KAMILA ROSAMILIA KANTOVITZ

"EFEITO DE MATERIAIS RESINOSOS E IONOMÉRICOS NA INIBIÇÃO DA DESMINERALIZAÇÃO DO ESMALTE DE FISSURAS OCLUSAIS - ESTUDO DA PERDA MINERAL DO ESMALTE E ADAPTAÇÃO MARGINAL"

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para a obtenção do título de Mestre em Odontologia – Área de Odontopediatria.

Piracicaba

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Orientadora: Prof[a] Dr[a] Regina Maria Puppin Rontani
Co-Orientadora: Prof[a] Dr[a] Marinês Nobre dos Santos Uchôa
Banca examinadora: Prof[a] Dr[a] Regina Puppin Rontani
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Prof[a] Dr[a] Cecília Gatti Guirado

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A Comissão Julgadora dos trabalhos de Defesa de Dissertação de MESTRADO, em sessão pública realizada em 23 de Fevereiro de 2006, considerou a candidata KAMILA ROSAMILIA KANTOVITZ aprovada.

PROFa. DRa. REGINA MARIA PUPPIN RONTANI

PROFa. DRa. JOSIMERI HEBLING COSTA

PROFa. DRa. CECILIA GATTI GUIRADO

DEDICATÓRIA

*Dedico esta dissertação à minha mãe **Tânia**, ao meu Pai **Walter**, ao meu irmão **Waltinho** e ao meu "namorido" **Chico**, que não mediram esforços para me incentivar e apoiar durante esses anos.*

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"...Muitos são os caminhos, porém outras tantas as escolhas. Cabe a cada homem a iniciativa de trilhar o seu caminho. Cabe a cada um de nós a coragem de assumir a própria escolha..."

SUMÁRIO

RESUMO

As superfícies oclusais são locais suscetíveis ao acúmulo de biofilme bacteriano favorecendo o desenvolvimento de lesões de cárie. Os selantes de fóssulas e fissuras oclusais vêm sendo proposto na prevenção desta doença multifatorial, principalmente em pacientes de alto risco. Desta forma, os objetivos deste estudo *in vitro* foram avaliar: 1 - a formação de fendas na interface esmalte/selante (*gaps*) de diferentes tipos de materiais usados como selantes (Selante resinoso, Cimento de ionômero de vidro, Cimento de ionômero de vidro modificado por resina e Sistemas adesivos) quando submetidos ao severo estresse físico e químico e 2 - o efeito de inibição da perda mineral do esmalte produzida pelos selantes oclusais que contém ou não fluoretos e verificar a capacidade de liberação de flúor destes materiais. Um total de 108 terceiros molares humanos inclusos foi aleatoriamente dividido em grupos de acordo com o material, e selados: Concise (C), FluroShield (F), Helioseal Clear Chroma (H), Vitremer (V), Fuji II-LC (FII), Ketac Molar (KM), Fuji IX (FIX), Single Bond (SB), e Clearfil Protect Bond (CF). Todos os grupos foram submetidos à ciclagem térmica (500 ciclos) e de pH (14 dias). Para a avaliação da formação de fendas e do efeito de inibição à cárie, os espécimes foram constituídos de fragmentos de fissuras oclusais, obtidos a partir de secções longitudinais, no sentido vestíbulo-lingual da fossa central para os molares inferiores, e da fossa mesial para os superiores. Por meio de microscopia eletrônica de varredura, da análise de microdureza (% de volume mineral) e da análise da liberação de flúor destes materiais nas soluções dês-remineralizadoras foram avaliados os efeitos dos materiais quanto à formação de "*gaps*" e inibição da perda mineral do esmalte dentário. Os resultados demonstraram que Single Bond e Vitremer foram efetivos na preservação da interface material selador/superfície oclusal do esmalte, suportando as condições de severo estresse físico e químico oferecidos pelo modelo *in vitro* proposto. Os selantes resinosos não foram capazes de prevenir a perda mineral do esmalte oclusal de dentes permanentes exposto ao desafio cariogênico. Já selantes ionoméricos revelaram os menores valores de perda mineral de esmalte na mesma situação experimental. Deve-se considerar que o flúor liberado pelos selantes ionoméricos foi capaz de prevenir a perda mineral do esmalte. Entretanto, apenas a presença de flúor na composição do material não foi capaz de interferir na inibição da perda mineral do esmalte.

