



FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS



BONIEK CASTILLO DUTRA BORGES

Cirurgião-Dentista

**EFEITO DA APLICAÇÃO DE FOSFOPEPTÍDIO DE CASEÍNA-FOSFATO DE CÁLCIO
AMORFO (CPP-ACP) ASSOCIADA AO PROTOCOLO ADESIVO NA RESISTÊNCIA
DE UNIÃO DE UM SELANTE DE FÓSSULAS E FISSURAS**

Dissertação apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do Título de Mestre em Clínica Odontológica, com área de concentração em Dentística.

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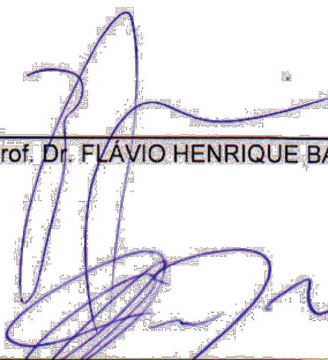
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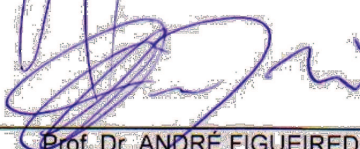
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*Dedico este trabalho aos
meus pais, Joílson e
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Resumo

O objetivo deste trabalho foi investigar, *in vitro*, o efeito da aplicação de uma pasta contendo fosfopeptídeo de caseína – fosfato de cálcio amorfo (CPP-ACP) (MI Paste – MI) previamente ao protocolo adesivo na resistência da união de um selante resinoso de fósulas e fissuras ao esmalte dental humano. Foram utilizados 98 espécimes de esmalte proximal de terceiros molares totalmente erupcionados, divididos em 14 grupos experimentais (n=7): G1/G8- condicionamento ácido (CA) (Scotchbond Etchant), sem a aplicação de sistema adesivo; G2/G9- CA + resina hidrófoba de um sistema adesivo convencional de três passos (Scotchbond Multipurpose Plus); G3/G10- CA + sistema adesivo convencional de dois passos (Single Bond 2); G4/G11 - CA + sistema adesivo autocondicionante de passo único (Clearfil S³ Bond); G5/G12- CA + sistema adesivo autocondicionante de dois passos (Clearfil SE Bond); G6/G13- sistema adesivo autocondicionante de dois passos; G7/G14- adesivo autocondicionante de passo único. MI foi aplicada previamente ao protocolo adesivo apenas nos grupos de G1 a G7. Após selamento das superfícies (FluroShield), foram confeccionados blocos retangulares (palitos) (~0,7mm² de área seccional) para o ensaio de microtração. O teste foi efetuado imediatamente após confecção das amostras por meio da Máquina de Ensaio Universal, na velocidade de 0,5 mm/min. Após, o padrão de fratura foi analisado por meio de Microscopia Eletrônica de Varredura (MEV). Os valores de resistência da união foram analisados por meio de ANOVA dois fatores com parcela subdividida. A aplicação da pasta contendo CPP-ACP previamente aos sistemas adesivos convencionais e àqueles autocondicionantes sem condicionamento ácido prévio aumentou os valores de resistência da união. Houve predomínio de fraturas mistas nos grupos que receberam MI Paste, ao passo que, naqueles em que não se aplicou MI Paste a maior prevalência foi de fraturas adesivas. O contato do esmalte com CPP-ACP anteriormente à aplicação

dos sistemas adesivos é um método eficaz para aumentar a resistência de união entre esmalte e selantes de fósulas e fissuras.

Palavras-chave: adesivos dentários, resistência à tração, odontologia preventiva.

Abstract

This study evaluated the effect of applying a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) containing paste (MI Paste - MI) previously to adhesive procedures on the microtensile bond strength of a pit and fissure sealant to human enamel *in vitro*. Ninety eight fragments were obtained from proximal surfaces of totally erupted third molars. Specimens were divided into 14 experimental groups (n=7): G1/G8- phosphoric acid conditioning (PA) (Scotchbond Etchant) and no adhesive system; G2/G9- PA + hydrophobic resin of a three step adhesive system (Scotchbond Multipurpose Plus); G3/G10- PA + etch-and-rinse two-step adhesive system (Single Bond2); G4/G11- PA + all-in-one adhesive (Clearfil S³-Bond); G5/G12- PA + two-step self-etching adhesive system (Clearfil SE-Bond); G6/G13- all-in-one adhesive; G7/G14- two-step self-etching adhesive system. MI was applied on the enamel before adhesive strategies only for groups from G1 to G7. After sealing (FluroShield), beams with approximately 0.7 mm² of sectional area were cut for microtensile test, which was executed through a universal test machine at a speed of 0.5 mm/min. Fractured specimens were analyzed by scanning electronic microscopy to determine failure modes. Bond strength data was statistically analyzed by two-way ANOVA with subparcels. Groups which MI was applied on the enamel followed by sealant bonding with etching-and-rinse and self-etching adhesives without additional enamel acid etching presented higher means than other which MI was not applied. Higher frequency of cohesive failures was observed for groups with MI, while adhesive fractures were more prevalent in groups without MI. Applying a CPP-ACP containing paste before adhesive systems to the enamel is an effective method to increase bond strength of the sealant tested.

Key-words: dental adhesives, tensile strength, preventive dentistry.

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1- Introdução

Passadas recentes décadas, a odontologia presenciou grande avanço nos materiais e técnicas utilizados, bem como uma mudança nos princípios adotados, emergindo condutas mais eficientes com ênfase na prevenção em saúde bucal (Krol, 2003). Entre as técnicas não-invasivas empregadas na prevenção de cáries em crianças e adolescentes, destaca-se o selamento de fósulas e fissuras, o qual consiste em uma manobra não invasiva, conservadora, introduzida na prática odontológica desde 1960, no intuito de obliterá-las, protegendo-as do ataque ácido bacteriano (Ahovuo-Saloranta *et al.*, 2008).

Diversos estudos comprovaram a eficácia do selamento de fósulas e fissuras na prevenção de cáries oclusais em pacientes de risco (Ahovuo-Saloranta *et al.*, 2008; Azarpazhooh e Main, 2008; Beauchamp *et al.*, 2009). Ademais, Borges *et al.* (2010) demonstraram que o selamento do esmalte com selante resinoso sobre lesões cariosas não cavitadas em dentina foi capaz de paralisá-las, em um procedimento realizado sem preparo de cavidades e, portanto, com maior conservação de tecido dental. Com isso, os selantes de fósulas e fissuras desempenham um importante papel na prevenção primária e controle da cárie.

Entretanto, a retenção do selante resinoso é de fundamental importância no seu sucesso clínico (Papacchini *et al.*, 2006; Yazici *et al.*, 2006). Alguns autores apontaram crescentes níveis de perda deste material em longo prazo, fato que está diretamente associado ao surgimento (Tianviwat *et al.*, 2008; Jodkowska, 2008) e progressão de cáries nos casos em que foi utilizado com finalidade terapêutica (Borges *et al.*, 2010). Com isso, torna-se importante pesquisar protocolos de aplicação que forneçam um maior tempo de retenção e integridade da união selante/esmalte, pois, dessa forma, seu efeito protetor contra o surgimento e progressão de cárie nas fósulas e fissuras seria prolongado.

A aplicação de uma camada adesiva entre o selante resinoso e o esmalte surgiu como um protocolo alternativo para aumentar a retenção deste material ao elemento dentário. Entretanto, estudos clínicos envolvendo adesivos convencionais mostram resultados controversos de retenção do selante após longos períodos de observação. Enquanto Feigal *et al.* (2000) obtiveram aumento na retenção utilizando-se uma camada intermediária, Mascarenhas *et al.* (2008) obtiveram resultados contrários. Quando comparada a efetividade dos agentes autocondicionantes, estudos clínicos também exprimem distintas observações. Venker *et al.* (2004) relataram que adesivos autocondicionantes propiciaram menor retenção ao material selador, comparada àquela fornecida apenas pelo condicionamento ácido separado. Já Feigal e Quelhas (2003) reportaram conclusões inversas. Desta forma, surge a necessidade de avaliar-se a real influência dos sistemas adesivos convencionais e autocondicionantes na retenção dos materiais seladores de fóssulas e fissuras.

