UNESP - UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE ODONTOLOGIA DE ARARAQUARA

INFLUÊNCIA DO TRATAMENTO DE SUPERFÍCIE NA ADAPTAÇÃO EXTERNA DE CAVIDADES CLASSE V HÍGIDA E AFETADA POR CÁRIE SUBMETIDAS À CICLAGEM TÉRMICA.

Mateus Rodrigues Tonetto

Araraquara
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Orientador:

Prof. Dr. Marcelo Ferrarezi de Andrade

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3º Examinador: Prof. Dr. Matheus Coelho Bandéca

Araraquara, 27 de Julho de 2012.

Mateus Rodrigues Tonetto

DADOS CURRICULARES

Nascimento	04/02/1988 – Junqueirópolis - SP
Filiação	Sebastião Aparecido Tonetto Sônia Magoga Rodrigues Tonetto
2006 a 2009	Graduação em Odontologia pelo Centro Universitário de Araraquara – UNIARA
2010 a 2012	Curso de Pós Graduação em Ciências Odontológicas, Área de Concentração Dentística, nivel Mestrado, na Faculdade de Odontologia de Araraquara – FOAr – UNESP

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À **Deus** por não ter me abandonado nos momentos mais difíceis dessa caminhada, sempre me iluminando e guiando em todas minhas decisões.

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SUMÁRIO

Resumo

Tonetto MR. Influência do tratamento de superfície na adaptação externa de cavidades classe V hígida e afetada por cárie submetidas à ciclagem térmica [Dissertação de Mestrado]. Araraquara: Faculdade de Odontologia, Universidade Estadual Paulista; 2012.

Resumo

O presente trabalho propôs avaliar a utilização de clorexidina e laser de Er,Cr:YSGG no processo de adaptação externa em restaurações de cavidades hígidas e afetadas por cárie submetidas a ciclagem térmica. Foram realizados preparos cavitários mistos de classe V vestibular em 36 molares humanos, dos quais metade foram induzidos artificialmente à cárie. Os dentes foram subdivididos em 6 grupos, cavidade hígida: sem tratamento prévio, utilização de clorexidina e utilização de laser de Er,Cr:YSGG e cariados: sem tratamento prévio, utilização de clorexidina e utilização de laser de Er,Cr:YSGG. Foi empregado o sistema adesivo Single Bond 2 (3M ESPE, St. Paul, MN, EUA) e resina composta Filtek Z350 XT (3M ESPE, St. Paul, MN, EUA). Os espécimes foram submetidos a 3000 ciclos térmicos (5-55° C em banhos de 60 segundos). Para avaliação da adaptação externa foram obtidas réplicas de epóxi pela impressão da superfície das restaurações. Foi realizada a análise quantitativa da adaptação externa, tanto em dentina hígida quanto afetada, por meio de réplicas de epóxi, utilizando MEV. Os valores porcentuais de margem contínua mostraram que não houve diferença estatisticamente significante em relação aos diferentes tratamentos de superfície e a ciclagem térmica pode influenciar nos valores de adaptação quando comparado os valores antes e após a ciclagem.

Descritores: Resinas compostas, clorexidina, laser, cárie dentária, Microscopia Eletrônica de Varredura.

Abstract

Tonetto MR. Influence of surface treatment on the external adaptation of class V healthy and caries affected cavities submited to thermal cycling [Dissertation]. Araraquara: Faculdade de Odontologia, Universidade Estadual Paulista; 2012.

Abstract

The present study proposed to evaluate the use of chlorhexidine and laser Er,Cr:YSGG in the process of external adaptation in mixed healthy and caries affected cavities subjected to thermal cycling. Mixed class V cavity preparations were performed on the buccal surface of 36 human molars, Half of them were artificially caries induced. After this procedure, the teeth received different forms of surface treatment: without previous treatment, use of chlorhexidine and the use of Er,Cr:YSGG. Adhesive system Single Bond 2 and Z350 XT composite resin were used to restoration. The specimens were subjected to 3000 thermal cycles (5-55°C 60 seconds). Epoxy replicas were obtained and then observed under SEM to verify the external adaptation. It was concluded that the application of laser and chlorhexidine did not affect the percentages of marginal adaptation of Class V restorations. Furthermore, thermocycling may influence adaptation values

Keywords: Composite resins, chlorhexidine, laser, dental caries, Scanning Electronic Microscopy.

Introdução

INTRODUÇÃO

A odontologia restauradora vem evoluindo continuamente com o surgimento de novos materiais e técnicas cada vez mais avançadas, porém, ainda existem limitações quando se refere a um bom selamento marginal. A busca por um material que promova um vedamento ideal da cavidade oral é tema de grande parte dos estudos, sendo esta propriedade uma das mais importantes para o sucesso clínico de uma restauração.

Quando nos referimos à adesão, a maior preocupação se deve ao substrato dentinário, devido sua composição aquosa em contrapartida às características hidrofóbicas que compõem os monômeros resinosos. Neste sentido, busca-se o aprimoramento da adesão dos materiais restauradores à dentina desde o estabelecimento da técnica por meio da formação da camada híbrida[24].

Fatores intrínsecos do substrato dentinário, como a quantidade de dentina intertubular e túbulos dentinários, a formação de dentina reacionária e umidade da dentina podem influenciar o mecanismo de união dos materiais restauradores[19]. Pesquisas mostram que após a remoção do tecido cariado, há permanência de bactérias na parede dos preparos cavitários[3,10,40], o que pode contribuir tanto para formação de cáries recorrentes quanto interferirem na adesão de materiais restauradores, aumentando a desadaptação e microinfiltração marginal das restaurações adesivas.

Apesar dos diversos métodos de prevenção, a cárie dental é uma das infecções mais prevalentes na cavidade oral[5], e quando acomete o substrato dentinário, ocorrem alterações nas características físicas, químicas e biológicas que podem influenciar a estabilidade de união resina-dentina ao longo do tempo[55], sendo esta, uma condição clínica frequênte. Embora muitos estudos de adesão serem realizados em dentina hígida, a maioria dos preparos cavitários apresentam substrato dentinário alterado pelo processo carioso[33], observando-se uma camada híbrida mais espessa e maior exposição de fibrilas colágenas na base da camada híbrida[17,53], o que afeta negativamente a adesão de materiais a esse substrato.

A heterogeneidade estrutural e composicional do substrato dentinário representam um grande desafio para a odontologia adesiva[12], principalmente no que se refere a estabilidade longitudinal das interfaces produzidas neste substrato[35]. Quando são comparados substratos diferentes, como dentina hígida e afetada por cárie, suas particularidades se tornam cada vez mais evidentes[25,55].

Devido a fenômenos químicos e fisiológicos que acontecem durante a evolução do processo carioso, a zona intertubular da dentina afetada por cárie apresenta menor conteúdo mineral[26,53,56,58], enquanto que cristais resistentes à dissolução ácida são depositados no interior dos túbulos dentinários, reduzindo a permeabilidade desse tecido[29,44]. Essa obstrução tubular representa um mecanismo de defesa contra a invasão bacteriana, além de limitar a penetração de monômeros resinosos e a formação dos tags de resina[56]. O conjunto dessas estruturas com a camada híbrida representa as principais e mais efetivas formas de adesão das resinas à dentina[31,51].

A adesão da resina à dentina ocorre por meio da infiltração e polimerização de resinas hidrofílicas na malha de colágeno exposta em função da aplicação de ácido[24], formando uma camada híbrida. A dentina afetada por cárie apresenta maior profundidade de desmineralização, dificultando a impregnação dos monômeros resinosos. Entretanto, tem sido observada a discrepância entre a profundidade de desmineralização e infiltração dos monômeros resinosos levando a manutenção de fibrilas de colágeno desprotegidas dos monômeros, resultando na penetração de flúidos nas porosidades, denominado de nanoinfiltração[38], fenômeno que acelera a degradação da união resina dentina. As restaurações adesivas devem promover além de um vedamento imediato da interface entre o material restaurador e a estrutura dental, garantir a durabilidade da união aos tecidos dentários. Dessa forma, existem várias alternativas que visam produzir interfaces adesivas menos susceptíveis à biodegradação imposta pela cavidade oral. Mesmo não havendo um total conhecimento em relação ao mecanismo de deterioração da interface adesiva, sabe-se que as metaloproteinases da matriz dentinária (MMPs) são famílias de enzimas proteolíticas capazes de degradar a matriz orgânica da dentina desmineralizada, atuando nas fibrilas de colágeno expostas na base da camada híbrida decorrentes da discrepância entre a profundidade de desmineralização ácida da dentina e a infiltração monomérica durante os procedimentos adesivos[13,42].

Tem sido demonstrado que soluções de clorexidina, além da ampla utilização como agente antimicrobiano, têm a capacidade de inibir a atividade de MM-2 (gelatinase-A) e MM-9 (gelatinase-B)[15]. A clorexidina a 2% em solução aquosa, sobre a dentina condicionada, previamente ao uso de adesivos, apresenta bons resultados como agente coadjuvante no processo de adesão polimérica ao substrato dentinário[6], e não interfere negativamente no desempenho adesivo imediato[7,11,32,36] e ao longo do tempo[4,7,11,18].