PALAVRA-CHAVE: selante de fissura, perda mineral do esmalte, microdureza, prevenção de cárie oclusal, sistemas adesivos, ionômero de vidro, ionômero de vidro modificado por resina e formação de *gaps*.

ABSTRACT

The occlusal surfaces are considered susceptible sites for the biofilm accumulation, which increases caries development. Pit and fissure dental sealants are recognized as an important adjunct approach for caries prevention in high caries risk patients. However, in the search for a material that has a good clinical performance, it should be considered the integration of retention and fluoride-releasing properties in sealant materials. The aims of this *in vitro* study were to quantitatively evaluate: 1 - the effect of different materials when used as sealants (Resin sealant, Glass-ionomer cements, Resin-modified glass-ionomer cements, and Adhesive systems) on the gap formation in the fissure submitted to physical and chemical stress, and 2 - the effect of enamel mineral loss of fluoride- and non-fluoride-containing occlusal sealants on permanent teeth at different distances from the sealant margin and verify the fluoride releasing capability of these materials. One hundred and eight impacted human third molars were sealed and randomly assigned into: Concise (C), FluroShield (F), Helioseal Clear Chroma (H), Vitremer (V), Fuji II-LC (FII), Ketac Molar (KM), Fuji IX (FIX), Single Bond (SB), and Clearfil Protect Bond (CF) groups. All groups were subjected to thermo cycling (500 cycles) and 14 days of pH cycling. Each tooth was longitudinally sectioned in order to obtain oclusal specimens. It was consist in a perpendicular slice to the fissure orientation in the central fossa of mandibular and mesial fossa of maxillary molars. Scanning Electron Microscopy and cross-section microhardness evaluations assessed marginal adaptation and enamel mineral loss, respectively. The results demonstrated that Single Bond and Vitremer sealants were effective in preserve the marginal adaptation in the enamel occlusal fissure. They were able to support the stress conditions offered by this *in vitro* model. On the other hand, resin sealant did not prevent the enamel mineral loss in permanent teeth in a situation that simulated a high cariogenic challenge. Considering glass ionomer cements, the fluoride release level of these materials were able to decrease the enamel mineral loss. Moreover, only the presence of fluoride on the material’s composition cannot predict the material’s behavior with regard to their capability to interfere with the enamel mineral loss on permanent teeth.

KEY WORDS: fissure sealing, enamel mineral loss, microhardness, prevention, adhesive systems, glass-ionomer cements, resin-modified glass-ionomer, resin sealants, gap formation.

INTRODUÇÃO GERAL

As superfícies dentárias lisas livres têm se beneficiado através dos efeitos de agentes fluoretados, em relação ao incremento de cárie. Entretanto, as superfícies oclusais continuam sendo os locais de maior experiência de cárie, representando 55 a 60% de todas as lesões na idade de 5 a 17 anos (Pereira *et al.*, 1996; Meneghin *et al.*, 1999; Mejare *et al.*, 2004).

É notório que fóssulas e fissuras apresentam uma característica morfológica singular, formando verdadeiros nichos para a retenção de biofilme e subseqüente colonização por microrganismos nesta superfície. Essas regiões se caracterizam por não se beneficiarem efetivamente do mecanismo tampão da saliva e pela ação tópica ou sistêmica do flúor presente nas águas de abastecimento, do uso de dentifrícios fluoretados ou da aplicação profissional de fluoretos, fato que potencializaria o processo de desmineralização do esmalte dentário (Nikiforuk, 1985). Além disso, deve-se considerar a dificuldade do debridamento mecânico na superfície oclusal (Tando *et al.*, 1989). Neste sentido, a maior suscetibilidade à cárie dentária parece estar relacionada ao acúmulo de biofilme na superfície oclusal dos molares, como demonstrado por Carvalho *et al*. (1989) que verificaram o maior acúmulo de biofilme na fossa mesial dos molares superiores e na fossa central dos molares inferiores.