Com propósito de prever o poder retentivo de materiais resinosos à estrutura dental após realização de protocolos adesivos, métodos laboratoriais *in vitro* de resistência da união são frequentemente conduzidos, entre os quais se menciona o teste de resistência à microtração (De Munck *et al.*, 2005). Apesar de estudos clínicos mostrarem resultados semelhantes ao que acontece no meio bucal, a vantagem dos testes laboratoriais é a possibilidade de controlar variáveis que podem possuir diferentes comportamentos no mesmo indivíduo ou num grupo de indivíduos, tais como ciclo mastigatório, forças oclusais, microbiota, hábitos de higiene bucal e dieta, levando a interpretações errôneas dos resultados quando se realiza trabalhos *in vivo* sem seu controle. No ensaio de novos materiais ou técnicas de aplicação, torna-se importante, primeiramente, eliminar variáveis que venham a modificar o comportamento destes, para que sejam obtidas informações apenas da interação destes materiais e/ou técnicas com os tecidos dentais.

Recentemente, compostos a base de CPP-ACP (casein phosphopeptide amorphous calcium phosphate) tem sido lançados no mercado, com promissor potencial anticariogênico e cariostático devido à liberação de íons

cálcio e fosfato (Cross, Huq e Reynolds, 2007). Sua utilização prévia ao selante poderia potencializar a ação protetora fornecida ao elemento dental e deve ser averiguada para analisar a interferência destes nanocomplexos na resistência da união selante/esmalte. Ademais, em relação à resina composta, Adebayo, Burrow e Tyas (2007) obtiveram um aumento da resistência de união de sistemas adesivos convencionais e autocondicionantes com prévio condicionamento ácido ao esmalte quando este tecido recebeu aplicação prévia de uma pasta constituída de CPP-ACP. Desta forma, torna-se de grande valor averiguar quantitativamente a resistência da união selante/esmalte quando do contato prévio da superfície dental com o CPP-ACP, associado ao posterior emprego de sistemas adesivos.

Portanto, este trabalho propôs-se a analisar a influência da aplicação prévia de uma pasta contendo CPP-ACP (MI Paste, GC, Tóquio, Japão) associada a sistemas adesivos convencionais e autocondicionantes com e sem condicionamento adicional do esmalte na resistência da união de um selante de fóssulas e fissuras ao esmalte dental humano.

2- Capítulo 1

TITLE:

Effect of the casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) associated to adhesive procedures on bond strength of a pit and fissure sealant

ABSTRACT

This study evaluated the effect of applying a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) containing paste (MI Paste - MI) previously to adhesive procedures on the microtensile bond strength of a pit and fissure sealant to human enamel *in vitro*. Ninety eight fragments were obtained from proximal surfaces of totally erupted third molars. Specimens were divided into 14 experimental groups (n=7): G1/G8- phosphoric acid conditioning (PA) (Scotchbond Etchant) and no adhesive system; G2/G9- PA + hydrophobic resin of a three step adhesive system (Scotchbond Multipurpose Plus); G3/G10- PA + etch-and-rinse two-step adhesive system (Single Bond2); G4/G11- PA + all-in-one adhesive (Clearfil S³-Bond); G5/G12- PA + two-step self-etching adhesive system (Clearfil SE-Bond); G6/G13- all-in-one adhesive; G7/G14- two-step self-etching adhesive system. MI was applied on the enamel before adhesive strategies only for groups from G1 to G7. After sealing (FluroShield), beams with approximately 0.7 mm² of sectional area were cut for microtensile test, which was executed through a universal test machine at a speed of 0.5 mm/min. Fractured specimens were analyzed by scanning electronic microscopy to determine failure modes. Bond strength data was statistically analyzed by two-way ANOVA with subparcels. Groups which MI was applied on the enamel followed by sealant bonding with etching-and-rinse and self-etching adhesives without additional enamel acid etching presented higher means than other which MI was not applied. Higher frequency of cohesive failures was observed for groups with MI, while adhesive fractures were more prevalent in groups without MI. Applying a CPP-ACP

containing paste before adhesive systems to the enamel is an effective method to increase bond strength of the sealant tested.

CLINICAL SIGNIFICANCE

Applying a CPP-ACP containing paste before adhesive systems to the enamel is an effective method to increase bond strength of pit and fissure sealants.

INTRODUCTION

At the last years, Minimally Invasive Dentistry approach has raised on scientific and clinical dentistry worldwide (Vila Verde, Ramos and Stoneham, 2009). In this sense, sealing pit and fissure is recognized as an effective method to prevent caries initiation and to arrest caries progression, providing a physical barrier that inhibits microorganisms and food particles from collecting in pits and fissures (Beauchamp *et al.*, 2009; Borges *et al.*, 2010).

However, the efficacy of sealants is directly related to their long-term retention (Papacchini *et al.*, 2006). The loss of sealant has been associated to subsequent caries development (Tianviwat, Chongsuvivatwong and Sirisakulveroj, 2008). Moreover, it was showed that sealing the enamel upper dentine in non-cavitated occlusal lesions could arrest them since the sealant was attached to the pits and fissures (Borges *et al.*, 2010). It is in this way evident the need to research in an attempt to find an adhesive protocol that can provide higher retention to the sealing material, since it would be increasing the benefic effect against caries development and progression of the sealing material.

The use of adhesive materials as an intermediary layer between the etched enamel and resin-based sealant has shown higher retention rates of pit and fissure sealants (Wadenya *et al.*, 2009). Nevertheless, results of clinical trials involving etch-and-rinse and self-etching adhesive systems before sealant placement are still controversial. While some authors (Feigal *et al.*, 2000; Feigal and Quelhas, 2003) reported increasing in retention of sealants when etch-and-rinse and self-etching adhesives were used, others (Mascarenhas *et al.*, 2008;

Venker *et al.*, 2004) showed that applying etch-and-rinse and self-etching adhesives before sealant placement did not affect its retention rates. So, there is the need to clarify the influence of adhesives on retention of pit and fissures sealants points in the literature.

Recently, casein phosphopeptide – amorphous calcium phosphate (CPP-ACP)-based composites, such MI Paste (GC Corporation, Tokio, Japan), have been introduced in the market worldwide, which contains bio-available calcium and phosphate (Cross, Huq and Reynolds, 2007; Reynolds, 2008). Increased amount of calcium on the enamel might provide a stronger chemical interaction between it and 10-MPD containing self-etching adhesives, since this monomer is capable of forming strong ionic bonds with calcium (Yoshida *et al.*, 2004). Moreover, Adebayo, Burrow and Tyas (2007) showed increased bond strength of etch-and-rinse and self-etching adhesives used with prior acid conditioning when a CPP-ACP containing paste was applied on the enamel before the adhesive procedure. So, it is of great concern to evaluate if a previous enamel conditioning with a CPP-ACP based composite followed by etch-and-rinse and self-etching adhesives may increase bond strength of a pit and fissure sealant to enamel.

This work tested the hypothesis that applying MI Paste, a CPP-ACP containing paste, before etch-and-rinse, self-etching adhesives with and without previous acid conditioning would increase microtensile bond strength between a pit and fissure sealant and human enamel.