Outro tratamento de superfície que poderia ser viável como um coadjuvante no procedimento adesivo é laser. O laser de érbio pode minimizar a susceptibilidade ao ataque ácido e ação cariogênica por conduzir à formação de compostos solúveis mais estáveis,[50] e além disso pode proporcionar restaurações com maior resistência de união e ser capaz de melhorar a longevidade pois, produz características adequadas para adesão, como irrugularidade superficial, ausência de *smear layer* e abertura dos túbulos dentinários. A aplicação do laser na odontologia vem sendo estudada quanto a sua interação com os tecidos dentais,[43] e as pesquisas revelam que os lasers de érbio podem ser empregados para condicionamento dos tecidos duros dentais, remoção seletiva de tecido cariado e preparo cavitário.[23,37] As características produzidas tanto pelo laser de Er;Cr,YSGG (2,78 µm) quanto Er,YAG (2,94 µm) são comparáveis, provocando alterações morfológicas no esmalte que o tornam mais ácido resistente, podendo prevenir a ação de cárie,[8,14,22] além do aumento da rugosidade da superfície dentinária, abertura dos túbulos dentinários sem remoção da *smear layer* e efeito de descontaminação cavitária.[2,39]

Para uma melhor reprodução da função clínica, o envelhecimento artificial por meio de ciclos térmicos é uma opção viável e relevante[52]. As amostras são submetidas às variações de temperatura, ao extremo frio e quente, como forma de simular o estresse térmico que ocorre na cavidade oral[21,28,47,49].

Quanto ao número de ciclos, temperatura e tempo de permanência em cada uma das temperaturas, varia muito entre os pesquisadores, não havendo um consenso geral sobre o emprego da ciclagem térmica[34].

Considerando o grande número de falhas em restaurações, que é maior no segmento posterior comparado ao anterior[20,48], é importante lembrar as condições a que estão expostas, o que inclui a alteração de temperatura[27,41,57]. Além disso, a ciclagem térmica influencia na microinfiltração marginal das restaurações pelo alto coeficiente de expansão térmica linear, o que promove a contração e a expansão da restauração de forma diferente do que ocorre na estrutura dentária natural, formando “*gaps*” na interface dente/restauração[9,28,30].

Dessa forma, após aprovação do Comitê de Ética em Pesquisa (Anexo), o presente estudo propôs investigar o efeito do laser Er,Cr:YSGG exerce na adaptação externa de restaurações classe V submetidas à ciclagem térmica, em dentina hígida e afetada por cárie.

Proposição

PROPOSIÇÃO

O objetivo deste trabalho é avaliar:

Artigo 1 - O efeito que o laser Er,Cr:YSGG e clorexidina exercem na adaptação externa de restaurações classe V submetidas à envelhecimento artificial, em dentina hígida e afetada por cárie.

Artigo 2 - Se o envelhecimento artificial por ciclagem térmica influencia na adaptação marginal de restaurações classe V mistas com/sem utilização de clorexidina no processo de adesão.

Capítulo 1

ARTIGO 1

Effects of Er,Cr:YSGG laser irradiation on external adaptation of restorations in caries-affected cavities.

Mateus Rodrigues Tonetto, DDS, MSc[a], Matheus Coelho Bandéca, DDS, PhD[b], José Roberto Cury Saad, DDS, PhD[c], Edson Alves de Campos, DDS, PhD[c], Sizenando de Toledo Porto Neto, DDS, PhD[c], Marcelo Ferrarezi de Andrade, DDS, PhD[c]

[a] PhD Student of Restorative Dentistry, School of Araraquara Dentistry, University of São Paulo State, Araraquara, Brazil.

[b] Professor of Dentistry, University of CEUMA, São Luíz, Brazil.

[c]Professor of Dentistry, School of Araraquara Dentistry, University of São Paulo State, Araraquara, Brazil.

ABSTRACT

This study evaluated the effect of Er,Cr:YSGG laser on the external adaptation of composite resin restorations in caries-affected cavities. Mixed class V cavity preparations were performed in 36 intact human third molars, in half of which caries was artificially induced. Both healthy and carious dentin were etched with 35% phosphoric acid (Ultradent Products Inc., South Jordan, Utah, USA), and the teeth were divided into three groups, i.e., (a) untreated etched dentin, (b) application of Er,Cr:YSGG laser and (c) use of chlorhexidine as an adjunct in the bonding process. Restorations were fabricated with Z350 XT FiltekTM composite resin (3M ESPE) and subsequently the specimens were subjected to thermocycling to simulate artificial aging. Quantitative analysis of external adaptation was performed by scanning electron microscopy (SEM) in both healthy and affected dentin using epoxy resin replicas. It was concluded that the application of laser and chlorhexidine did not affect the percentages of marginal adaptation of Class V restorations. Furthermore, thermocycling may influence adaptation values.

Keywords: dentin adhesives, dental caries, chlorhexidine, laser, dentin

INTRODUCTION

Restorative dentistry is constantly evolving thanks to the emergence of increasingly advanced new materials and techniques, but there are still limitations when it comes to good marginal sealing. The search for materials and techniques capable of producing optimal sealing of the cavity is the subject of many studies given that this property is of paramount importance to ensure clinically successful restorations. Adhesive restorations should not only produce immediate sealing of the interface between restorative material and tooth structure, but should also ensure a long-term bond strength to dental tissues.

In this context, there are some materials and techniques that minimize the degradation caused by the oral environment. The literature is replete with studies that evaluate chlorhexidine, which has proven effective as an adjuvant agent in the process of polymeric adhesion to the dentin substratum [1]. Moreover, it does not impair immediate and long-term adhesive performance [2-4]. Erbium laser can reduce susceptibility to etching and cariogenic action, and lead to the formation of soluble compounds with increased stability [5]. Furthermore, it can also produce restorations with greater bond strength, besides enhancing longevity, as it features adhesion-inducing properties, such as surface irregularities, absence of smear layer and opening of dentinal tubules.

Since 1964 the application of lasers in dentistry has been studied as regards its interaction with dental tissues [6], with research showing that erbium lasers can be used for etching hard dental tissues, obtaining cavity preparation selectively removing decayed tissue [7, 8]. The features produced by both Er;Cr:YSGG (2.78 mM) and Er:YAG (2.94 mM) are comparable, leading to morphological changes that render the enamel more acid resistant. Both lasers can also avert the effects of decay [9], in addition to increasing the roughness of the dentin surface and opening dentinal tubules without removing the smear layer or producing a cavity decontamination effect [10]. Another benefit provided by erbium laser is that it does not cause pulp heating or vibration, making this technique even more attractive clinically [11]. The temperature rise occurring inside the pulp chamber after application of erbium laser may be lower than 5.5°C [12]. Er;Cr:YSGG and Er:YAG lasers are the most resonant with hydroxyapatite and water, allowing cavities and surface etching to be performed with minimal injury to surrounding tissues and pulp [13], a microretentive pattern similar to what occurs in acid etching .

The safest method to ensure adequate bond strength is the micro-mechanical retention of resin materials in micro-porosities created by acid etching [14, 15]. According to studies that evaluate the bond strength and quality of the restorations, erbium laser shows favorable results [16]. Others have shown that laser does not facilitate adhesive procedures and may even prove harmful [17]. This study suggests a better external adaptation with increased long-term stability of cavities prepared with the Er,Cr:YSGG, after etching with phosphoric acid, as well as chlorhexidine.

Thus, the present study aimed to investigate the effects that Er,Cr:YSGG and chlorhexidine have on the external adaptation of class V restorations - in healthy vs. carious dentin - subjected to artificial aging.

MATERIAL AND METHODS:

Tooth selection and Cavity preparation:

Thirty-six extracted human third molars of human teeth were used. All teeth were caries-free and kept in 0.1% thymol at 4ºC until ready for use.

Initially, standard mixed class V cavities were prepared on the buccal surfaces of all 36 teeth involving the cervical third of each tooth, using a spherical diamond bur. Each point was replaced by a new one after preparation of four cavities. Cavity dimensions were 2.0 mm depth, 2.0 mm gingivoincisal and 4.0 mm mesiodistal.

The cavities were standardized with the aid of an adhesive template, millimeter probe, rubber stop and digital caliper. Each was checked for marginal imperfections, such as fractures or cracks using a stereoscopic microscope (Model SZXL, Oympus, Sao Paulo, Brazil) with 20x magnification.

Artificial induction of carious lesions:

The 36 specimens were divided into two groups, and one of them was covered with a layer of epoxy adhesive (ARALDITE, Ciba Especialidades Químicas Ltda., São Paulo, Brazil) and nail polish (Colorama, CEIL Com. Ind. Exp. Ltda, São Paulo, Brazil) along their entire length, except for 1 mm around the cavity. After sterilization, the specimens were suspended in a cariogenic solution comprised of 3.7 g of BHI broth (Brain Heart Infusion, Becton Dickinson and Company - USA), 2 g of sucrose (Synth; Labsynth, Sao Paulo, Brazil), 1 g of glucose (Synth; Labsynth, São Paulo, Brazil) and 0.5 g of yeast extract (Becton Dickinson and Company, Sparks, MD, USA) per 100 ml of distilled water, and autoclaved at 120^{o} C for 20 minutes. Thereafter, strains of Streptococcus mutans were inoculated (5 mL for every 108 CFU/mL), and the teeth were sterilized and placed in a solution (25 mL/tooth) in a dry heat oven for 14 days, with the cariogenic medium being changed every 48 hours. After caries incubation, all plaque on the teeth was removed with sterile gauze and the epoxy adhesive and enamel were removed with a scalpel blade. The teeth were then rinsed thoroughly with water.

