Brazilian Dental Journal (2013) 24(5): 508-512
http://dx.doi.org/10.1590/0103-6440201302292
ISSN 0103-6440

Microhardness of Enamel Adjacent to Orthodontic Brackets After CO_2 Laser Irradiation and Fluoride Application

Leonardo Pucci Stangler[1], Fábio Lourenço Romano[1], Mariana Umekita Shirozaki[1], Rodrigo Galo[2], Alessandra Marques Correa Afonso[1], Maria Cristina Borsatto[1], Mírian Aiko Nakane Matsumoto[1]

[1]Department of Pediatric Clinic, Ribeirão Preto School of Dentistry, USP - University of São Paulo, Ribeirão Preto, SP, Brazil
[2]Department of Dental Materials, Ribeirão Preto School of Dentistry, USP - University of São Paulo, Ribeirão Preto, SP, Brazil

Correspondence: Prof. Dr. Fábio Lourenço Romano, Avenida do Café, S/N, Monte Alegre, 14040-904 Ribeirão Preto, SP, Brasil. Tel: +55-16-3602-4146, 3602-4146. e-mail: fabioromano@forp.usp.br

This study evaluated the effectiveness of carbon dioxide (CO_2) laser combined or not with fluoride application on the surface microhardness of enamel adjacent to orthodontic brackets. Fifteen human molars were selected from which 30 enamel fragments measuring 4 mm^2 were obtained. The fragments were embedded in PCV tubes with acrylic resin and prepared using water abrasive paper, felt disks and alumina. Orthodontic brackets cut in half were bonded to enamel and 3 microhardness readings were performed on the adjacent surface, as follows: initial, after cariogenic challenge and final. The specimens were divided into the following 3 groups (n=10): Group C: control, Group L: irradiated with CO_2 laser, and Group FL: topical fluoride application and CO_2 laser irradiation. After initial reading, the specimens were placed in a demineralizing solution for 32 h and the second reading was to verify if demineralization was uniform in all groups. After the treatments, the specimens were submitted to DES-RE cycling for 8 days followed by final surface microhardness reading. The data were analyzed statistically using ANOVA and Duncan test (α=0.05). At the final measurement Group FL obtained higher microhardness value than Groups C and L ($p<0.05$). Groups L and FL were statistically superior to Group C ($p<0.05$). Irradiation with CO_2 laser around orthodontic brackets combined or not with topical fluoride application was effective to increase the surface microhardness of enamel.

Key Words: CO_2 laser, fluoride, demineralization, enamel.

Introduction

Direct bonding of orthodontic brackets to enamel has become an important procedure in orthodontics. This evolution has brought benefits to the orthodontist by simplifying and increasing the effectiveness of clinical procedures and to the patient by providing better esthetics and facilitated oral hygiene.

After acid etching of enamel was introduced in dentistry, all dental specialties underwent positive changes and needed adapting to the new reality (1,2). Orthodontics proposed direct bonding of orthodontic brackets to enamel surface to replace the banding.

This new technique has brought many advantages; however, it caused one problem: the increase in white spots on the enamel surface adjacent to orthodontic brackets. White spots are the result of the accumulation of biofilm around the brackets due to poor oral hygiene (1). White spot lesion is defined as sub-surface enamel porosity from carious demineralization that presents a milky-white opaque color when located on smooth surfaces (2).

White spot lesions are seen more frequently in patients who have undergone orthodontic treatment than in those receiving no treatment, and they become an esthetic problem for years after completion of treatment (3).

The main preventive measure against these lesions is good oral hygiene and the use of fluoride toothpaste. Other preventive measures are oral mouthwashes, varnishes, and adhesives. It is also important to assess the patient's risk of caries to implement an efficient oral hygiene regimen adjusted for each case in particular (2).

One way to prevent white spot lesions is to apply pit and fissure sealants on the enamel surface around the orthodontic brackets. Several studies have shown that the lack of patient cooperation is a critical factor in the control of white spots (1,2,4,5).

A few new methods have appeared to assist the orthodontist, such as the fluoride-releasing orthodontic adhesives. According to Passalini et al. (6), certain composites, such as those containing fluorides, are effective to prevent white stains around orthodontic brackets and they are indicated for patients with a high level of susceptibility to caries lesions.