METHODS AND MATERIALS

Experimental design and materials used

The factors under study were: enamel contact with a CPP-ACP containing paste (MI Paste – MI) at two levels – with application of MI on the enamel previously to the adhesive protocols and without MI application; and adhesive procedure at seven levels - no adhesive system (phosphoric acid conditioning only – Schotchbond Etchant – PA); PA + bond agent of a three step

adhesive (Schotchbond Multipurpose Plus – MP); PA + etch-and-rinse two step adhesive (Single Bond 2 – SB); PA + all-in-one adhesive (Clearfil S³ Bond – S3); PA + two-step self-etching adhesive (Clearfil SE Bond – SE); S3 only and SE only. Chemical compositions, manufacturers and batch numbers of the materials used in this work are listed in Table 1.

Teeth selection

Twenty-five extracted erupted human third molars were obtained under a protocol (121/2009) approved by the institutional review board from the Piracicaba Dental School, State University of Campinas (FOP/UNICAMP). Mesial and distal surfaces of these teeth were cleaned with a slurry of pumice and water and examined under a stereomicroscope (Meiji 2000, Meiji Techno 2000, Tokio, Japan) at 10X to ensure that they were free of surface cracks, decalcification, or any sign of previous grinding. Teeth were sectioned bucco lingually at a distance of 3 mm from the approximal surfaces using a double-face diamond saw (KG Sorensen, Barueri, SP, Brazil) to obtain an enamel/dentin fragment. Specimens which presented a rectangular flat enamel surface lower than 8 mm x 4 mm were not included in this study.

After selecting the specimens, 49 rectangular flat enamel fragments of 8 mm x 4 mm were cut and sectioned in half, resulting in 49 pairs of sub-fragments with a 4 mm x 4 mm enamel surface area. The pairs of sub-fragments were stored in distilled water at 4°C (Lupi-Pégurier *et al.*, 2007; Foxton *et al.*, 2008) no more than 3 weeks.

Sub-fragments randomization and sealing

The pairs of sub-fragments were randomly assigned to 7 pairs of groups, which were divided considering each adhesive strategy (7 levels) with or without previous enamel CPP-ACP exposition (Table 2).

A square-chapped silicon (Express, 3M ESPE, St Paul, MN, USA) mould with 1 mm of height was made surrounding all sub-fragments before sealing

in an attempt to provide a barrier for restraining the sealant. Materials were applied according to the following protocol:

MI: sub-fragment was dried, MI Paste (0.016 ml) was left undisturbed for 3 min, water rinsed (10 s), and sub-fragment was air dried (10 s).

PA: Scotchbond Etchant was applied on the enamel for 30 s, water rinsed (30 s), and air dried until the surface appears chalky.

MP: bond agent of Scotchbond Multipurpose Plus was applied on the acid conditioned enamel, gently air thinned (10 s at 20 cm), and adhesive was light cured for 10 s.

SB: two coats of Single Bond 2 were applied on the acid conditioned enamel with slight agitation, gently air dried (10 s at 20 cm) after each one, and the adhesive was light cured for 10 s.

S3: two coats of Clearfil S³ Bond were applied on the enamel (previously acid conditioned or not) with slight agitation (20 s), air dried (10 s at 20 cm), and light cured for 10s.

SE: one coat of the primer of Clearfil SE Bond was applied on the enamel (previously acid conditioned or not) with slight agitation (20 s), air dried (10 s at 20 cm), one coat of the bond agent of Clearfil SE Bond was applied (15 s), air thinned (10 s at 20 cm), and light cured for 10s.

FS: Fluroshield was placed in the silicon mould to build up a sealant block with 1 mm of height, and was light cured for 20 s.

Photoactivation was executed with the curing-light tip set 3 mm from the enamel surface in order to reproduce *in vivo* conditions which the curing-tip light may be set on this distance from the sealant surface due to cusps morphology. XL3000 halogen lamp (3M ESPE, St Paul, MN, USA – 500 mW/cm²) was use to photocure adhesives and sealant in this study. Radiance levels at 3mm (340 mW/cm²) and 2 mm (450 mW/cm²) from the enamel surface were accessed through the device Demetron Radiometer (Demetron Research Corp., Model 100, serial 105415, Kerr Corporation – Orange, CA 92867) to obtain the light intensity that reached the adhesives and sealant, respectively.

After sealant cure, composite increments were placed over the sealant and on the dentin to build up a block with 8 mm of height enough to obtain the beams (Perdigão *et al.*, 2005). Two composite increments (Evolu-X A2 shade, Dentsply, Petrópolis, RJ, Brazil) were filled and photoactivated through XL3000 (40 s). Before placement of the resin on dentinal tissue, Clearfil SE Bond was applied according to the previously described protocol.

Microtensile bond strength (MTBS) test

After storage in distilled water at 37°C for 24 hours, sealed sub-fragments were sectioned with a water-cooled low-speed diamond saw (Buehler, São Paulo, SP, Brazil) in x and y directions to obtain sticks with a cross section of approximately 0.7 mm². A maximum of 4 beams were obtained per sub-fragment and the number of prematurely debonded ones was recorded. The cross-sectional area of each stick was measured with a digital caliper (Mitutoyo, Tokio, Japan) and recorded for the calculation of the bond strength.

Beams were affixed to the grips of a microtensile device coupled to a universal test machine (Emic, São José dos Pinhais, PR, Brazil). The MTBS test was performed at 0.5 mm/min.

After the microtensile test, beams were affixed to aluminum stubs (Procind Ltda, Piracicaba, SP, Brazil) with the fractured interfaces facing upward, metalized (SDC 050 Suptter Coater, Baltec) and evaluated by scanning electronic microscopy (JEOL, Tokyo, Japan) to determine the failure modes (Perdigão *et al.*, 2006): predominantly adhesive failure (Figure 2A); predominantly cohesive failure (Figures 2B.1 and 2B.2) or mixed failure (Figure 2C).

Statistical analysis

Two-way ANOVA with subdivided parcels and Tukey's test at a 5% level of significance were executed to analyze bond strength results. Absolute and relative frequencies were calculated for failure modes.

RESULTS

ANOVA showed that there was a significant difference between applying and not applying MI before the adhesive protocols ($p < 0.01$); among the adhesive protocols ($p < 0.01$) and towards the interaction between the above factors ($p < 0.01$).

Table 3 shows Tukey's test results. Comparing the application of MI into each adhesive protocol, contact of the enamel substrate with the CPP-ACP containing paste before all etch-and-rinse (G2 and G3) and both self-etching adhesives without prior phosphoric acid enamel conditioning (G6 and G7) resulted in higher means than homologous groups which MI was not applied on the enamel. On the other hand, no adhesive (G1), PA + S3 (G4) and PA + SE (G5) were not affected by the previous contact of MI with the enamel.

By analysis of all adhesive strategies into each group with or without previous MI enamel exposition, Clearfil SE Bond (G7) showed the highest MTBS among all adhesive protocols executed after enamel contact with MI (G1 to G7), while the lowest MTBS were obtained by groups: no adhesive (G1), MP (G2) and SB (G3). However, PA + SE was the adhesive strategy which showed the highest MTBS when enamel sub-fragments did not have any contact with MI (G8 to G14). No adhesive (G8), SB (G9) and SE (G14) resulted in the lowest MTBS among groups without applying of MI (G8 to G14).

The data obtained in the fracture pattern analysis were analyzed by frequency distribution (Figures 2 and 3). Adhesive fractures were more present in groups which MI Paste was not applied on the enamel before adhesive strategies. On the other hand, cohesive failures were more prevalent in groups which MI Paste reached enamel before adhesive procedures.

DISCUSSION

Results of MTBS test showed that MI applied on the enamel before etching-and-rinse and self-etching adhesives only without additional enamel acid conditioning had a beneficial effect. Therefore, the hypothesis that applying a CPP-ACP containing paste before etch-and-rinse and self-etching adhesives with and

without previous acid conditioning would increase microtensile bond strength between a pit and fissure sealant and human enamel was partially accepted.