Both groups - healthy teeth (n=18) and carious teeth (n=18) - were randomly divided into three groups: Without surface treatment, application of Er,Cr:YSGG laser and application of chlorhexidine.

Chlorhexidine group

` The groups that received chlorhexidine as a surface treatment after etching with 35% Ultra-Etch phosphoric acid (Ultradent Products Inc., South Jordan, Utah, USA) for 15 s on the dentin and 30 s on the enamel had their surface washed with distilled water for 10 seconds and were then dried with absorbent paper to ensure a moist surface. Subsequently, 20 µL of a solution (2% chlorhexidine) was applied passively with the aid of a micropipette, covering the whole dentin surface for 60 seconds. All excess was removed with absorbent paper to ensure a moist appearance.

Laser group

After phosphoric acid etching, rinsing the surface with distilled water and drying with absorbent paper to obtain a moist surface, application of Er,Cr:YSGG laser (Waterlase, Biolaser - USA) was performed at the Center for Lasers and Applications, set at a wavelength of 2780 nm. This system features beam delivery by flexible crystal-based optic fiber, and a handpiece which allows several sapphire point options for the output beam. The system also features a water jet and air jet to cool the samples during irradiation when desired.

Thus, the group samples were irradiated by a single operator for 20 seconds using G4 output point with a diameter of 600 μm, according to manufacturer's specifications, and was positioned 1 mm away from the sample during irradiation. The output energy of the beam was set in the apparatus at 7.6 mJ, resulting in an energy density of 2.7 J/cm^2.

Adhesive procedures

After the treatments described above and a control group that received no further treatment, Single Bond 2 adhesive system (3M ESPE, St. Paul, MN, USA) was employed following manufacturer's recommendations. With the aid of a microbrush, two layers of adhesive were applied, all excess was removed, and a gentle air stream was applied for 5 seconds at a distance of 10 cm to evaporate the solvents. Light cure was then performed with a LED curing light (Radii Plus, power intensity 1200mW/cm^2, 440 ~ 480nm) for 10 seconds.

After the adhesive procedure, the cavities were restored with restorative resin composite Z350 XT, shade A3 (3M ESPE, St. Paul, MN, USA). The composite was inserted in three increments with each increment being light cured for 20 seconds. Light cure was performed with the same LED unit described above.

After curing, the restorations were finished and polished with diamond points and abrasive discs of different granulations (SofLex Popon

- 3M ESPE). Final polishing was performed with a silicon carbide brush (Brush Jiffy - Ultradent) and then the samples were stored in water at 37° C in a dark environment.

Table 1 - Materials, manufacturers and key components.

Material (Manufacturer)	Key components	Application method
Adper Single Bond 2 (3M ESPE, St Paul, MN, USA)	Bis-GMA, HEMA, diurethane dimethacrylate, polialcenoic acid copolymer, camphorquinone, water, ethanol and 1.3 glycerol dimethacrylate, 10% by weight of silica nanoparticles	Apply two layers, air stream for 5 s individually, and light cure for 10 s
Filtek Z350XT (3M ESPE, St Paul, MN, USA)	Bis-GMA, UDMA, Bis-EMA, zirconia, silica	Incrementally in three individual layers and individual light cure for 20 s
Ultra-Etch (Ultradent Products INC, South Jordan, Utah, EUA)	35% Phosphoric Acid	Applied to dentin for 15 s and to enamel for 30 s, washed with water for 10 seconds and dried with absorbent paper
Clorexidina s (FGM Produtos Odontológicos. Joinvile, SC, Brasil)	2% Chlorhexidine digluconate	Passive application of 20 µL for 60 sec

External adaptation analysis

Twenty-four hours after storage, external adaptation was analyzed in the 36 specimens.

Impressions of the external margin of each restoration were made with a polyvinyl siloxane-based material (President light body; Coltène Whaledent-AG Altstätten, Switzerland) and epoxy replicas (Epofix, Stuers, Rødovre, Denmark) were fabricated. Subsequently, the specimens were subjected to thermocycling consisting of 3000 cycles of water with temperatures ranging from 5°C to 50°C with a dwell time of 2 min by temperature.

After cycling, impressions were once again taken of the external margin of each restoration using polyvinyl siloxane (President light body; Coltène Whaledent-AG Altstätten, Switzerland). Epoxy replicas were then fabricated from the impressions, and the quantitative analysis of the external margin was performed by scanning electron microscopy (XL20, Philips, Eindhoven, Netherlands) with a magnification of up to 200x. Results were recorded as a percentage of "continuous margin" and "non-continuous margin".

To assess external adaptation, differences found in the percentages of "continuous margin" were statistically analyzed and the conditions of homogeneity of variance and normality of residuals were assessed by Levene's test and Shapiro-Wilk test, respectively. Once a non-normal distribution was found to exist, Kruskal-Wallis test was applied to compare the different treatments between the groups. Wilcoxon test was employed to compare the groups before and after thermocycling. Calculations were performed using SPSS 13.0 computer software for Windows.

RESULTS

Kruskal-Wallis test results are shown in table 2 and table 3, with means being presented along with standard deviations. As can be observed, no significant difference was found, indicating that the groups behaved similarly with regard to the different surface treatments, both in the healthy substratum and the decayed margins of enamel and dentin.

Table 2 - Percentages and SD of continuous margins at the enamel margin length in Class V Cavities before and after thermal and mechanical loading.

Substratum	Treatment	Before thermocycling	After thermocycling
Intact	Without Treat.	98,05 (1,85)	92.42 (1.63)
	Chlorhexidine	98,09 (2,59)	92.90 (5.01)
	Laser	94.24 (4.66)	91.72 (4.30)
Decayed	Without Treat.	95.19 (4.45)	89.72 (4.09)
	Chlorhexidine	96.09 (2.94)	93.03 (3.31)
	Laser	89.69 (8.13)	78.61 (6.70)

No significant differences were found between groups (Kruskal–Wallis test - $p>0.05$).

Table 3 – Percentages and SD of continuous margins at the dentin margin length in Class V cavities before and after thermal and mechanical loading.

Substratum	Treatment	Before thermocycling	After thermocycling
Intact	Without Treat.	87.74 (5.17)	82.06 (3.64)
	Chlorhexidine	89.38 (5.47)	85.13 (5.20)
	Laser	82.66 (8.62)	69.99 (9.20)
Decayed	Without Treat.	88.98 (3.10)	73.75 (5.54)
	Chlorhexidine	90.74 (4.51)	86.34 (4.83)
	Laser	84.50 (6.66)	67.79 (6.88)

No significant differences were found between groups (Kruskal–Wallis test - $p>0.05$).

In comparing the percentage values of marginal adaptation in the region of enamel and dentin, Wilcoxon test showed a significant difference before and after thermocycling. After evaluation, the region of enamel showed no statistically significant difference between applying laser to healthy teeth vs. applying chlorhexidine to carious teeth (Fig. 1). Assessment of the dentin region disclosed that differences were not significant between treating healthy and carious teeth with chlorhexidine vs. treating decayed teeth with laser (Fig. 2).

Figure 1. Percentage values of marginal adaptation in the region of enamel.

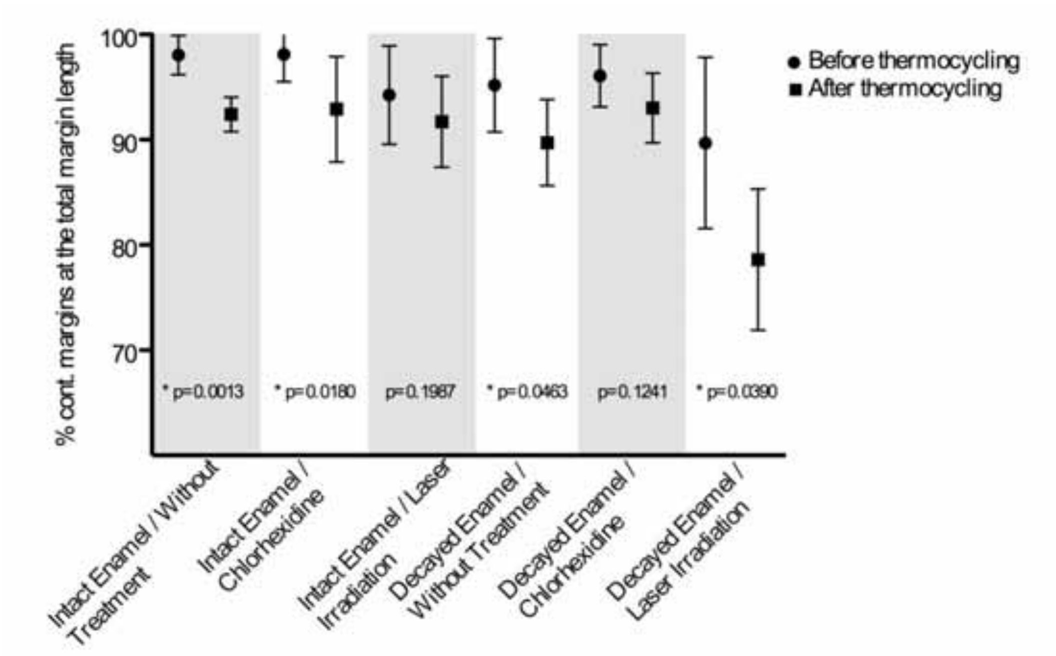
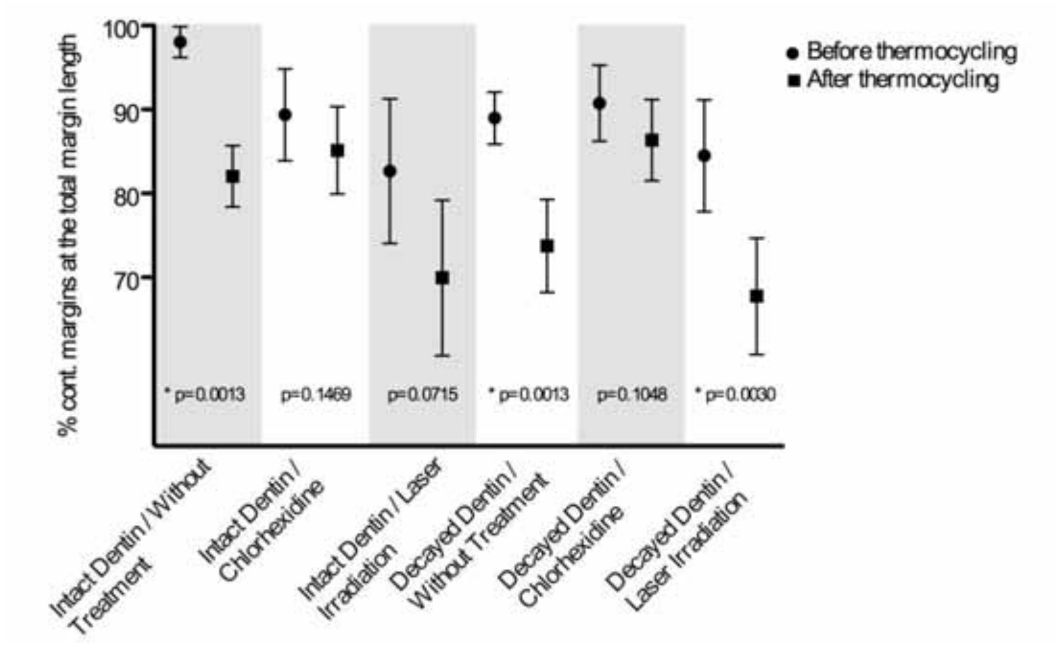