Another method is the carbon dioxide (CO_2) laser, developed by Patel in 1964 (7). According to Rodrigues et al. (8), this type of laser seems to be more appropriate to prevent caries. Previous studies have shown that the CO_2 laser increases enamel and dentin resistance to caries by reducing demineralization (9,10). However, further studies must be made to evaluate the effectiveness of the device to act as a caries-preventive or remineralizing agent against

white spots around orthodontic brackets.

The aim of this study was to test the null hypothesis that CO_2 laser irradiation combined or not with fluoride application influences surface microhardness of enamel adjacent to orthodontic brackets after cariogenic challenge.

Material and Methods

This study was approved by the institutional Ethics Committee under protocol #03158312.4.0000.5419.

Fifteen healthy human permanent maxillary and mandibular molars, without any cracks or fractures, which had not been submitted to any chemical, physical or orthodontic treatment, were selected. Thirty enamel slabs (4 mm wide, 4 mm high and 2 mm thick) were obtained from these teeth and embedded in self-curing acrylic resin in the center of PVC cylinders (20 mm in diameter and 4 mm high), as follows: the buccal surface of the fragments was placed as close as possible and fixed with wax on the glass plate. Then the plastic tubes were placed on the fragments and acrylic resin was poured until the tube was completely full.

After resin polymerization, the buccal surface of the tooth fragments was flattened with wet abrasive papers of increasing grits (#500, #600 and #1200; Buehler Ltd., Lake Bluff, IL, USA) and polished with felt discs embedded in aluminum oxide pastes (1 µm, 0.3 µm and 0.05 µm) in a polishing machine (Politriz DP-9U2; Struers A/S, Copenhagen, Denmark), until the surface was smooth and free of scratches. After this stage, the bonding area of the bracket was delimited using adhesive tape (Adelbras, Vinhedo, SP, Brazil).

The test specimens were placed in a microhardness tester (HMV-2, Shimadzu, Kyoto, KY, Japan) where the initial surface hardness of enamel was measured previously in determined areas. The microhardness tester was calibrated for a load of 25 g for 10 s.

A total of three microhardness readings were performed: initial, after cariogenic challenge and final. On each test specimen, three readings were performed at different pre-determined points adjacent to the bracket. These points were at 1.5 mm from the upper margin of the enamel with a gap of 0.5 mm from each other in the vertical direction and 1 mm from the bonding area along the enamel surface. The three values obtained in each specimen were averaged and a mean value was obtained for each test specimen.

After the tape was removed, prophylaxis was performed on the protected sanded and polished surface with a rubber cup driven by a low speed motor and pumice paste and fluoride-free water for 10 s, followed by rinsing for 10 s and drying with an oil- and moisture-free triple syringe for 10 s. Then enamel was etched with 37% phosphoric acid (37 Condac; FGM, Joinville, Brazil) for 15 s, washed for 15 s and dried for 15 s. A thin layer of bonding agent XT primer (3M Unitek, Monrovia, CA, USA) was applied followed by light jets of air to spread the material. The mandibular central incisor edgewise bracket (Slim; Morelli, Sorocaba, SP, Brazil) cut into the middle to occupy only a small area of the tooth fragment, was bonded onto this surface with Transbond XT (3M Unitek). Prior to the bonding procedures, the area around the bracket was isolated using adhesive tape (Adelbras), leaving only the exposed enamel received the adhesive to prevent the prophylactic procedure, enamel etching and excess of the bonding agent and composite from invading the reading area.

After bonding procedure, the test specimen received a layer of synthetic enamel (Niasi, Taboão da Serra, SP, Brazil) in the region of the PVC tube and acrylic resin, leaving exposed only the reading area. The purpose of the enamel was to delimit the same area exposed for all the test specimens, as the amount of demineralizing solution is calculated by the area exposed to demineralization, and to make it impermeable to prevent ion exchange with the solution. The specimens were placed individually in a plastic container with the demineralizing solution containing 1.4 mM Ca, 0.91 mM P, 0.06 µg F/mL, pH 5.0 and kept in an oven at 37 °C for 32 h. After this period, they were washed in distilled water, the brackets were removed and the specimens were placed in the microhardness tester to measure enamel surface microhardness in the same region where the initial reading was performed. The aim of the intermediate reading was to verify whether the specimens in all groups showed similar leveling of microhardness values.