In view of the continuous introduction of materials into dental practice, knowing their chemical composition make that unusual but scientifically assisted application protocols can be suggested and tested, finding relevant benefits. MI Paste tested in this study is a CPP-ACP containing product utilized in oral health mainly as an anticariogenic agent. CPP-ACP is a closed complex that releases bio-available calcium and phosphate ions, helping to prevent enamel demineralization and remineralization (Cross, Huq e Reynolds, 2007).

Regarding chemical composition of contemporary adhesives, it was demonstrated that the monomer 10-MDP (originally synthesized by Kuraray, Osaka, Japão), is capable of forming strong ionic bonds with calcium due to the low dissolution rate of the resulting Ca-salt in its own solution (Yoshida *et al.*, 2004). So, possibly the higher amount of calcium reached the enamel after MI Paste application provided strong adhesion between the self-etching 10-MDP containing adhesives tested and enamel.

The fact observed in this study that SE showed higher MTBS than S3 is not in accordance with the results of a previous investigation involving composite resin on unground enamel without application of any calcium available material before these adhesive systems (Perdigão *et al.*, 2006). The two hydroxyl groups in the 10-MDP molecule present in SE and S3 chelate favorably to calcium (Perdigão and Geraldini, 2003). So, one can suggest that a higher amount of calcium on the enamel after application of MI in the present investigation promoted a stronger chemical interaction between this substrate and SE, which has higher 10-MDP content than S3, resulting in superior MTBS for SE than for S3.

Moreover, the results obtained in this study using self-etching adhesives with prior acid enamel conditioning differ from those showed by Adebayo, Burrow and Tyas (2007). The above mentioned authors obtained improved bond strength when Tooth Mouse, a similar of MI, was applied on the enamel, and a phosphoric acid conditioning followed by application of SE was realized. In the present study,

the previous contact of enamel with MI did not increased MTBS for SE preceded by acid etching. This may have been due to the different methods adopted in the present work, which bonded a pit and fissure sealant to unground enamel and specimens were submitted to tensile stress. The aforementioned authors bonded a composite resin to ground enamel and bonds were stressed in shear. Several investigations (Inoue *et al.*, 2003; Lopes *et al.*, 2004; Reis *et al.*, 2009) report different bond strength results between ground and unground enamel for self-etching adhesives. Differences in composition of pit and fissure sealants and composite resins may also influence bond strength values, besides the type of stress loaded to the samples, such microtensile or microshear, explaining controversial results found in both investigations.

Regarding the etching-and-rinse adhesives tested, even though they do not claim chemical interaction with tooth substrates, the application of MI before enamel acid etching left to increased MTBS of MP and SB. The contact between enamel and MI can change the acid etching pattern (Adebayo, Burrow and Tyas, 2007; Adebayo, Burrow and Tyas, 2009), offering a more receptive substrate to the adhesive system after phosphoric acid conditioning. Subsequently, MP and SB samples that received MI application before adhesive procedures (G2 and G3, respectively) had increased MTBS than other which did not (G9 and G10, respectively).

Although the beams tested in the study have several bonding interfaces, this fact did not influence the results obtained, since adhesive bonding interface was placed correctly in half of metallic grips, so that ruptures occurred in this interface only. The absence of adhesive failures in groups which MI was utilized before adhesion strategy can presume that a stronger interaction between enamel and adhesives/sealant occurred. Although MI had no influence on bond strength of enamel in G1, G4 and G5 in comparison with G8, G11 and G12, it could improve longevity of sealants placed according to adhesive strategies used for these groups. Cohesive fractures in the adhesive/sealant were more frequents in G1, G4 and G5 and represent the integrity of the subjacent adhesive layer protecting, the

dental substrate. Moreover, cohesive enamel fractures indicate that bond strength between the adhesive/sealant and enamel was properly higher than enamel strength. Since enamel rupture hardly occurred in *in vivo* conditions, the sealant would possibly be maintained for a long time on fissures.

Among groups which had no CPP-ACP containing paste applied on the enamel, enamel acid conditioning before SE and S3 application provided the highest MTBS. Although literature is scarce in studies evaluating phosphoric acid etching prior SE and S3 on unground enamel, authors have demonstrated that acid etching prior to application of other self-etching adhesives provided higher penetration of the adhesive into the unground enamel surface compared to that achieved solely by application of self etching adhesive (Sundfeld *et al.*, 2005). So, since no calcium available material was applied before SE and S3 in these groups, bonding was possibly more dependent of micromechanical interlocking between adhesives and surface, which is obtained strongly by a previous acid conditioning of unground enamel. This supposed sentence may be confirmed since SE and S3 applied without prior acid etching could not provide similar MTBS to groups that an acid conditioning before self-etching adhesives was performed. On the other hand, the fact SB had the lower MTBS than MP among groups without enamel application of MI may be justified due to the higher hydrophilic content and lower pH of SB (a simplified one-bottle adhesive). The high initial pH of more acidic systems appear to dramatically weaken the bonding performance, either via chemical interacting with the adhesive layer placed next (Sanares *et al.*, 2001) or via the presence of solvents within the polymer, which render the adhesive layer thinner and may weaken the polymer formed (Cho and Dickers, 2004), thus compromising their bond strength to enamel.

The low amount of beams obtained in this study may be possibly explained by poor polymerization of FS on bottom surface, which is a crucial request for obtaining stable bond strength of sealants to enamel (Christopher *et al.*, 2009). Although unground enamel used in this study has a superficial layer less reactive to self-etching adhesives (Reis *et al.*, 2009), it is important to employ this

substrate to investigate bonding performance of pit and fissure sealants instead of the ground one. It should be taken into account that sealants are applied into fissures with intact, aprismatic enamel instead of ground, prismatic enamel (Papacchini *et al.*, 2006) and the MTBS results found in unground enamel are closely to the ones that would occur in *in vivo* conditions.

CONCLUSION

Within the limitation of this study, one can affirm that MI Paste applied on unground enamel before etching-and-rinse and self-etching adhesives without prior acid etching improved bond strength of the pit and fissure sealant tested to the enamel. Clearfil SE Bond, a two-step self-etching adhesive, was the best among all tested.

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TABLES

Table 1. Materials used in this study.

Material and manufacturer	Batch number	Chemical composition
MI Paste (GC Corporation, Tóquio, Japão)	090406M	Pure water, CPP-ACP ¹ , D-sorbitol, Propylene glycol, Silicon dioxide, Titanium dioxide, Xylitol, Phosphoric acid, Favoring, Zinc oxide, Sodium saccharin, Ethyl p-HB ² , Magnesium oxide, Guar gum, Propyl p-HB ² , Butyl p-HB ²
Scotchbond Etchant (3M, St Paul, MN, USA)	9NL	35% H ₃ PO ₄
Scotchbond Multi-Purpose Plus (3M ESPE, St Paul, MN, USA)	8RF	Bond: Bis-GMA ⁴ , HEMA ³ , dimethacrylates, initiators
Adper Sinlge Bond 2 (3M ESPE, St Paul, MN, USA)	8PT	Bis-GMA ⁴ ; HEMA ³ ; dimethacrylates, polyalkenoic acid copolymer, initiators, water, ethanol
Clearfil S ³ Bond (Kuraray, Okayama, Japão)	00116A	HEMA ³ , Bis-GMA ⁴ , MDP ⁵ , colloidal silica, D1-Canphoroquinone, ethanol, water, photoinitiators, silanated colloidal silica
Clearfil SE Bond (Kuraray, Okayama, Japão)	00896A	Primer: Water, HEMA ³ , canphoroquinone, hydrophilic dimethacrylate
	01321A	Bond: MDP; Bis-GMA; HEMA; canphoroquinone; hydrophobic dimethacrylate, N,N-diethanol p-toluidine bond; colloidal silica
Fluroshield (Dentsply/Calk, Milford, DE,)	182017B	⁶ UDM-Bis-GMA, ⁷ BBASG, dimethacrylates, ⁴ Bis-GMA, ⁸ NaF, ⁹ DPEPAP

1: Casein phosphopeptide-amorphous calcium phosphate; 2: Hydroxybezoate; 3: 2-hydroxyethyl methacrylate; 4: bisphenol-glycidyl methacrylate; 5: 10-methacryloyloxydecyl dihydrogen phosphate; 6: Urethane modified Bis-GMA dimethacrylate; 7: Barium boron alumino silicate glass; 8: Sodium fluoride; 9: Dipentaerythritol pentaacrylate phosphate

Table 2. Pairs of experimental groups of this study.