Figure 2. Percentage values of marginal adaptation in the region of dentin.



DISCUSSION

As demand grows for cosmetic procedures, adhesive techniques evolve significantly to enable new protocols capable of achieving increased long-term durability of restorations. This study evaluated the external adaptation of class V restorations subjected to different treatments. It should be emphasized that successful restorations depend not only on a high bond strength value, but above all on efficient marginal sealing. Moreover, proper sealing of the cavity margins must withstand immediate effects, such as polymerization shrinkage, in addition to the effects of degradation of the adhesive interface [1, 18], such as hydrolytic degradation, enzymatic degradation and plasticization effects.

More often than not, the performance of a given material or adhesive technique is evaluated by microtensile tests [19, 20] carried out with static load until fracture. However, clinical microleakage is commonly observed, as well as marginal pigmentation and gaps due to interface failures [21]. This study assessed the adaptation of the margins of cavities restored with composite resin (Fig. 3; Fig. 4), whereby percentages of marginal gaps were subtracted from the total. Gaps between restorative material and dental structure are caused by deficiency in compensating for the stress generated in the polymerization process [22, 23], and may occur in the region of enamel, dentin or even in the adhesive layer. They can extend into the cavity and undermine the restoration . These gaps result from a deficiency in compensating for the initial polymerization shrinkage stress which occurs in the restoration before any effort is exerted [22, 24].

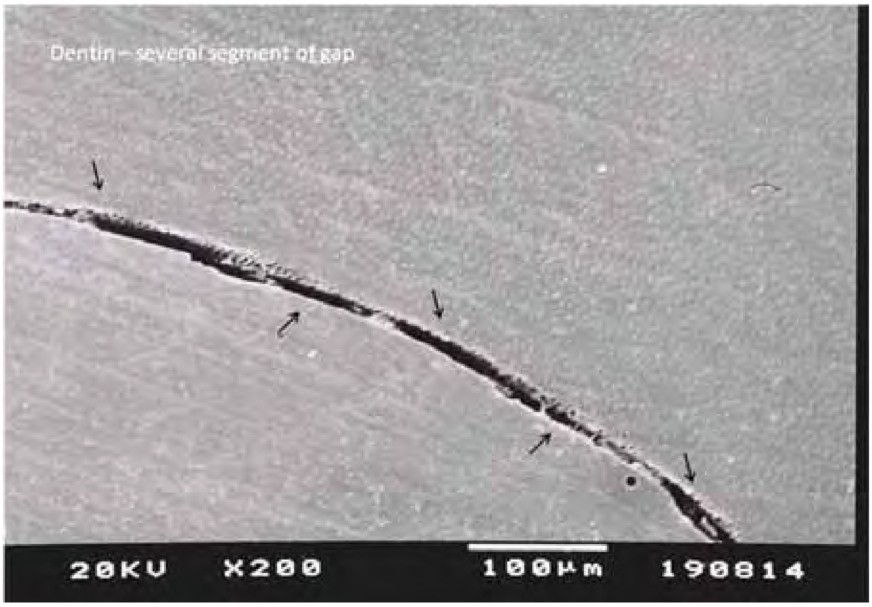


Fig 3 – Representative image of the external adaptation after thermal cycling.

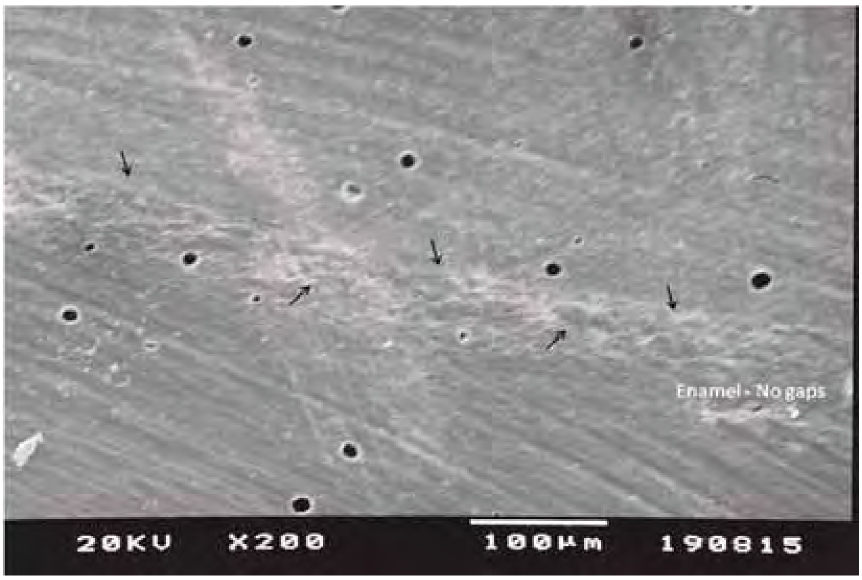


Figure 4 – Representative image of continuous margin.

The method employed to analyze the gaps and marginal continuity consisted in the use of epoxy resin replicas analyzed by scanning electron microscopy (SEM). An illustrative image can be seen, representing the marginal sealing of mixed class V cavity (Fig. 5). This method allows a quantitative analysis, which counts the continuous margin (%). It also allows for an evaluation of the entire adhesive interface morphology including its micromorphology. Furthermore, it is a non-destructive examination given the use of a replica, which allows the reassessment of the same sample as described in this study, where the same specimen was evaluated before and after thermocycling . Additionally, by making use of replicas, the method allows in-vivo tests to be performed with longevity control. For this reason this method was employed in this study.

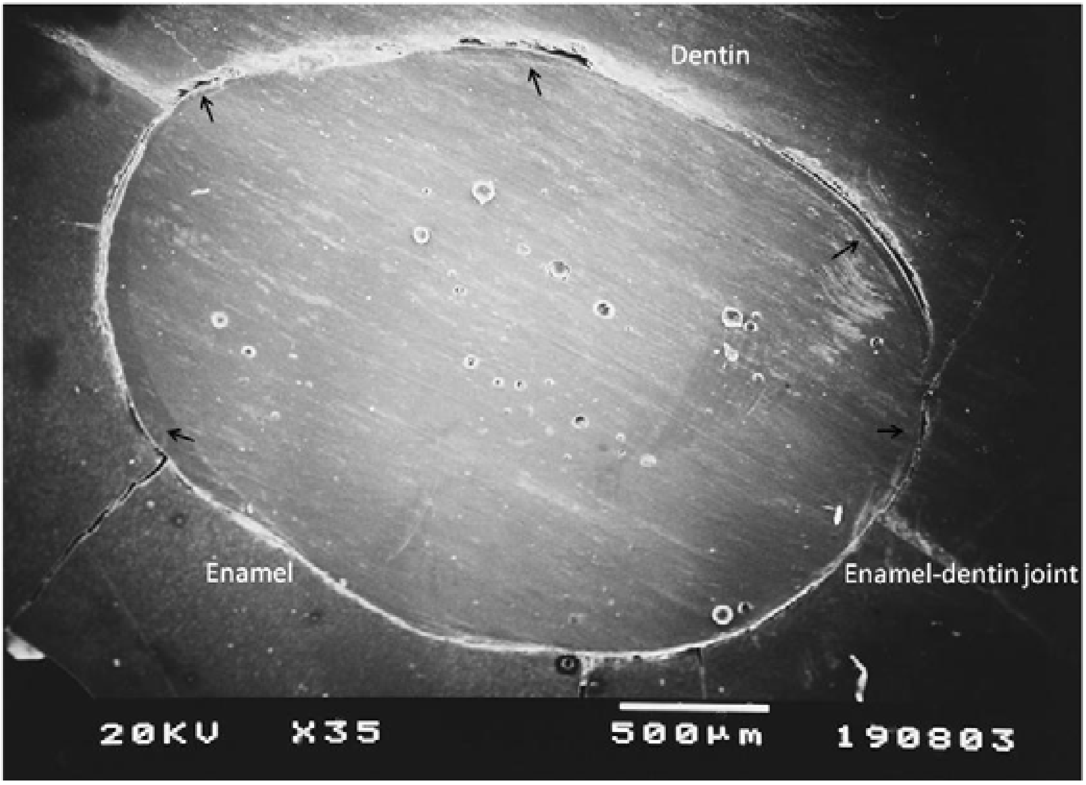


Figure 5 - SEM micrograph (35× magnification). Representative frontal view of a class V restoration with margins located on enamel and dentin. Gaps can be observed in several segments of the dentinal margin while few gaps are visible on enamel margins.