The specimens were randomly divided into 3 groups (n=10): Group 1 - control (C) received no treatment; Group 2 (L) received CO_2 laser irradiation (Shanghai Jue Hua Technology Development, Shanghai, China) at an output power of 0.5 W for 20 s, and Group 3 (FL) received topical fluoride (1.23% acidulated phosphate fluoride) applied with cotton swabs for 1 min, followed by the removal of excess fluoride with a paper towel and CO_2 laser irradiation at output power of 0.5 W for 20 s.

After the treatment performed in each group, the specimens were subjected to DES-RE cycling regimen for 8 days. This procedure was performed to simulate oral conditions. Each specimen was stored individually in a plastic container and placed in a demineralizing solution containing 1.4 mM Ca, 0.91 mM P, 0.06 mg F/mL, pH 5.0, for two hours every day and in a remineralizing solution containing 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 mg F/mL, 0.1 M TBS, pH 7.0, for 22 h. On the fourth day of cycling, the demineralizing and remineralizing solutions were replaced by new solutions. The solution was changed for each specimen, and at the end of cycling (8^{th} day) the specimens were washed in distilled water. After this,

final microhardness measurements of the enamel surface adjacent to the bracket were performed exactly in the same area as the previous readings. ANOVA and Duncan's test were used for statistical analysis (α=0.05). All the methods used are shown in Figure 1.

Results

The mean microhardness values of enamel adjacent to the orthodontic bracket in the different groups (C, L and FL) - measured during the 3 stages of the experiment – initial, after cariogenic challenge, and final - are described in Table 1.

Table 1 shows a statistically significant reduction of the mean values of the initial surface microhardness of enamel microhardness after cariogenic challenge. This reduction was not maintained in the final readings, where