With CPP-ACP exposition	Without CPP-ACP exposition
G1 – MI + PA + FS	G8 – PA + FS
G2 – MI + PA + MP + FS	G9 – PA + MP + FS
G3 – MI + PA + SB + FS	G10 – PA + SB + FS
G4 – MI + PA + S3 + FS	G11 – PA + S3 + FS
G5 – MI + PA + SE + FS	G12 – FA + SE + FS
G6 – MI + S3 + FS	G13 – S3 + FS
G7 – MI + SE + FS	G14 – SE + FS

MI – MI Paste; PA – Phosphoric acid; FS – FluroShield; MP – Scotchbond Multipurpose Plus; SB – Single Bond 2; S3 – Clearfil S3 Bond; SE – Clearfil SE Bond.

Table 3. Microtensile bond strength means in MPa (standard deviations), number of prematurely Debonded Beams (DB) and Intact Beams tested (IB) considering all group tested.

With CPP-ACP applying			Without CPP-ACP applying		
Groups	MTBS	DB/IB	Groups	MTBS	DB/IB
G1 – MI + PA + FS	35.78 (9.44)Ac	16/12	G8 – PA + FS	30.62 (9.55)Ab	9/19
G2 – MI + PA + MP + FS	38.23 (9.41) Ac	12/16	G9 – PA + MP + FS	27.76 (7.16)Bb	20/8
G3 – MI + PA + SB + FS	35.56 (9.25)Ac	16/12	G10 – PA + SB + FS	22.5 (3.3)Bc	17/11
G4 – MI + PA + S3 + FS	44.25 (13.4)Ab	15/13	G11 – PA + S3 + FS	34.29 (13.79)Aa	20/8
G5 – MI + PA + SE + FS	43.52 (8.98)Ab	10/18	G12 – FA + SE + FS	41.89 (13.58)Aa	15/13
G6 – MI + S3 + FS	49.56 (11.83)Ab	14/14	G13 – S3 + FS	28.6 (7.08)Bb	19/9
G7 – MI + SE + FS	55.23 (8.9)Aa	19/9	G14 – SE + FS	25.01 (3,35)Bc	17/11

MI – MI Paste; PA – Phosphoric acid; FS – FluroShield; MP – Scotchbond Multipurpose Plus; SB – Single Bond 2; S3 – Clearfil S3 Bond; SE – Clearfil SE Bond.

Mean values with the same letter were not statistically different by Tukey's test ($p>0.05$). (Lowercase letters for vertical and capital letters for horizontal).

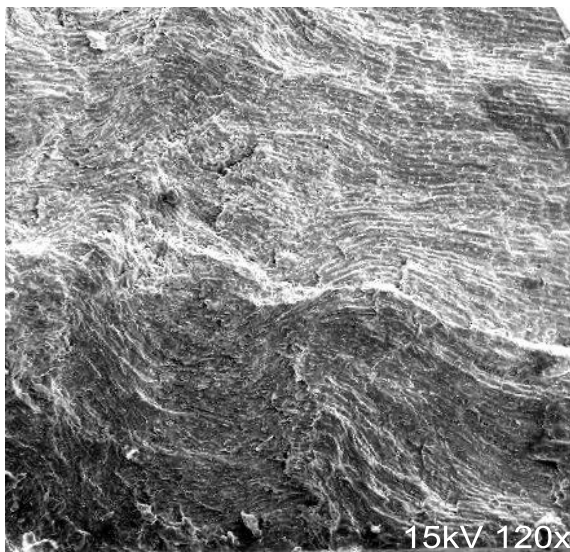


Figure 1A- Predominantly adhesive failure between the sealant and enamel.

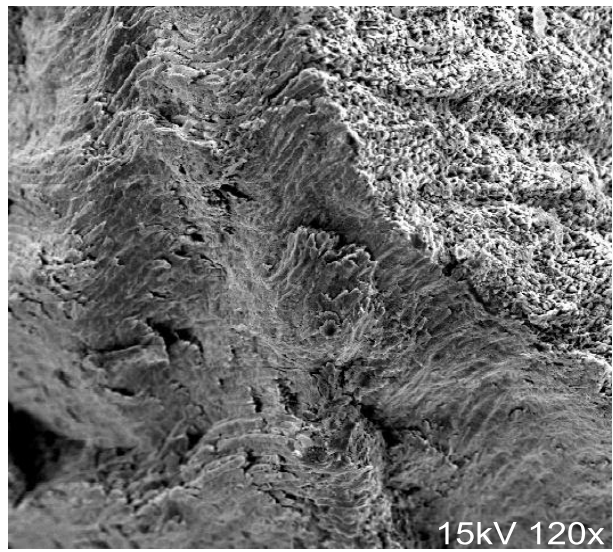


Figure 1B.1- Predominantly cohesive fracture in the enamel.

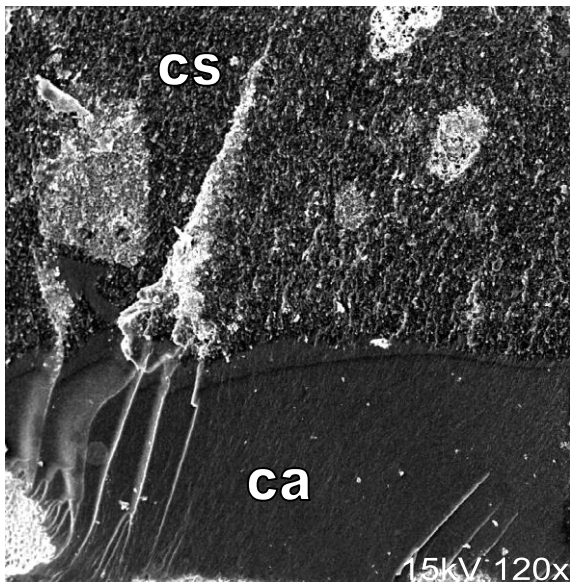


Figure 1B.2- Cohesive failure in the sealant (cs) and in the adhesive system (ca).

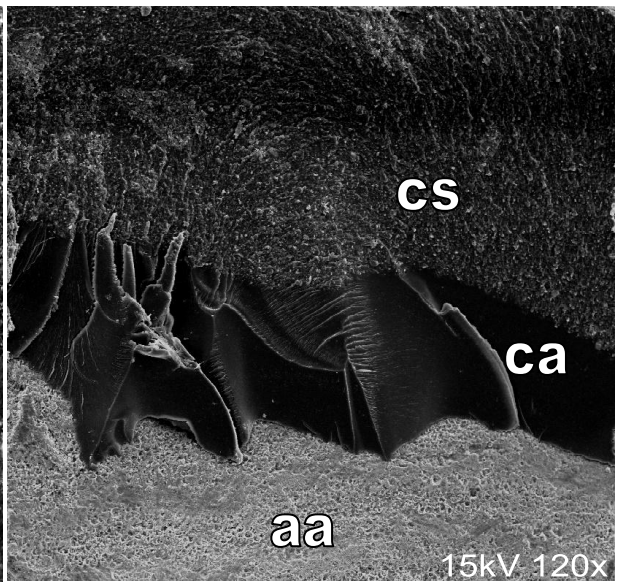


Figure 1C- Mixed fracture: adhesive failure in the adhesive system (aa), cohesive failure in the sealant (cs) and in the adhesive (ca).