Na busca por uma alternativa preventiva que impeça o início e/ou interrompa a progressão das lesões de cárie na superfície oclusal, o emprego de materiais que promovam a obliteração mecânica destas áreas têm sido utilizados desde 1967 (Cueto & Buonocore, 1967). A efetividade desse procedimento está intimamente relacionada às propriedades dos materiais seladores de fóssulas e fissuras oclusais. Estas incluem: biocompatibilidade, capacidade retentiva, resistência à abrasão e ao desgaste, resistência da união esmalte dentário/material, tensão superficial, viscosidade, adaptação marginal e penetração do material (Barrie *et al.*, 1990).

Diversos materiais vêm sendo desenvolvidos e propostos para o selamento de fóssulas e fissuras oclusais. Dentre eles estão os cimentos de ionômero de vidro (McLean & Wilson, 1977), os cimentos de ionômero de vidro modificados por resinas (Johnson *et al.*, 1995; Winkler *et al.*, 1996), materiais resinosos (Bowen, 1965), e os sistemas adesivos (Grande *et al.*, 2000).

Os selantes resinosos se unem mecanicamente a superfície de esmalte através dos monômeros polimerizados no interior dos poros do esmalte condicionado (*resin tags*), e dependendo do material podem ou não liberar fluoretos, podendo auxiliar o processo de remineralização. No entanto, estes apresentam propriedades mecânicas inferiores aos selantes resinosos (Kilpatrick *et al.*,1996; Forss & Halme,1998; Tyas *et al.*, 2000; Poulsen *et al.*, 2001). Já os cimentos de ionômero de vidro modificados por resina apresentam propriedades físicas similares às resinas compostas, enquanto mantêm as características básicas dos cimentos de ionômero de vidro (Almuammar *et al.*, 2001). Recentemente, os sistemas adesivos vêm sendo utilizados como selantes de fóssulas e fissuras devido à facilidade de aplicação e aos bons resultados clínicos e laboratoriais quando estes foram usados como uma camada intermediária entre o substrato dentário e o material selador ou isoladamente (Feigal *et al.*, 1993; Grande *et al.*, 2000).

É sabido que selantes podem ser utilizados eficientemente em crianças de alto risco à cárie desde que estes estejam retidos na superfície oclusal (Weintraub, 2001). A retenção dos materiais nesta superfície por um longo período de tempo, ou pelo menos durante o período crítico imediatamente após a irrupção dental é um dos fatores mais importantes para o bom desempenho clínico dos mesmos. No entanto, muitos dos materiais empregados têm demonstrado diferentes taxas de retenção (Hitt & Feigal, 1992; Forss & Halme,1998; Poulsen *et al*,2001; Weintraub, 2001). A baixa taxa de retenção poderia ser compensada pela presença de flúor residual do material remanescente no fundo da fissura, que liberado para a superfície de esmalte promoveria um efeito adicional na inibição da desmineralização (Seppä & Forss,1991; Hicks, 1998). Assim, a adição de fluoretos aos materiais seladores representaria uma opção viável para prevenir lesões de cáries em crianças de alto risco (Ripa, 1991).

Além disso, a incorporação dos fluoretos, presentes nos materiais seladores de fóssulas e fissuras, ao esmalte dentário poderia ser capaz de reduzir a solubilidade do esmalte e aumentar o potencial da estrutura dentária no processo de remineralização. A presença de fluoretos na superfície oclusal contribuiria ainda para a prevenção à cárie devido aos efeitos antimicrobianos (Featherstone *et. al.*, 1986).