The preparation of Class V-type cavities limits the release of stress occurring at the time of polymerization shrinkage by showing a high C factor, which constitutes a challenge to the integrity of the adhesive interface. Such threat can be minimized by the incremental technique employed in this study [25]. Another important feature is the possibility of performing a single restoration involving enamel and dentin in the same cavity, which allows the behavior of the restoration to be assessed in the two substrate [26]. In addition, cavity size and preparation were standardized, while the same type of point was used to ensure that the role of comparison factors was the most relevant in terms of interference with the results.

In this study the effect of artificial aging was minimized when chlorhexidine was applied on the acid-etched cavity walls. Chlorhexidine may inhibit matrix metalloproteinases (MMPs) and thereby reduce the degradation of collagen fibrils present at the resin/dentin interface, in addition to featuring antimicrobial properties [27].

The findings revealed that when Er,Cr:YSGG laser was applied, the continuous margin percentage values were lower compared to other groups. However, these values were not statistically significant. This finding could be explained by the morphological changes that occur on the laser-irradiated surface, which hinders the formation of adequate hybrid layer [28]. Even if the laser were to produce roughness on the irradiated surface, with open dentinal tubules, no adequate penetration of resin monomers occurs [29, 30].

Another likely outcome might be an increased bond strength in the bottom wall, where the laser is supposed to have been more active as it is a convergent beam. At the time of the polymerization shrinkage, the stress generated at the tooth/restoration interface might have led to an increased formation of gaps. ‘This relationship between marginal adaptation and bond strength is quite controversial, with some studies reporting an increase in bond strength in laser-irradiated cavities.

Dentin irradiation with laser followed a 0.25 W power protocol, which allows the evaluation of tissue changes and produces a bactericidal effect while increasing the dentinal resistance to the action of acids. When irradiation is performed, a spectrum of wavelength of the light emitted by the laser is absorbed by water. Providing high absorption of light energy by the water molecules and concentrating a high amount of energy in small amounts of tissue, thereby preventing heat damage in the deeper portions of the dental tissue [31]. The high concentrations of energy in water molecules evaporate quickly, leading to micro-explosions in the dental tissue, also called thermomechanical ablation, whereby the hard tissue is abraded [32].

The decision to compare the effects of surface treatments in healthy vs. carious teeth is due to the fact that restorative treatment is predominantly performed when the dentin is already affected by caries [33]. This leads to physiological and chemical changes, such as a reduction in mineral content in the intertubular area of the dentin affected by decay [34]. Meanwhile, acid dissolution-resistant crystals are deposited in the dentinal tubules as a defense against bacterial invasion, which reduces the permeability of resin monomers and the formation of resin tags [35]. These findings are in line with this study, which found a trend towards lower values of marginal adaptation of teeth affected by caries, mainly in the dentin, as can be seen in fig. 2.

CONCLUSIONS

Both Er,Cr:YSGG laser and chlorhexidine behaved similarly to the extent that no significant difference was found in percentages of marginal adaptation between the dentin surface treatment methods used in healthy vs. carious teeth. After comparing the values found before and after cycling, it was concluded that thermocycling exerts an impact on marginal adaptation values.

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Capítulo 2

ARTIGO 2

Influence of artificial aging in marginal adaptation of mixed Class V cavities.

Mateus Rodrigues Tonetto, DDS, MSc[a], Hélida Gomes de Oliveira Barud, DDS, MSc[a], Keren Cristina F. Jordão Basso, DDS, MSc[a], Matheus Coelho Bandéca, DDS, PhD[b], DDS, PhD[c], Edson Alves de Campos, DDS, PhD[c], Sizenando de Toledo Porto Neto, DDS, PhD[c], Marcelo Ferrarezi de Andrade, DDS, PhD[c]

[a] PhD Student of Restorative Dentistry, School of Araraquara Dentistry, University of São Paulo State, Araraquara, Brazil.

[b] Professor of Dentistry, University of CEUMA, São Luíz, Brazil.

[c]Professor of Dentistry, School of Araraquara Dentistry, University of São Paulo State, Araraquara, Brazil.

Address:

Pio Correa Pinheiro Avenue, Apt 651 31b

District: Melhado

Araraquara - SP

CEP: 14807-031

Phone: +55 16 81354693

E-mail: mateus.tonetto@foar.unesp.br

ABSTRACT

The aim of this study was to investigate whether the artificial aging by thermal cycling had influenced the marginal adaptation of class V restorations with / without chlorhexidine application in the bond process. Twelve intact human third molars were used. Class V cavity preparations were performed on the buccal surface and the teeth received 35% phosphoric acid-etching procedure (Ultradent Products Inc., South Jordan, Utah, USA). Subsequently, the samples were divided in two groups: untreated acid etched dentin and chlorhexidine application as an adjunct in the bond process. The adhesive Single Bond 2 (3M ESPE, St. Paul, MN, USA) was used after 2% chlorhexidine application, and the restorations were performed with Filtek TM Z350 XT (3M ESPE) composite resin. The specimens were submitted to artificial aging by thermal cycling with 3000 cycles. Analyzes were performed on scanning electron microscopy using replicas of marginal adaptation in percentage of continuous margin before and after the artificial aging. The data were analyzed by paired test and the results showed statistically significant differences in the percentage of continuous margin with / without chlorhexidine treatment before and after thermal cycling. This study concluded that the artificial aging by thermal cycling influenced the marginal adaptation of mixed Class V composite restorations.

Keywords: artificial aging, chlorhexidine, dental marginal adaptation.

INTRODUCTION

In the recent times the aesthetics concept has been widespread in dental practice, a fact that is not restricted only to the dentists, but also reaches the patients. Thus, the light cured composite resins have become the first choice in most of all restorations due to its aesthetic, bonding properties and faster polymerization[1]. However, even the most perfect criteria restorations can be influenced by factors that act in the degradation of the bond interface. And this fact can produce failures in the adaptation, resulting in cracks between the restorative material and tooth tissue, resulting in improper restorations[2,3].

The clinical success and the durability of adhesive restorations depend on the quality of the bond interface between the tooth and restoration, which depend on the correct use of the adhesive system. There are several alternatives procedures to promote the bond interfaces less susceptible to the biodegradation inside the oral cavity. The chlorhexidine has been shown effective to reduce the collagen fibers degradation, when it is applied to the etched dentin prior to bond system[4-7]. Studies have demonstrated that the hybrid layer integrity is preserved for a longer period after chlorhexidine pre treatment. This fact occurs once it works as an adjuvant for the bonding process[6,8] and does not interfere with the adhesive performance for short or long term[5,7,9].

The artificial aging by thermal cycles is a viable and relevant option[5]. The samples were submitted to temperature variations, from the coldest to the hottest, in order to simulate the thermal stress that happens in the oral cavity[10,11]. The thermal cycling can influence the microleakage because there is a difference in coefficient of linear thermal expansion between the tooth structure and the restorative material. Furthermore, it allows the formation of "gaps" in the interface tooth/restoration[12,13].

The purpose of this study was to evaluate the thermal cycling influenced the marginal adaptation of Class V composite restorations with / without application of chlorhexidine in the bonding process.

MATERIALS AND METHODS

Tooth selection and cavity preparation

Twelve caries-free human third molars were selected and stored in 0.1 % thymol solution at 4° C until its used. Standardized cavities Class V were prepared at the buccal surface of each tooth and located in enamel and dentin using spherical diamond burs.

The dimensions of the cavities were 2.0 mm in depth, 2.0 mm in height, and 4.0 mm in width. Each bur was replaced with a new one after four cavity preparations. The standardization of the cavities preparation was performed with the aid of an adhesive template, probe millimeter, rubber stop, digital caliper and a stereoscopic magnifying glass (Model SZXL, Oympus, Sao Paulo, Brazil) in order to check the marginal imperfections analysis, such as fractures or cracks.