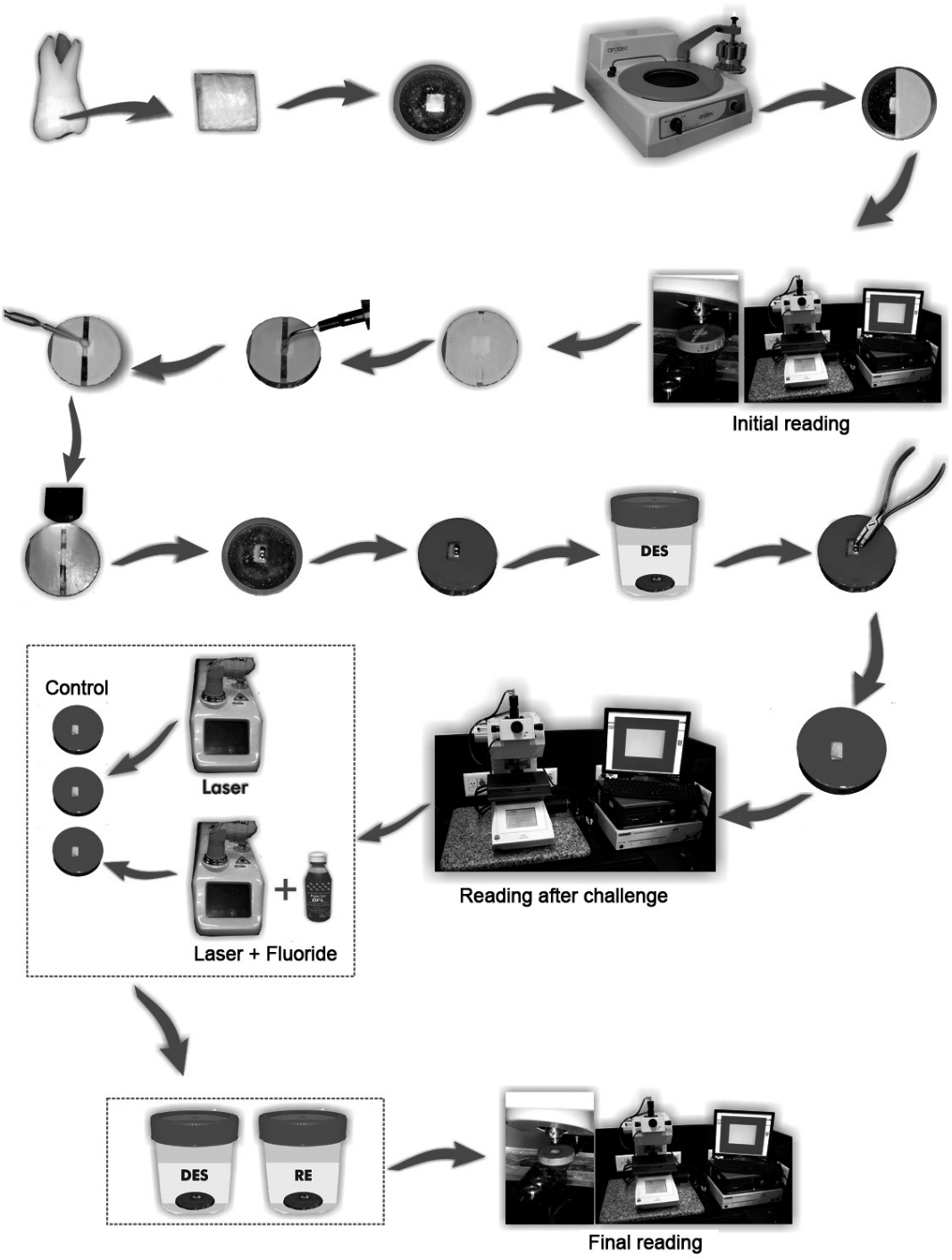

Figure 1. Flowchart of the methodology.

the microhardness values increased in the group FL and remained similar in group L.

The initial enamel microhardness was compared among the groups and no statistically significant difference was observed (p=0.770). It also occurred after cariogenic challenge (p=0.985). The lack of difference shows a uniform pattern of enamel in all specimens during the two readings, which confirms the leveling of the sample. However, this was not repeated in the final microhardness values and statistically significant differences were found among the groups (p=0.042).

The last measurement showed that the Group FL was statistically superior to the other groups (p<0.05). The groups that received treatment - Groups L and FL - were statistically superior to Group C (p<0.05).

Discussion

It is known that orthodontic treatment increases the risk of white spot lesions. According to Gorton and Featherstone (11), approximately 50% of patients present clinically visible white spot lesions during treatment for approximately 2 years. There is a considerable number of studies in the area of prevention (1,5,12-14) and treatment of white spot lesions (15,16). One way to assess changes in enamel is by surface microhardness analysis.

The use of fluoride is one of the most studied, known and effective methods to prevent dental caries (5). Much of the success attributed to fluoride is due to its capacity of reversing the beginning and progression of caries (17).

The application of CO_2 laser on the enamel surface has been studied with great interest since the 1970s. Studies show that this type of laser causes structural and ultrastructural changes in enamel (18,19). There are several explanations in the literature about how reactivity occurs in the enamel treated with CO_2 laser (20). One explanation is that the lower permeability of enamel results from the fusion of microparticles on its surface (9). Another explanation is that the relation between decreased permeability with melting, fusion and recrystallization of the enamel particles creates a barrier on the tooth surface (21). Thus, the ideal procedure is that the CO_2 laser be used before the lesion is established.

The efficacy of fluoride in combination with CO_2 laser to prevent demineralization is being extensively studied (10). This interaction must be indicated for patients at high risk of developing caries (22). However, further studies must be conducted to evaluate its effectiveness to treat white spot lesions around orthodontic brackets.

The results of this study showed that CO_2 laser irradiation combined with the topical fluoride application was effective to increase enamel microhardness. The same occurred when only laser was used, but the fluoride and laser association was superior. These results support previous studies that concluded that the combination of the CO_2 laser and fluoride is more effective in inhibiting caries than when applying the CO_2 laser only (8,23,24). However, there is no uniformity in the methodology and evaluation of the characteristics of enamel. A previous study (23) found that the combination of laser and fluoride inhibits caries by the percentage of mineral loss. The present study evaluated effectiveness of the treatments by measuring enamel surface microhardness after the lesion established. Souza-Gabriel et al. (24) also measured enamel microhardness by comparing the effects of CO_2 laser and other sources of fluoride to inhibit the progression of lesions in enamel using other methodology. Tepper et al. (25) evaluated the effect of combining CO_2 laser with amine fluoride solution for inhibiting demineralization and, although no statistically significant difference was found, the authors believe that there was synergy among treatments. According to Steiner-Oliveira et al. (22), CO_2 laser alone or combined with fluoride produces effective protection against demineralization but, on the other hand, the laser treatment associated with fluoride showed no significant inhibitory effect on demineralization.