Figure 1. Failure modes found in this study.

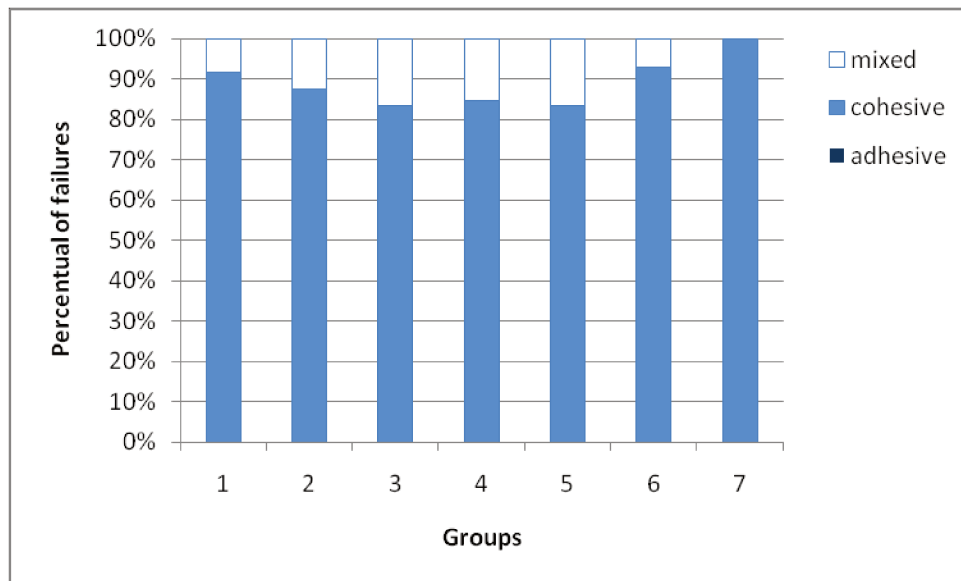


Figure 2. Distribution of failure modes among groups which MI Paste was applied on the enamel before adhesive strategies.

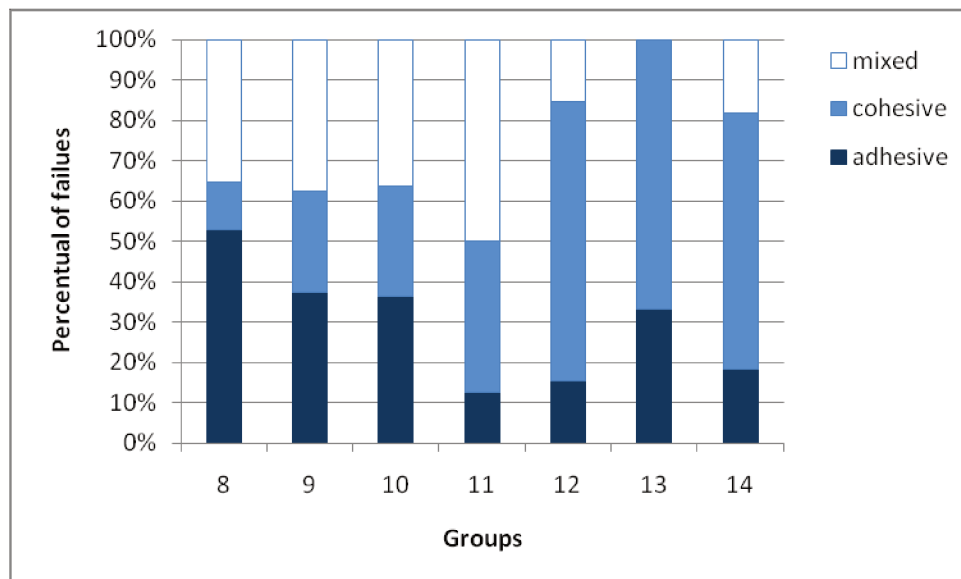


Figure 3. Distribution of failure modes among groups which MI Paste was not applied on the enamel before adhesive strategies.

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3- Considerações gerais

Os resultados de investigações envolvendo a paralisação de lesões cariosas mediante selamento das mesmas (Paris, 2006; Gomez *et al.*, 2005; Martignon, Ekstrand e Ellwood, 2006) tem ampliado as indicações dos selantes de cicatrículas e fissuras. Antes considerado apenas agentes coadjuvantes na prevenção de cárie, atualmente estes materiais desempenham importante papel no controle da doença já instalada, demonstrado por meio de ensaios clínicos randomizados e controlados (Gomez *et al.*, 2005; Martignon, Ekstrand e Ellwood, 2006; Borges *et al.*, 2010).

O trabalho de Mertz-Fairhurst *et al.* (1998) evidenciou ser possível paralisar lesões cariosas cavitadas em dentina sem qualquer remoção de tecido cariado, a partir do vedamento cavitário com resina composta. Os autores atribuíram este fato ao isolamento das bactérias do ambiente bucal, que as tornam inviáveis pela ausência de substrato, e o processo de progressão é paralisado. Seguindo-se este princípio, Borges *et al.*, (2010) confirmaram que o selamento do esmalte sobre lesões oclusais não cavitadas em dentina com selante de cicatrículas e fissuras pode paralisá-las sem a remoção de dentina infectada por meio de instrumentos odontológicos. Estes fatos trazem grandes avanços dentro de um contexto de mínima intervenção, uma vez que qualquer preparo de cavidades em lesões não cavitadas acarreta destruição de tecido dental sadio.

Entretanto, como a efetividade dos selantes de fóssulas e fissuras, quando utilizados com finalidade terapêutica ou preventiva, está ligada a sua permanência na região onde estes foram aplicados, a busca por protocolos de aplicação que possam aumentar a retenção destes materiais surge como necessidade imprescindível.

Nesse sentido, protocolos adesivos que forneçam maiores valores de resistência da união do que os convencionais podem emergir como promissores

no aumento da retenção *in vivo* destes materiais seladores. Uma união mais forte entre selantes e esmalte pode resistir mais firmemente às condições bucais, propiciando maior tempo de permanência onde foram aplicados. Assim, os resultados deste trabalho sugerem um novo protocolo de aplicação dos selantes de cicatrículas e fissuras, resultante da interação entre os nanocomplexos a base de CPP-ACP e sistemas adesivos, principalmente aqueles autocondicionantes contendo o monômero 10-MDP.

Vale salientar que esta interação responsável por aumentar a resistência de união do selante de cicatrículas e fissuras testado ao esmalte pode propiciar também comportamento semelhante às resinas compostas. A união mais forte entre compósitos e estruturas dentais, além de poder aumentar a retenção destes materiais ao elemento dental, pode tornar a interface adesiva mais resistente ao desafio cariogênico. Portanto, pesquisas laboratoriais devem ser conduzidas para validar esta hipótese, ao passo que, quanto aos selantes, investigações *in vivo* devem ser procedidas para confirmar os resultados obtidos *in vitro* neste estudo.

4 Conclusão

De acordo com os resultados da presente investigação, pode-se afirmar que:

- 1) A aplicação da pasta contendo CPP-ACP previamente a sistemas adesivos convencionais e autocondicionantes sem condicionamento adicional do esmalte aumentou a resistência da união ao selante testado.
- 2) A aplicação da pasta contendo CPP-ACP previamente a todos os protocolos adesivos aumentou o índice de fraturas coesivas.
- 3) O adesivo autocondicionante de dois passos Clearfil SE Bond sem condicionamento ácido adicional mostrou-se o melhor entre todos os testados após aplicação da MI Paste.
- 4) Quando a pasta contendo CPP-ACP não foi aplicada no esmalte, o condicionamento adicional do mesmo seguido da aplicação de sistemas adesivos autocondicionantes mostrou o melhor desempenho.
- 5) O contato do esmalte com a pasta contendo CPP-ACP sem a posterior aplicação de sistemas adesivos não gerou aumento na resistência de união do selante testado.