Dentin treatment and restorative procedures

The teeth were randomly divided in two groups according to chlorhexidine treatment/without chlorhexidine treatment and adhesive system Single Bond 2(3M ESPE, St. Paul, MN, USA) application. The adhesive system was applied following the manufactures recommendations. Groups with chlorexidine treatment for the dentin surface were conditioned with 35% phosphoric acid Ultra-Etch (Ultradent Products Inc., South Jordan, Utah, USA) for 15 s on dentin and 30 s on enamel. The cavities were rinsed for 15 s and dried with absorbent paper and received 20 mL of 2% chlorhexidine for 60 seconds and then the adhesive system was applied in two layers and dried with an air spray for 5 seconds and light-cured for 10 seconds. The group without chlorhexidine treatment received the adhesive system according to the same humidity conditions described above. The Z350 XT (3M ESPE, St. Paul, MN, USA) composite resin, shade A3 was inserted into the cavities according to the incremental technique. The increments were light-cured with LED (Radii Plus, with output intensity of 1200 mW/cm^2) for 20 seconds each. All restorations were finished and polished by using abrasive wheels with different grits (SofLex PopOn - 3M ESPE). Final polishing was performed by carbide brush (Brush Jiffy - Ultradent). The teeth were stored in water at 37° C in the dark.

Evaluation of the external adaptation:

After storage, impressions with a polyvinylsiloxane material (President light body, Coltène-Whaledent AG, Altstätten, Switzerland) were made and epoxy resin replicas were prepared (Epofix, Stuers, Rodovre, Denmark). After the confection of the replicas, the teeth were submitted to artificial aging. The Thermal cycling was performed in flushing water with temperatures changing 3.000 × from 5° C to 55° C with a dwelling time of 2 min each. After the thermal cycling, another impression of the outer margin of each restoration and epoxy replicas were prepared for the computer assisted quantitative analysis in a scanning electron microscope (XL20, Philips, Eindhoven, The Netherlands) at 200× magnification.

RESULTS

The differences between the percentages of continuous margin before and after Thermal cycles loading in enamel and in dentin were evaluated using the Tukey´s test pairs comparison. There was significant difference before and after the thermal cycles and a lower value of continuous margin can be observed after thermal cycles loading. The data are shown in Table 1 and 2, and the means demonstrated significant differences that are shown by different letters in the same line.

Table 1 - Analysis of the enamel in pairs before and after thermal cycling with the averages and standard deviations (mean ± SD) of non-continuous margin in % (5 % significance level, by Tukey´s test).

Substratum	Treatment	Marginal adaptation (%)	
		Before thermocycling	After thermocycling
Intact	Without Treat.	92.43 (4.76) [a]	85.16 (4.02) [b]
	Chlorhexidine	93.91 (5.29) [a]	84.25 (3.86) [b]
Decayed	Without Treat.	88,35 (3.67) [a]	79.41 (5.71) [b]
	Chlorhexidine	89.40 (5.08) [a]	80.94 (5.32) [b]

Table 2 - Analysis of the dentin in pairs before and after thermal cycling with the averages and standard deviations (mean ± SD) of non-continuous margin in % (5 % significance level, by Tukey test).

Substratum	Treatment	Marginal adaptation (%)	
		Before thermocycling	After thermocycling
Intact	Without Treat.	87.98 (5.16) [a]	80.09 (5.27) [b]
	Chlorhexidine	88.14 (5.29) [a]	82.55 (4.09) [b]
Decayed	Without Treat.	79,56 (4.72) [a]	71.86 (5.82) [b]
	Chlorhexidine	77.80 (4.86) [a]	71.48 (4.75) [b]

DISCUSSION

The microleakage between tooth / restoration interface is one of the most critical factors of aesthetic restoration. The shrinkage polymerization, the stress provoked by masticatory efforts and the coefficient of linear thermal expansion lead to degradation of sealing marginal[3,4]. The involved and the uninvolved collagen fibers with the adhesive system degraded with the same proportion to the restoration aging[14,15].Thus, collagen fibers can affect and influence the pathogenesis of the carious process because it destabilizes the acid etched fibers through the action of metalloproteinases (MMPs).

These activated enzymes are responsible for the hydrolytic degradation of collagen which interfere with the integrity of the hybrid layer[14,16]. The pH values ranging from 2.3 to 5 can activate the MMP, this process is known as acid-activation. Bacterial metabolism and acid etching result in acids which may activated the MMPs. However, studies have been demonstrated that the chlorhexidine application to adhesive systems can prevent the degradation of collagen fibers and present antimicrobial properties[4,6,7].

Carrilho et al, 2007[10] had shown that the hybrid layer was preserved in the specimens pre treated with 2% chlorhexidine after comparison to their respective controls without treatment. So the chlorhexidine has an antimicrobial function already recognized and also acts as a important potent MMP inhibitor[4]. The results obtained in this study revealed that the chlorhexidine treatment group showed no statistical differences in comparison to without treatment group. The minimal chlorhexidine concentration capable of inhibit MMP type 9 is 0,002 %, while the MMP type 2 shows the more sensitive response and being inhibited as low as 0.0001 %, and MMP type 8 is inhibited by 0,02 % [4]. Pasheley et al., 2004[5] demonstrated that concentration of 0.2 % chlorhexidine is sufficient to inhibit the collagenolytic activity to near-zero levels.

However, there is no standardization and most studies in the literature reports the use of 2% chlorhexidine[6-10], as the concentration used in this study. *In vivo* evaluation is most appropriated way to obtain information regarding the oral conditions[11],but this procedure consumes time, financial resources and the rapid evolution of restorative materials and adhesives lead to seek alternatives to simulate clinical conditions through *in vitro* studies[12]. Another condition to be simulated *in vitro* studies is the intra-oral temperature variation, which happens during ingestion of food and beverages and can influence the restorations. The difference in the coefficient of thermal expansion between the tooth structure and restorative materials can induce stresses in the bond interface[13]. In order to simulate the oral conditions, the thermal cycling is the ideal test to be performed[13-15]. In these tests, the specimens are alternately dipped in solutions made of saliva or water and exposed to different temperatures (hot/cold). This study used the thermal fatigue by thermal cycling (3000 cycles, from 5° C to 55° C) with a dwelling time of 2 min each temperature according to ISO TR 11450 standardization.

The standard recommends that the thermal cycling is performed with at least 500 cycles in water from 5° C and 55° C. This study showed that the thermal cycling influenced the external adaptation in enamel/dentin in all groups in comparison to the initial situation.

Lucena-Martín *et al.,* 2001[13] found that dye leakage at the gingival margin in Class V restoration was significantly increased after thermal cycling (500 cycles, from 5 ° to 55° C). However, according to these authors the same fact is not observed at coronary margins in enamel, which shows that the adhesive bond to enamel is more resistant to thermal stress. In this study was concluded that the class V composite restorations had an initial cracks and after artificial aging by thermal cycling, the cracks increased, independent of the application of chlorhexidine.

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Discussão

DISCUSSÃO

O sucesso clínico de uma restauração depende, sobretudo, do efetivo selamento marginal que um material restaurador proporciona e principalmente os efeitos decorrentes da contração de polimerização e degradação da interface adesiva. Dessa forma, o estresse pode induzir forças de tracionamento sobre as paredes laterais e de fundo de um preparo cavitário e levar ao surgimento de fendas pelo deslocamento do material restaurador[46]. Estas fendas podem causar manchamento marginal, sensibilidade pós-operatória e cáries recorrentes.

Fatores como presença de dentina cariada, profundidade do preparo, presença de dentina esclerótica, estão presentes na maioria dos preparos cavitários e devem ser considerados, podendo ser desfavorável no processo de adesão[16,25,54,55]. Sendo assim, optamos por realizar os tratamentos em dentina hígida e afetada por cárie. As pesquisas mostram que após a remoção do tecido cariado, há permanência de bactérias na parede dos preparos cavitários[3,10,40], e isso pode contribuir tanto para formação de cáries recorrentes quanto interferirem na adesão de materiais restauradores, aumentando a desadaptação e microinfiltração marginal das restaurações adesivas.

Os ensaios de resistência de união são os mais comumente realizados a fim de avaliar os comportamentos físicos-mecânicos das interfaces[45]. Entretanto, devemos lembrar que um material que possibilita um bom comportamento físico-mecânico não garante um bom selamento das margens das restaurações, principalmente quando analisamos a adaptação externa, onde ficamos limitados de concluirmos sobre o sucesso clínico da restauração se não considerarmos a qualidade da interface adesiva ou até mesmo a microinfiltração marginal.

Considerando que a clorexidina, alem de agente antimicrobiano com amplo espectro que atua sobre bactérias, fungos e leveduras e indicada para a desinfecção de preparos cavitários[32], também pode servir na preservação da camada híbrida quando utilizada após o condicionamento ácido[7], pois tem a capacidade de inibir a atividade das enzimas MMP-2 (gelatinase-A) e MMP-9 (gelatinase-B)[15]. Observando nossos resultados, em relação à utilização da clorexidina como coadjuvante no processo adesivo, não houve diferença quando comparado com o controle. Uma possível explicação poderia ser a realização do envelhecimento por ciclagem térmica que levou 5 dias, o que poderia não ter sido suficiente para ocorrer a degradação da camada híbrida.

Quanto a irradiação da dentina com laser de Er,CR:YSGG sem refrigeração ar-água, sabe-se que um protocolo com a mesma potência utilizada em nosso trabalho, 0,25W, é capaz de promover um efeito bactericida além de aumentar a resistência da dentina à ação dos ácidos[1]. Isso poderia ter interferido para a formação da camada híbrida, já que foi visto que, apesar de não significativa, houve uma piora na adaptação externa em relação aos outros grupos. O que também poderia ocorrer é uma ação da irradiação do Laser na parede de fundo da cavidade e este efeito estar relacionado indiretamente com o selamento das margens cavitárias.