Direct comparisons between this study and studies in the literature could not be made due to the differences in the methodology used to assess the effects of CO_2 laser combined or not with fluoride in the treatment of surfaces submitted to cariogenic challenge. The published studies emphasize the effectiveness of CO_2 laser and fluoride in preventing structural damages to the enamel, but not as a remineralizing agent.

The null hypothesis was not confirmed. The CO_2 laser treatment alone or associated with fluoride was effective in increasing surface microhardness of enamel adjacent to orthodontic brackets.

Table 1. Mean values (standard deviation) and statistical comparison of enamel microhardness

Groups	Initial reading	Reading after cariogenic challenge (leveling)	Final reading
Control (C)	264.80 (4.61)^{Aa}	99.20 (11.05)^{Ba}	84.00 (11.6)^{Ca}
Laser (L)	263.40 (3.94)^{Aa}	100.7 (10.3)^{Ba}	98.10 (6.1)^{Bb}
Fluoride + Laser (FL)	260.20 (5.14)^{Aa}	101.80 (10.9)^{Ba}	129.60 (16.8)^{Cc}

Same lowercase letters in rows and uppercase letters in columns indicate no statistically significant difference at p<0.05.

Resumo

Este estudo avaliou a eficácia do laser de CO_2, associado ou não à aplicação de flúor na microdureza superficial do esmalte

dentário adjacente a bráquetes ortodônticos. Foram selecionados 15 molares humanos, dos quais 30 fragmentos de esmalte com 4 mm^2 foram obtidos. Os fragmentos foram incluídos em tubos de PVC, contendo resina acrílica, preparados usando lixas d'água e discos de feltro e alumina. Bráquetes ortodônticos cortados ao meio foram colados no esmalte e 3 leituras de microdureza foram realizadas na superfície adjacente: inicial, após desafio cariogênico e final. Os espécimes foram divididos em 3 grupos (n=10): Grupo C - Controle, Grupo L - irradiado com laser de CO_2 e Grupo FL - aplicação tópica de flúor e irradiação com laser de CO_2. Após leitura inicial, os espécimes foram colocados em solução desmineralizadora por 32 h e a segunda leitura foi realizada para verificar se desmineralização foi uniforme em todos os grupos. Após os tratamentos, os espécimes foram submetidos a ciclagem DES-RE durante 8 dias seguida da leitura da microdureza superficial final. Os dados foram analisdos estatisticamente utilizando ANOVA e o teste de Duncan (α=0,05). Na mensuração final o grupo FL obteve maior valor de microdureza que os grupos C e L ($p<0,05$). Os grupos L e FL foram estatisticamente superiores ao grupo C ($p<0,05$). A irradiação de laser de CO_2 ao redor de bráquetes ortodônticos combinadas ou não à aplicação tópica de flúor foi eficaz no aumento da microdureza superficial do esmalte.