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*De acordo com a norma da UNICAMP/FOP , baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

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Apêndice

Anexo 1: MATERIAIS E MÉTODOS

ARMAZENAMENTO E PREPARO DOS DENTES

Os dentes selecionados (**Figura 1A**) foram submetidos ao debridamento com lâminas de bisturi nº 15 (Wuxi Xanda Medical Device CE LTDA, Xishan, China) e curetas periodontais e polidos com escovas de Robinson (Microdont, São Paulo, SP, Brasil), pedra pomes (SS White, Rio de Janeiro, RJ, Brasil) e água em baixa rotação. Em seguida, estes elementos foram armazenados em água destilada a 4°C até sua utilização no trabalho, por até três semanas.



Figura 1A- Terceiros molares.

As faces proximais dos 25 elementos foram utilizadas para obtenção dos fragmentos de esmalte não preparado. Cortes foram realizados nas faces mesial, distal e oclusal com discos diamantados dupla-face (KG Sorensen, Barueri, SP, Brasil) em peça de mão para a obtenção dos espécimes de espessura aproximada em 3 mm. Seccionou-se os elementos a 3 mm das faces proximais, sendo geradas dois fragmentos nas dimensões de 8 x 4 x 3 mm, das quais foram cortados dois sub-fragmentos dimensionados em 4 x 4 x 3mm de cada elemento dental (**Figuras 1B a F**).



Figura 1B- Marcação da espessura do fragmento.



Figura 1C- Marções para secção dos fragmentos.



Figura 1D- Disco diamantado dupla face.



Figura 1E- Corte dos fragmentos proximais.



Figura 1F- Fragmento proximal.

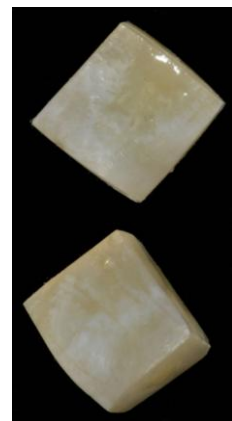


Figura 1G- Subfragmentos.

Desta forma, de cada elemento, foram confeccionados quatro subfragmentos, totalizando 100 espécimes, dos quais 98 foram utilizados neste experimento. Cada par de subfragmentos foi armazenado em água destilada a 4°C até sua utilização.

RANDOMIZAÇÃO DAS SUPERFÍCIES

Duas randomizações para os subfragmentos foram procedidas. Primeiramente, sete pares de subfragmentos foram, aleatoriamente, escolhidos para cada par de grupos (G1/G8; G2/G9; G3/G10; G4/G11; G5/G12; G6/G13; G7/G14); então, a cada par, dividiu-se os subfragmentos cegamente entre os grupos pré-selecionados, de forma que o efeito da aplicação ou não da MI Paste (CPP-ACP) fosse averiguado na mesma superfície de esmalte, de acordo com cada protocolo adesivo.

SELAMENTO DOS SUBFRAGMENTOS

Anteriormente à aplicação dos materiais, uma matriz de silicona de adição (Express, 3M ESPE, St Paul, MN, USA) foi confeccionada, posicionando-se o material ao redor dos fragmentos para que a matriz obtivesse a forma quadrada dos espécimes (**Figura 1H**). Após polimerização da silicona e obtenção da matriz, esta foi deslocada em direção ao esmalte para que fosse fornecido o espaço quadrado (4 mm^2) correspondente a 1 mm de altura, regulada através de sonda milimetrada (**Figura 1I**). Este espaço conteve o selante posteriormente. Os espécimes receberam o material selador de acordo com o protocolo designado a cada grupo. Os materiais foram aplicados de acordo com as instruções dos fabricantes:

MI: secou-se os subfragmentos, aplicou-se MI (16 ml) por três minutos e lavou-se os subfragmentos com jato de água por 10 segundos (**Figuras 1J e K**).

PA: Scotchbond Etchant foi aplicado no esmalte por 30 segundos, lavado por 30 s com jato de água, e seco até surgir uma aparência opaca (**Figura 1L**).

MP: o *bond* do Scotchbond Multipurpose Plus foi aplicado no esmalte condicionado; gentilmente, a camada de adesivo foi espalhada com jato de ar (10 s a 20 cm) e, após, polimerizada por 10 segundos (**Figura 1M e N**).

SB: duas camadas do Single Bond 2 foram aplicadas na superfície condicionada. Um leve jato de ar foi procedido entre estas camadas e após a última (10 s a 20 cm); então, o adesivo foi polimerizado por 10 segundos.

S3: duas camadas do Clearfil S³ Bond foram aplicadas no esmalte (previamente condicionado com ácido fosfórico ou não) com leve agitação (20 s), as quais foram secas com jato de ar (10 s a 20 cm) e fotopolimerizadas por 10 segundos.

SE: uma camada do primer do Clearfil SE Bonde foi aplicada no esmalte (previamente condicionado com ácido fosfórico ou não) com leve agitação (20 s), a qual foi seca com jato de ar (10 s a 20 cm); aplicou-se uma camada do *bond* do Clearfil SE Bond (15 s), espalhou-a com jato de ar (10 s a 20 cm), a qual, então, foi fotopolimerizada por 10 segundos.

FS: Fluroshield foi aplicado no molde de silicone para construir um bloco de selante de 1 mm em altura; polimerizou-se o selante por 20 segundos (**Figura 10 e 1P**).



Figura 1H- Matriz de silicone.

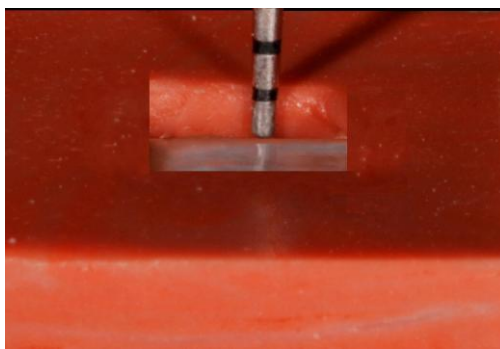


Figura 1I- Posicionamento da matriz.



Figura 1J- MI Paste.



Figura 1K- Aplicação da MI Paste.

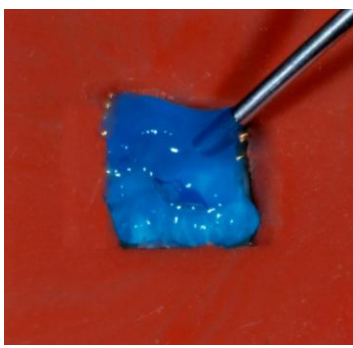


Figura 1L- Aplicação do ácido fosfórico.

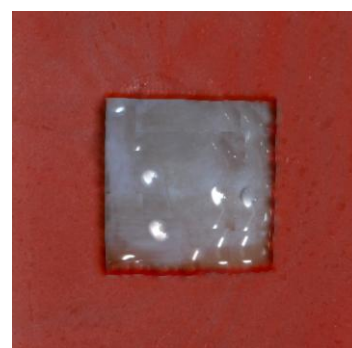


Figura 1M- Aplicação do *bond* do adesivo Scotchbond Multipurpose Plus.

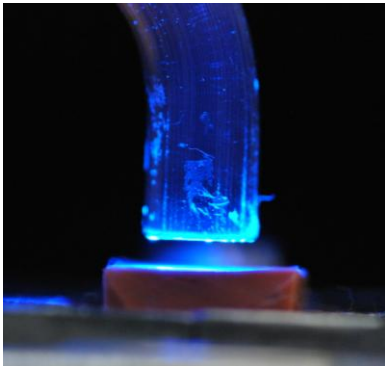


Figura 1N- Polimerização do adesivo.