Diversas pesquisas vêm sendo realizadas diante da variedade de materiais para selamento oclusal disponível no mercado. (Seppa & Forss, 1991; Hicks, 1998; Hicks *et al.*, 2000; Torii *et al.*, 2002; Hanning & Grafe, 2004). Dentre estes estudos Seppa & Forss (1991), Hicks (1998) e Hicks *et al.* (2000) investigaram a ação anticariogênica dos materiais indicados para o selamento oclusal simulando as condições orais de pacientes com alto risco à cárie. Entretanto, esses estudos analisaram a formação da lesão de cárie através da profundidade e da área da lesão (avaliações qualitativas). Esse método não traduz a variação do conteúdo mineral decorrente da inibição ou da formação da desmineralização do esmalte. Além disso, esses estudos, exceto o de Seppa & Forss (1991) avaliaram a perda mineral das superfícies de esmalte vestibular ou lingual, ou seja, superfícies lisas, onde a orientação dos prismas de esmalte acontece de maneira diferenciada em relação à superfície oclusal.

Neste contexto, na busca por um material específico que se adaptasse à superfície do esmalte, que possuísse as melhores propriedades retentivas e ainda que prevenisse o desenvolvimento da desmineralização do esmalte, em pacientes de alto risco, evidencia-se a necessidade da realização de uma avaliação quantitativa da perda de mineral da superfície oclusal e, portanto do desenvolvimento da lesão de cárie, e da degradação da união esmalte-material em situação de alto estresse, avaliando-se o desempenho de diferentes materiais seladores.

PROPOSIÇÃO GERAL

Esta Dissertação foi dividida em dois artigos que estão contemplados nos capítulos 1 e 2.

Os objetivos deste estudo foram:

1. Avaliar a formação de fendas na interface (adaptação marginal) de diferentes materiais utilizados como selantes de fóssulas e fissuras (selantes resinosos, cimentos de ionômero de vidro, cimentos de ionômero de vidro modificados por resina e sistemas adesivos), submetidos a severo estresse físico (termociclagem) e químico (ciclagem de pH)[1];

2. Avaliar *in vitro* o efeito da inibição da perda mineral do esmalte em dentes permanentes produzida pelos selantes oclusais que contém ou não fluoretos e verificar a capacidade destes materiais em liberar flúor[2].

Este trabalho foi realizado no formato alternativo, com base na deliberação da CCPG 001/98, da Universidade Estadual de Campinas (Unicamp).

[1] O artigo "Marginal adaptation of pit and fissure sealer materials after severe physical and chemical stress. A SEM study" foi enviado ao *Journal of Dentistry* (Capítulo 1).

[2] O artigo "Mineral loss inhibition of enamel sealed with fluoride- and non-fluoride-containing dental materials" foi enviado a *Caries Research* (Capítulo 2).

CAPÍTULO 1

MARGINAL ADAPTATION OF PIT AND FISSURE SEALER MATERIALS AFTER SEVERE PHYSICAL AND CHEMICAL STRESS. A SEM STUDY[3].

Short title: MARGINAL ADAPTATION OF SEALER MATERIALS INTERFACES

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[3] Enviado para publicação no *Journal of Dentistry***.**

Marginal adaptation of pit and fissure sealer materials after severe physical and chemical stress. A SEM study.

Summary

Objectives. This in vitro study evaluated the marginal adaptation (gap formation) in the fissure of different sealer materials (Resin sealant, Glass-ionomer cement, Resin-modified glass-ionomer cement, and adhesive system) submitted to physical and chemical stress, using Scanning Electron Microscopy evaluation (SEM).

Methods. Eighty impacted human third molars were randomly assigned to the following experimental groups (n=10): FluroShield (F), Helioseal Clear Chroma (H), Vitremer (V), Fuji II-LC (FII), Ketac Molar (KM), Fuji IX (FIX), Single Bond (SB), and Clearfil Protect Bond (CF). All groups were subjected to thermo cycling and 14 days of pH cycling. A blinded and calibrated examiner performed SEM analysis. Gap formation was scored according to: 0 - no sealant marginal gaps; 1 - sealant marginal gaps present or total sealant loss. The score 0 was considered a success, while scores 1 represented failure. Data were analyzed by Kruskal-Wallis and Bonferroni tests ($p<0.05$).

