nurs Res*, 2016; 65(1): 68-75.
19. Nammour S, Demortier G, Florio P, Delhaye Y, Pireaux JJ, Morciaux Y, Powell L. Increase of enamel fluoride retention by low fluence argon laser in vivo. *Lasers Surg Med*, 2003; 33(4): 260-263.
20. Colucci V, do Amaral FL, Pécora JD, Palma-Dibb RG, Corona SA. Water flow on erbium:yttrium-aluminum-garnet laser irradiation: effects on dental tissues. *Lasers Med Sci*, 2009; 24(5): 811-818.

21. Ramalho KM, Hsu CY, de Freitas PM, Aranha AC, Esteves-Oliveira M, Rocha RG, de Paula Eduardo C. Erbium lasers for the prevention of enamel and dentin demineralization: a literature review. *Photomed Laser Surg*, 2015; 33(6): 301-319.
22. Ying D, Chuah GK, Hsu CY. Effect of Er:YAG laser and organic matrix on porosity changes in human enamel. *J Dent*, 2004; 32(1): 41-46.
23. Mathew A, Reddy NV, Sugumaran DK, Peter J, Shameer M, Dauravu LM. Acquired acid resistance of human enamel treated with laser (Er: YAG laser and Co2 laser) and acidulated phosphate fluoride treatment: An in vitro atomic emission spectrometry analysis. *Contemp Clin Dent*, 2013; 4(2): 170-175.
24. Kwon YH, Lee JS, Choi YH, Lee JM, Song KB. Change of enamel after Er: YAG and CO2 laser irradiation and fluoride treatment. *Photomed Laser Surg*, 2005; 23(4): 389-394.
25. Delbem AC, Cury JA, Nakassima CK, Gouveia VG, Theodoro LH. Effect of Er: YAG laser on CaF<sub>2</sub> formation and its anti-cariogenic action on human enamel: an in vitro study. *J Clin Laser Med Surg*, 2003; 21(4): 197-201.
26. Hara AT, Queiroz CS, Paes Leme AF, Serra MC, Cury JA. Caries progression and inhibition in human and bovine root dentine in situ. *Caries Res*, 2003; 37(5): 339-344.
27. Klein AL, Rodrigues LK, Eduardo CP, Nobre dos Santos M, Cury JA. Caries inhibition around composite restorations by pulsed carbon dioxide laser application. *Eur J Oral Sci*, 2005; 113(3): 239-244.
28. Rodrigues LK, Nobre Dos Santos M, Featherstone JD. In situ mineral loss inhibition by CO<sub>2</sub> laser and fluoride. *J Dent Res*, 2006; 85(7): 617-621.
29. Souza-Gabriel AE, Colucci V, Turssi CP, Serra MC, Corona SA. Microhardness and SEM after CO (2) laser irradiation or fluoride treatment in human and bovine enamel. *Microsc Res Tech*, 2010; 73(11): 1030-1035.
30. Featherstone JDB, O'Really MM, Shariaty M. Enhancement of remineralization in vitro and in vivo. In: Leach AS (ed). *Factors relating to demineralization and remineralization of teeth*, IRL, Oxford, 1986; 23-34.
31. Serra MC, Cury JA. The in vitro effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. *Quintessence Int*, 1992; 23(2): 143-147.
32. Duarte AR, Peres MA, Vieira RS, Ramos-Jorge ML, Modesto A. Effectiveness of two mouth rinses solutions in arresting caries lesions: a short-term clinical trial. *Oral Health Prev Dent*, 2008; 6(3): 231-238.
33. Zimmer BW, Rottwinkel Y. Assessing patient-specific decalcification risk in fixed orthodontic treatment and its impact on prophylactic procedures. *Am J Orthod Dentofacial Orthop*, 2004; 126(3): 318-324.
34. Geraldo-Martins VR, Lepri CP, Faraoni-Romano JJ, Palma-Dibb RG. The combined use of Er,Cr:YSGG laser and fluoride to prevent root dentin demineralization. *J Appl Oral Sci*, 2014; 22(5): 459-464.
35. Braga SR, de Oliveira E, Sobral MA. Effect of neodymium:yttrium-aluminum-garnet laser and fluoride on the acid demineralization of enamel. *J Investig Clin Dent*, 2015; *in press*
36. Nemezio MA, Carvalho SC, Scatolin RS, Colucci V, Galo R, Corona SA. Effect of fluoride varnish combined with Er:YAG laser on the permeability of eroded dentin: an in situ study. *Braz Dent J*, 2015; 26(6): 671-677.
37. Salehzadeh Esfahani K, Mazaheri R, Pischevar L. Effects of treatment with various remineralizing agents on the microhardness of demineralized enamel surface. *J Dent Res Dent Clin Dent Prospects*, 2015; 9(4): 239-245.
38. Ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. *Eur J Oral Sci*, 1997; 105(5 Pt 2): 461-465.
39. Apel C, Meister J, Götz H, Duschner H, Gutknecht N. Structural changes in human dental enamel after subablative erbium laser irradiation and its potential use for caries prevention. *Caries Res*, 2005; 39(1): 65-70.
40. Karandish M. The efficiency of laser application on the enamel surface: a systematic review. *J Lasers Med Sci*, 2014; 5(3): 108-114.
41. Liu Y, Hsu CY, Teo CM, Teoh SH. Subablative Er: YAG laser effect on enamel demineralization. *Caries Res*, 2013; 47(1): 63-68.
42. Maung NL, Wohland T, Hsu CY. Enamel diffusion modulated by Er: YAG laser (Part 2). Organic matrix. *J Dent*, 2007; 35(10): 794-799.
43. White DJ, Featherstone JD. A longitudinal microhardness analysis of fluoride dentifrice effects on lesion progression in vitro. *Caries Res*, 1987; 21: 502-512.