Para melhor reprodução da função clínica, o envelhecimento artificial por meio de ciclos térmicos é uma opção viável e relevante[7]. Em nosso estudo observamos que em todos os grupos a ciclagem térmica exerceu influência sobre a adaptação externa, tanto em esmalte quanto em dentina, sendo que após a termociclagem os valores de adaptação marginal foram menores em relação à situação inicial (Apêndice).

Talvez seja interessante a continuação do trabalho com a realização de análises microscópicas da qualidade da interface adesiva, já que as amostras não foram destruídas e nem preparadas para realização da microscopia, o que seria um ponto positivo da utilização de réplicas.

Conclusão

CONCLUSÃO

1. A cilcagem térmica foi capaz de reduzir a porcentagem de margem contínua para todos os grupos avaliados;

2. O tratamento com clorexidina e Laser não apresentaram resultados estatisticamente significante em relação a adaptação marginal.

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Apêndice

MATERIAL E MÉTODO

Seleção dos dentes:

Neste estudo foram utilizados 36 terceiros molares humanos extraídos (Banco de dentes humanos da FOAr - Unesp), isentos de cárie, mantidos em solução de timol 0,1% a 4° C até o momento de sua utilização.

Preparo cavitário:

Inicialmente, em todos os 36 dentes foram preparadas cavidades padronizadas de classe V nas superfícies vestibulares de cada dente envolvendo o 1/3 cervical utilizando ponta diamantada esférica. Cada ponta foi substituída por uma nova após quatro cavidades preparadas e as dimensões das cavidades foram de 2,0 mm de profundidade, 2,0 mm de altura gengivo-incisal e 4,0 mm de largura mésio-distal.

Os preparos cavitários foram padronizados com auxílio de um gabarito adesivo, sonda milimetrada, *stop* de borracha e paquímetro digital, (Figura 1) e verificado para análise de imperfeições marginais, tais como fraturas ou trincas, utilizando uma lupa estereoscópica (Modelo SZXL, Oympus, São Paulo, Brasil) com aumentos de até 20 vezes.

FIGURA 1 - Padronização e confecção dos preparos cavitários com auxílio de paquímetro digital e caneta de alta rotação.

Indução artificial da lesão de cárie:

As 36 amostras selecionadas foram divididas em 2 grupos e foram cobertas com uma camada de adesivo epóxi (ARALDITE, Ciba Especialidades Químicas Ltda., São Paulo, Brasil) e esmalte de unha (Colorama, CEIL Com. Exp. Ind. Ltda, São Paulo, Brasil) em toda a extensão, exceto 1 mm ao redor do preparo cavitário, e após esterilizados, os espécimes foram suspensos dentro de uma solução cariogênica composto de 3,7 g de BHI caldo (Brain Heart infusion, Becton Dickinson and Company - USA), 2 g de sacarose (Synth; LabSynth, São Paulo, SP, Brazil), 1 g de glicose (Synth; LabSynth, São Paulo, SP, Brazil) e 0,5 g de extrato de levedura (Becton Dickinson and Company, Sparks, MD, USA) para cada 100 mL de água destilada, e autoclavado a 120°C durante 20 minutos. Em seguida foi realizada a inoculação de cepas de streptococus mutans (5 mL a cada 10^8 UFC/mL), e os dentes foram esterilizados e mantidos na solução (25 mL/dente) em estufa durante 14 dias, sendo que a cada 48 horas o meio cariogênico foi trocado. Decorrido o período de incubação da cárie, o biofilme sobre os dentes foi removido com gazes esterilizadas e o adesivo epóxi e esmalte foi removido com auxílio de uma lâmina de bisturi e posteriormente os dentes foram lavados abundantemente com água corrente (Figura 2).

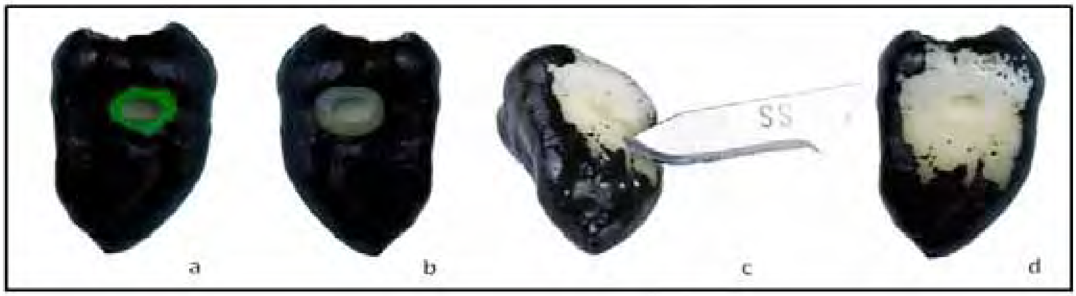

FIGURA 2 – Preparo das amostras para indução artificial de cárie: Utilização de esmalte de unha e remoção com lâmina de bisturi.

Tanto o grupo de dentes hígidos (n=18) quanto o grupo de dentes afetados por cárie (n=18) foram aleatoriamente subdivididos em três grupos: sem tratamento da superfície; aplicação do laser de Er,Cr:YSGG e aplicação da clorexidina.

Aplicação da clorexidina

Para os grupos que receberam a clorexidina como tratamento da superfície, após condicionamento com ácido fosfórico a 35% Ultra-Etch (Ultradent Products INC, South Jordan, Utah, EUA) por 15 s na dentina e 30 s no esmalte, a superfície foi lavada com água destilada por 10 segundos e secas com papel absorvente para a obtenção de uma superfície úmida e posteriormente foi aplicado passivamente 20 mL de uma solução de clorexidina a 2% (Chlorhexidina 2%), com o auxílio de uma micropipeta, cobrindo toda a superfície de dentina por 60 segundos. Os excessos foram removidos com papel absorvente sendo mantido o aspecto úmido.

Irradiação das amostras

Após condicionamento com ácido fosfórico, lavagem da superfície com água destilada e secagem com papel absorvente para a obtenção de uma superfície úmida, a aplicação do laser de Er,Cr:YSGG (Waterlase, Biolaser – USA) foi realizada no Centro de Lasers e Aplicações (IPEN – CNEN/SP), regulado com comprimento de onda de 2780 nm. Este sistema possui entrega de feixe por fibra óptica cristalina flexível, e uma peça de mão que permite diversas opções de pontas de safira na saída do feixe. O sistema também possui um jato d’água e de ar para resfriar as amostras durante a irradiação quando desejado.

Dessa forma, as amostras dos grupos foram irradiadas por um mesmo operador durante 20 segundos utilizando ponta de saída G4, com diâmetro de 600 μm, segundo especificações do fabricante, e foi posicionada a 1 mm da amostra durante a irradiação. A energia de saída do feixe potência ajustada no aparelho foi de 7,6 mJ, o que resultou em uma densidade de energia de 2,7 J/cm^2.

Procedimentos adesivos:

Após os devidos tratamentos descritos anteriormente e grupo controle que não recebeu tratamento adicional, foi empregado o sistema adesivo Adper Single Bond 2 (3M ESPE, St. Paul, MN, EUA), seguindo as recomendações do fabricante. Com auxílio de um microbrush, foram aplicadas duas camadas do adesivo, removendo-se excessos, e depois um leve jato de ar por 5 segundos com uma distância de 10 cm foi aplicado para evaporação dos solventes e a polimerização realizada com o aparelho fotopolimerizador LED (Radii Plus, intensidade de potência de 1200mW/cm^2, 440~480nm) durante 10 segundos. O aparelho foi verificado com o radiômetro Curing Radiometer P/N 10503-Model 100 (Demetron Researcb Scorp., USA) antes de cada utilização (Figura 3).

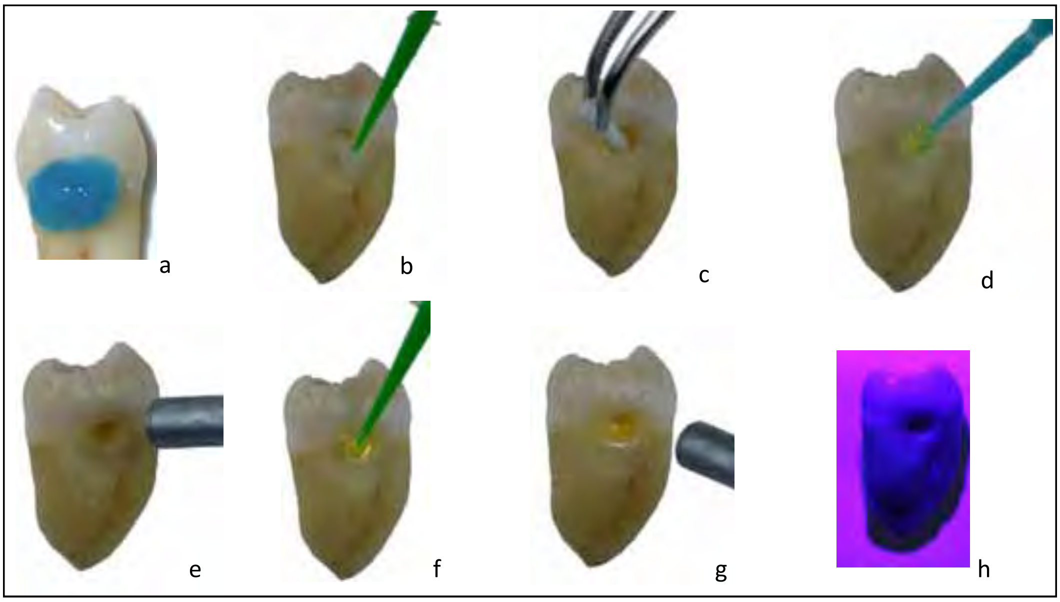

FIGURA 3 – Realização dos procedimentos adesivos.