Results. The success rates of SB (100%) and V (90%) were statistically superior to CF (0%) ($p<0.05$). Materials such as FII (70%), KM (60%), F (30%), H (20%), and FIX (20%) presented no difference among themselves ($p>0.05$). The worst results were found for CF.

Conclusions. Single Bond and Vitremer materials provided the highest success rate of marginal adaptation to occlusal fissure walls and were able to support the stress conditions offered by this in vitro model.

Introduction

The anatomical pits and fissures of occlusal surfaces have been recognized as susceptible areas for the initial development of dental caries.[1] Their complex morphology is considered a site for the retention of bacteria and food remnants, rendering mechanical debridement inaccessible.[2] Another factor responsible for the high prevalence of occlusal caries is the lack of salivary access to fissures, reducing the effectiveness of fluoride.[3] Moreover, Brow et al.[4] and Kaste et al.[5] showed that, in fluoridated communities, over 90% of dental caries are exclusively pit and fissure lesions. Keeping in mind the proneness of occlusal surfaces towards caries, the maintenance of oral hygiene in conjunction with fluoride therapy and prudent use of pit and fissure sealants seems to be the best preventive strategy for children and adolescents at a high risk of caries, who present a high frequency of carbohydrate ingestion, and with oral environment conditions that oscillate between high and low pH levels.[1]

Resin-based fissure sealants have been introduced to prevent occlusal caries since 1967[6] and they demonstrated high protection against caries development on these surfaces.[7] Caries risk assessment in the child population is important for decision-making regarding sealant use. The benefit of sealant is increased by its placement on surfaces judged to be at a high-risk of caries development.[8] This factor is the most important when considering the dental material effectiveness.

An ideal fissure sealant should present biocompatibility, retention capacity, resistance to abrasion and wear, low surface tension, low viscosity, and provide marginal adaptation.[9] The key consideration to the success of the sealing procedures is adequate adhesion (e.g. adaptation and penetration of the material into the previously etched system of fissures). The penetration/adaptation in turn depends on the geometric configuration of fissures, the deposition of the material into the latter, and the physico-chemical characteristics of the sealer used.[9] It can be assumed that the sealant should penetrate reliably into the enamel, rendering this region beneath the sealant less prone to demineralization or caries attack in the event of sealant loss.[10]

Since their approval by the American Dental Association in 1971,[11] sealants have experienced a series of modifications in the materials used (cyanoacrylates, polyurethanes, polycarboxylates, Bis-GMA, glass ionomer cements, resin-modified glass-ionomer, adhesive systems),[12-16] surface treatment (conventional acid etching, acidic primer resin, and self-etching primer) and curing systems (chemical and light-curing systems). The sealants presently used in clinical practice are usually Bis-GMA based resins.[17] Resin sealants are usually placed after cleansing and phosphoric acid etching of the fissure enamel. The enamel etching removes surface contaminants and increases surface energy, turning this surface easily wettable. Enamel etching also creates an irregular surface topography of micropores and microprojections, where monomers can penetrate and polymerize creating a mechanical bonding with the tooth surface.[18,19]

An important parameter in the evaluation of the clinical success of sealant materials is the marginal adaptation, mainly at the sealant margin. Etching procedures might increase adhesion to enamel of sealant materials, allowing better marginal adaptation.[20] The presence of a marginal gap can lead to marginal staining, which can be considered the first sign of sealant failure.[21] Marginal gap may also imply that there is no occlusal surface isolation from oral microorganisms and, consequently, an increased risk for the development of dental caries.[7] Furthermore, the lack of internal adaptation might generate interfacial stresses that potentially cause de-bonding of the sealant to the tooth.[22] This marginal deficiency can be influenced by the high challenges in the oral environment in which the bonding is kept. Thermal stress has been highlighted as an important test for analyzing the material and its long-term performance.[23]

The aim of this in vitro study was to evaluate the effect of different material types when used as sealants (Resin sealant, Glass-ionomer cements, Resin-modified glass-ionomer cements, and Adhesive systems) on the gap formation on the marginal sealant submitted to physical and chemical stress, using Scanning Electron Microscopy (SEM). The null hypothesis is that there is no difference among the materials used in this study, concerning gap formation on sealant margin.