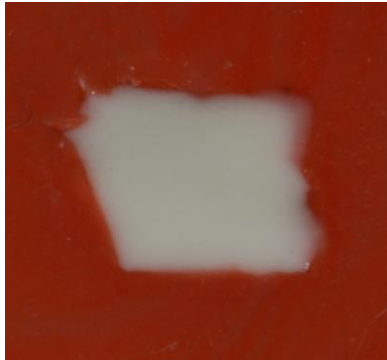


Figura 1O- Inserção do selante.

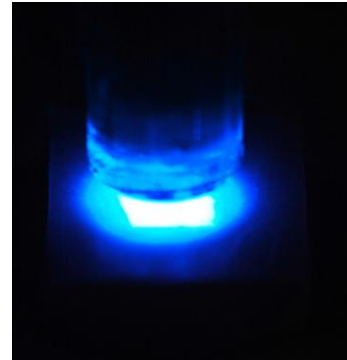


Figura 1P- Polimerização do selante.

ARMAZENAMENTO DOS SUBFRAGMENTOS E CONFECCÃO DOS PALITOS

Após aplicação do selante e confecção do bloco de resina composta, todos os subfragmentos foram armazenados durante 24 horas em água destilada a 37°C. Passado este período, os espécimes foram fixados em placa de acrílico com auxílio de cera pegajosa e palitos de, aproximadamente, 0,7 mm² foram cortados de todos os espécimes (Loguercio et al, 2008) com um disco diamantado (Buehler, São Paulo, SP, Brasil), em máquina cortadeira (Isomet 1000, Buehler, São Paulo, SP, Brasil) (**Figuras 1Q-S**).

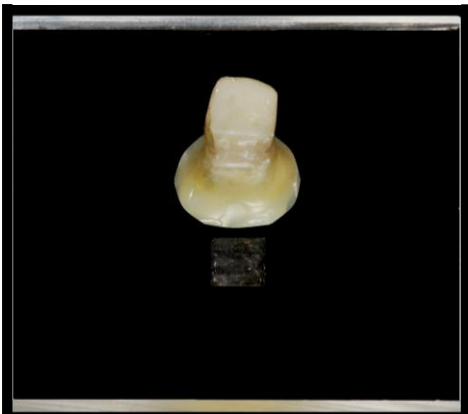


Figura 1Q- Espécime fixado na placa de acrílico com cera pegajosa.

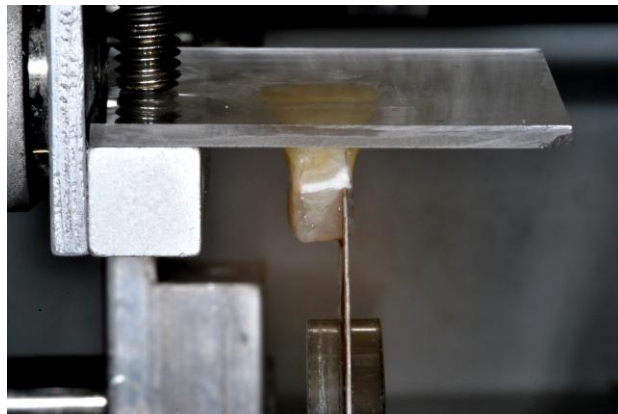


Figura 1R- Corte dos palitos.

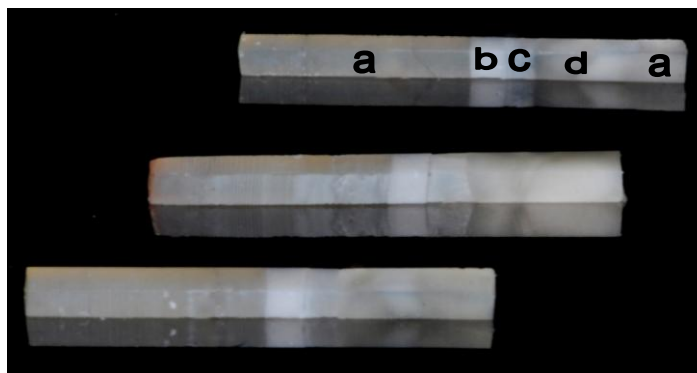


Figura 1S- Palitos cortados: (a) resina, (b) selante, (c) esmalte, (d) dentina.

ENSAIO DE MICROTRAÇÃO

Para realizar o teste de microtração, fixou-se os palitos com cola de cianoacrilato (Super Bond Gel, Loctite, Henkel, Brasil) em placas metálicas (**Figura 1T**), as quais foram encaixadas no dispositivo de microtração e este acoplado à Máquina de ensaio universal Emic (Emic Equipamentos e Sistemas de Ensaio LTDA, São José dos Pinhais, PR, Brasil) (**Figura 1U**). O ensaio foi conduzido com célula de carga de 200 N (20 kgf), na velocidade de 0,5 mm/min, até a ruptura da amostra. Foram anotados os valores de resistência máxima fornecidos pela máquina em Kgf. Depois de calculada a área da interface de união, em cada palito, com um paquímetro digital (Mitutoyo, Tóquio, Japão), os valores de resistência de união foram convertidos em MPa através da fórmula: $MPa = Kgf * 9,8 \text{ (constante)} / \text{área (expressa em mm}^2\text{)}$.

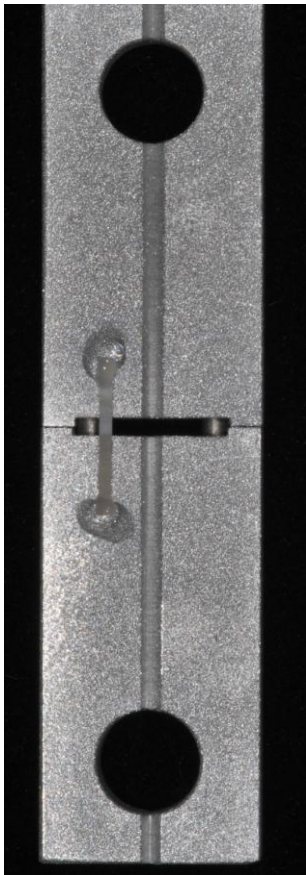


Figura 1T- Palito fixado ao dispositivo metálico.

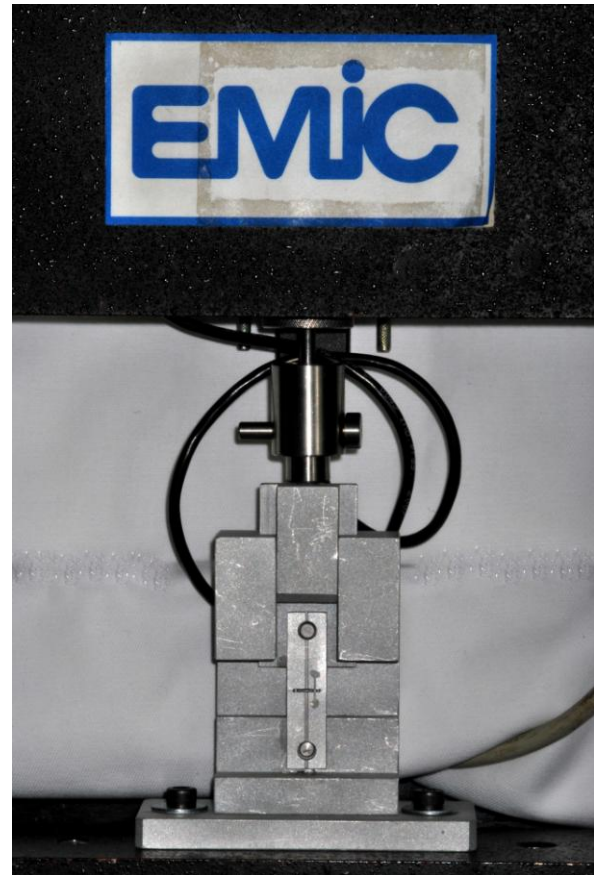


Figura 1U- Dispositivo metálico acoplado à Máquina de Ensaio Universal.