Após o procedimento de adesão, as cavidades foram restauradas com resina composta Z350 XT na cor A3 (3M ESPE, St. Paul, MN, EUA). A inserção da resina composta foi em três incrementos, e fotopolimerizados por 20 segundos cada incremento. A polimerização foi realizada com a mesma unidade de luz LED descrita anteriormente.

Após a polimerização, as restaurações receberam acabamento e polimento com pontas diamantadas e discos abrasivos de diferentes granulações (SofLex PopOn – 3M ESPE) e polimento final com escova de carbeto de silício (Jiffy Brush – Ultradent) e posteriormente os dentes restaurados foram armazenados em água a 37°C em ambiente escuro (Figura 4).

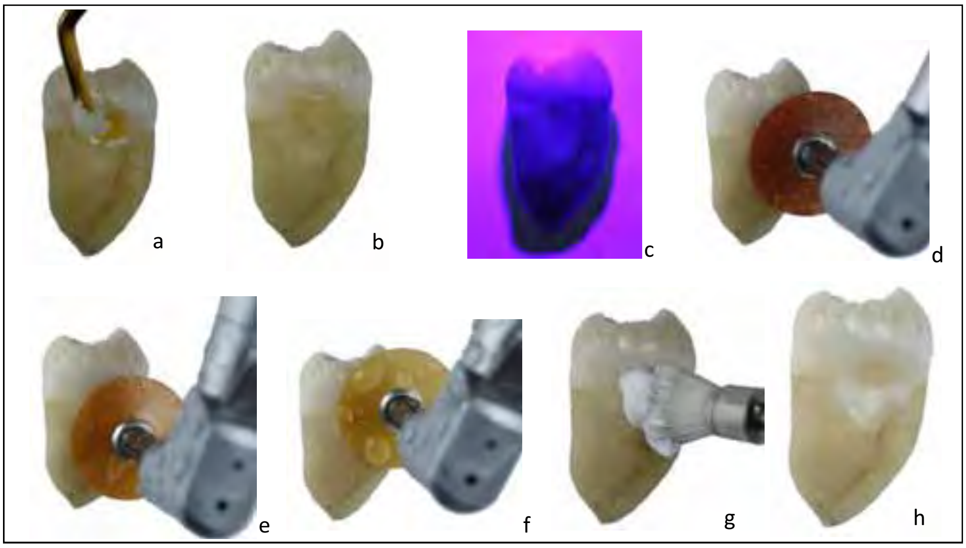

FIGURA 4 – Seqüência restauradora com acabamento e polimento com discos abrasivos.

Tabela 1 - Materiais utilizados, respectivos fabricantes e principais componentes

Material (fabricante)	**Principais componentes**	**Modo de aplicação**
Adper Single Bond 2 (3M ESPE, St Paul, MN, USA)	Bis-GMA, HEMA, diuretano dimetacrilato, copolímero do ácido polialcenóico, canforoquinona, água, etanol e glicerol 1.3 dimetacrilato, 10% em peso de nanopartículas de sílica	Aplicar duas camadas, jatos de ar por 5 s individualmente e fotoativação por 10 s
Filtek Z350XT (3M ESPE, St Paul, MN, USA)	Resinas Bis-GMA, UDMA, Bis-EMA, zircônia, sílica	Forma incremental em 3 camadas e fotoativação individual por 20s
Ultra-Etch (Ultradent Products INC, South Jordan, Utah, EUA)	Ácido fosfórico 35%	Aplicado por 15 s em dentina e 30 s em esmalte, lavado com água por 10 s e seco com papel absorvente
Clorexidina s (FGM Produtos Odontológicos. Joinvile, SC, Brasil)	Digluconato de Clorexidina a 2%	Aplicação passiva de 20 µL por 60 s

Análise da adaptação externa:

Decorrida 24 horas de armazenagem, a análise da adaptação externa foi realizada nos 36 espécimes constituintes.

Impressões da margem externa de cada restauração foram realizadas com um material à base de polivinil-siloxano (President light body; Coltène-Whaledent AG, Altstätten, Suíça) e preparadas réplicas de epóxi (Epofix, Stuers, Rodovre, Dinamarca). Posteriormente, os espécimes foram submetidos à ciclagem térmica constituída por 3.000

ciclos de água com temperaturas mudando de 5°C a 50ºC, com um tempo de permanência de 1 min por temperatura.

Após a ciclagem, novamente foram realizadas impressões da margem externa de cada restauração utilizando polivinil-siloxano (President light body; Coltène-Whaledent AG, Altstätten, Suíça). Em seguida, foram preparadas as réplicas de epóxi a partir das impressões e realizou-se a análise quantitativa da margem externa utilizando microscopia eletrônica de varredura (XL20, Philips, Eidhoven, Holanda) com uma ampliação de até 200x. Os resultados foram registrados em porcentagem de "margem contínua" e “não contínua" (Figura 5).

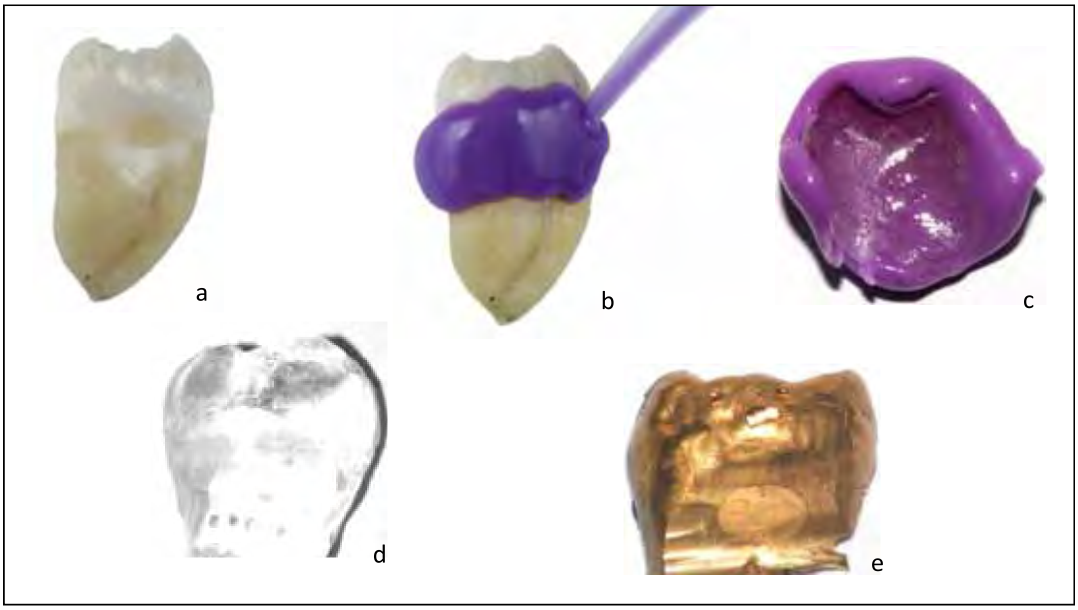

FIGURA 5 - Preparo das réplicas de epóxi a partir de impressões com polivinil-siloxano.

Anexo

UNIVERSIDADE ESTADUAL PAULISTA " JÚLIO DE MESQUITA FILHO"

FACULDADE DE ODONTOLOGIA DE ARARAQUARA

Comitê de Ética em Pesquisa

Certificado

Certificamos que o projeto de pesquisa intitulado *"EFEITO DA CLOREXIDINA NA ADAPTAÇÃO EXTERNA E CARACTERIZAÇÃO DA INTERFACE ADESIVA NA UNIÃO RESINA/DENTINA HÍGIDA E AFETADA POR CÁRIE APÓS CICLOS TERMOMECÂNICOS"* sob o protocolo nº **86/11**, de responsabilidade do Pesquisador (a) *MARCELO FERRAREZI DE ANDRADE* está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa–FOAr, com validade de 01 (um) ano, quando será avaliado o relatório final da pesquisa.

Certify that the research project titled *" EFFECT OF CHLORHEXIDINE ON THE EXTERNAL ADAPTATION AND CHARACTERIZATION OF THE ADHESIVE INTERFACE IN THE UNION RESIN / HEALTHY DENTIN AND A CARIE-AFFECTED DENTIN AFTER THERMOMECHANICAL CYCLES "*, protocol number **86/11**, under *Dr MARCELO FERRAREZI DE ANDRADE* responsability, is under the terms of Conselho Nacional de Saúde/MS resolution # 196/96, published on May 10, 1996. This research has been approved by Research Ethic Committee, FOAr-UNESP. Approval is granted for 01 (one) year when the final review of this study will occur.

Araraquara, 8 de fevereiro de 2012.

Prof Dr Maurício Meirelles Nagle

Coordenador

Autorizo a reprodução deste trabalho.

Araraquara, 27 de Julho de 2012.

MATEUS RODRIGUES TONETTO