Materials and Methods

Eight materials used for sealing procedures, were tested. Their brand names, type, composition, manufacturers, and batch numbers are listed in Table 1.

This study was conducted after approval from the Ethical Committee of Piracicaba Dental School, University of Campinas (Protocol 089/2004). Eighty impacted human third molars, extracted for orthodontic reasons and free from apparent caries, macroscopic cracks, abrasions and staining on the occlusal surface (assessed by visual examination) were selected. The teeth were cleaned and stored in 0.5% Chloramine T solution for up 2 months after extraction. Their roots were sectioned off 1 mm under the cement enamel junction using a double-face diamond saw (KG Sörensen, São Paulo, SP, Brazil), and the pulpal chambers of all teeth were filled with resin composite.

Teeth were submitted to a prophylaxis using pumice slurry and randomly assigned to eight groups (n=10), according to the sealant material used. All materials were applied according to the manufacturer's instructions, as follows: **FluroShield Group - F**: The enamel surface was etched using 37% phosphoric acid (H_3PO_4) gel for 30 s, rinsed for 10 s, and dried. The material was applied and light cured for 40 s; **Helioseal Clear Chroma Group - H**: The enamel surface was etched using 37% phosphoric acid (H_3PO_4) gel for 30 s, rinsed for 10 s, and dried. The material was applied and light cured for 20 s; **Vitremer Group - V**: The enamel surface was treated with Vitremer Primer. The primer was applied using a brush for 30 s, air-dried, and light cured for 20 s. The material was applied, light cured for 40 s, and the surface was protected with Vitremer Finish Gloss and light cured for 20 s; **Fuji II-LC Group - FII**: The enamel surface was treated with GC cavity conditioner for 10 s, rinsed thoroughly with water, and dried. The material was applied and light cured for 20 s; **Ketac Molar Group - KM**: The enamel surface was treated with polyacrylic acid conditioner for 30 s, rinsed for 10 s, dried, and the material was applied. The surface was protected with appropriate varnish; **Fuji IX Group - FIX**: The enamel surface was treated with polyacrylic acid conditioner for 30 s, rinsed for 10 s, dried, and the material was applied. The surface was protected with appropriate varnish; **Single Bond Group - SB**: The enamel surface was etched using 35% phosphoric acid (H_3PO_4) gel for 15 s, rinsed for 10 s, and dried. The material was applied and light cured for 10s; **Clearfil Protect Bond Group - CF**: The enamel surface was etched using Clearfil Protect Bond primer for 20 s and dried with mild airflow. The Clearfil Protect Bond bond was applied and light cured for 10 s.

All sealing materials were applied with a sharp explorer in order to avoid excessive spreading of sealant, and light curing within the recommended time using the Elipar Tri-light unit (ESPE – America Co., Seefeld 82229 - Germany). Light intensity was periodically checked in the unit and was set on 580 mW/cm^2. The specimens were stored for 24 hours at 37°C and 100% humidity.

Using a digital caliper (Mitutoyo, Suzano, Brazil), an occlusal enamel exposed area was set with 16 mm^2 in the main occlusal fissure, at the center on each tooth (Fig.1). The tooth was identified and isolated with double coats of acid-resistant nail varnish (Colorama, São Paulo, Brazil), except for the occlusal-delimited area.