References

1. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spots lesions in orthodontic practice: a contemporary review. Aust Dent J 2006;51:284-289.
2. Bishara SE, Otsby AW. White spot lesions: Formation, prevention and treatment. Semin Orthod 2008;14:174-182.
3. Ogaard B. Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment. Am J Orthod Dentofacial Orthop 1989;96:423-427.
4. SantosRL, Pithon MM, Fernandes ABN, Carvalho FG, Cavalcanti AL, Vaitsman DS. Fluoride release/uptake from different orthodontic adhesives: a 30-month longitudinal study. Braz Dent J 2013;24:410-414.
5. Geiger AM, Gorelick L, Gwinnett AJ, Groswold PG. The effect of a fluoride program on white spot formation during orthodontic treatment. Am J Orthod Dentofacial Orthop 1988;93:29-37.
6. Passalini P, Fidalgo TKS, Caldeira EM, Gleiser R, Nojima MCG, Maia LC. Preventive effect of fluoridated orthodontic resins subjected to high cariogenic challenges. Braz Dent J 2010;21:211-215.
7. Patel CKN. Selective excitation through vibrational energy transfer and optical maser action in N_2-CO_2. Phys Rev Lett 1964;13:617-619.
8. Rodrigues LKA, dos Santos MN, Pereira D, Assaf AV, Pardi V. Carbon dioxide laser in dental caries prevention. J Dent 2004;32:531-540.
9. Stern RH, Vahl J, Sognnaes RF. Lased enamel: Ultraestructural observations of pulsed carbon dioxide laser effects. J Dent Res 1972;51:455-460.
10. Featherstone JDB, Barret-Vespone NA, Fried D, Kantorowitz Z, Seka W. CO_2 laser inhibition of artificial caries-like lesion progression in dental enamel. J Dent Res 1998;77:1397-1403.
11. Gorton J, Featherstone JDB. *In vivo* inhibition of demineralization around orthodontic brackets. Am J Orthod Dentofacial Orthop 2003;123:10-14.
12. Wenderoth CJ, Weinstein M, Borislow AJ. Effectiveness of a fluoride–releasing sealant in reducing decalcification during orthodontic treatment. Am J Orthod Dentofacial Orthop 1999;116:629-634.
13. 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;126:318-324.
14. Kronenberg O, Lussi A, Ruf S. Preventive effect of ozone on the development of white spot lesions during multibracket appliance therapy. Angle Orthod 2009;79:64-69.
15. Willmot D. White spot lesions after orthodontic treatment. Semin Orthod 2008;14:209-219.
16. Bröchner A, Christensen C, Kristensen B, Tranaeus S, Karlsson L, Sonnesen L, et al.. Treatment of post-orthodontic white spot lesions with casein phosphopeptide-stabilised amorphous calcium phosphate. Clin Oral Invest 2011;15:369-373.
17. ten Cate JM, Featherstone JDB. Mechanistic aspects of the interactions between fluoride and dental enamel. Crit Rev Oral Bio Med 1991;2:283-296.
18. Tagliaferro EPS, Rodrigues LKA, Soares LES, Martin AA, Nobre-dos-Santos M. Physical and compositional changes on demineralized primary enamel induced by CO_2 laser. Photomed Laser Surg 2009;27:585-590.
19. González-Rodríguez A, López-González J, del Castillo JDL, Villalba-Moreno J. Comparison of effects of diode laser and CO_2 laser on human teeth and their usefulness in topical fluoridation. Lasers Med Sci 2011;26:317-324.
20. Corrêa-Afonso AM, Bachmann L, Almeida CA, Corona SAM, Borsatto MC. FTIR and SEM analysis of CO_2 laser irradiated human enamel. Arch Oral Biol 2012;57:1153-1158.
21. Nelson DG, Wefel JS, Jongebloed WL, Featherstone JBD. Morphology, histology and crystallography of human dental enamel treated with pulsed low-energy infrared laser radiation. Caries Res 1987;21:411-426.
22. Steiner-Oliveira C, Rodrigues LKA, Lima EB, Nobre-dos-Santos M. Effect of the CO_2 laser combined with fluoridated products on the inhibition of enamel demineralization. J Contemp Dent Pract 2008;9:1-10.
23. Tagliaferro EPS, Rodrigues LKA, dos Santos MN, Soares LES, Martin AA. Combined effects of carbon dioxide laser and fluoride on demineralized primary enamel: an in vitro study. Caries Res 2007;41:74-76.
24. Souza-Gabriel AE, Colucci V, Turssi CP, Serra MC, Corona SAM. Microhardness and SEM after CO_2 laser irradiation or fluoride treatment in human and bovine enamel. Microsc Res Tech 2010;73:1030-1035.
25. Tepper SA, Zehnder M, Pajarola GF, Schmidlin PR. Increased fluoride uptake and acid resistance by CO_2 laser-irradiation through topically applied fluoride on human enamel *in vitro*. J Dent 2004;32:635-641.

Received July 13, 2013
Accepted September 3, 2013