The teeth were subjected to thermo cycling (500 cycles of 5ºC and 55ºC with a dwelling time of 30 s) in distilled water. Afterwards, the acid-resistant varnish coating was reapplied. Then, the samples were subjected to a 14-day pH-cycling model, simulating a high cariogenic challenge according to Featherstone et al.[24] Each cycle consisted of a 6-hour immersion in demineralizing solution (DE) followed by an 18-hour immersion in remineralizing solution (RE). Each tooth was individually immersed in 40 mL of DE solution (2 mM calcium, 2 mM phosphate in 0.075 M acetate buffer, 0.02 μg F/mL, pH 4.3, 37°C), applied in the proportion of 2.5 mL/mm^2 of exposed enamel area. Teeth were then washed in deionized water for 30 s, dried with absorbent paper and individually immersed in 20 mL of RE solution (1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl in 0.1 M Tris buffer, 0.01 μg F/mL, pH 7.0, 37°C) used in the proportion of 1.25 mL/mm^2. Both solutions contained thymol crystals to avoid microbial growth. The solutions (DE and RE) were changed after 7 days.

For evaluation of the gap formation and sealant adaptation into the fissure systems, each tooth was longitudinally sectioned (Isomet, Buheler, Lake Bluff, IL, USA) in order to obtain a slice of 3 mm wide (including the occlusal-delimited area) perpendicular to the fissure orientation. One side of the slices was randomly selected. Impressions of the slices were taken with a low-viscosity polyvinyl siloxane material (Aquasil, Dentsply DeTrey, Konstanz, Germany). The impressions were poured with epoxy resin (Buehler, Lake Buff, IL, USA), gold-sputter coated (Balzers-SCD 050 Sputter Coater, Liechtenstein) and observed by SEM (JEOL- JSM 5600LV, Tokyo, Japan) at an accelerating voltage of 15kV, a working distance of 20 mm, and with a magnification of 60x.

A blind calibrated examiner evaluated the gap formation in the fissure twice, with a one-week interval between evaluations. Data were submitted to Sperman's correlation test and the intra-examiner coincidence level was 85%. The different groups were scored according to the ordinal scale: 0 - no sealant marginal gaps; 1 - sealant marginal gaps present or total sealant loss. The score 0 was considered a success, while score 1 was considered a failure.

The Kruskal-Wallis test was used for statistical analysis to determine differences among groups. The level of significance was set at $p<0.05$. A pair wise multiple comparison was performed by the post-hoc Bonferrroni test ($p<0.05$) (Analyse-it™ software program Ltd. - General + Clinical Laboratory Statistics VSM 1.71© 1977-2000).

Results

The score percentages of gap formation in the fissure for the eight groups are shown in Figure 2. It may be observed that Single Bond (100%) and Vitremer (90%) showed the highest success rate compared with Clearfil Protect Bond (0%) ($p<0.05$). The worst results were found for Clearfil Protect Bond, which demonstrated no specimens without gaps. The SEM photomicrographs (Fig.3A,B) illustrate a success score for Single Bond and Vitremer respectively, and a failure score for Clearfil Protect Bond (Fig.3C).

Discussion

Fissure sealants are currently one of the most effective tools available for protection against caries development in occlusal surfaces. The integrity of the tooth-sealer interface depends on a number of factors, such as the mechanical and chemical properties of the materials, the anatomy of the pit and fissures, the physical-chemical conditions of the oral cavity, and the operator technique.[25,26] The main aim of pit and fissure sealants is to ensure good retention of the sealant to the dental surface with a reduction in gaps at the material-enamel interface. Another important factor concerning sealants is the fluoride releasing ability of these materials when submitted to challenges in caries risk patients.[27] In this study, a caries risk situation was simulated by thermo - pH cycling models in order to submit materials to a high stress. These kinds of challenges can predict the long-term performance of the materials in margin sealing. SEM observation through tooth replicas can reveal gap formation, small fractures and degradation of sealant adhesion, indicating their failures.[2]

In this study, the null hypothesis was not accepted; there was a difference among the materials used as sealants, with respect to gap formation. Thus, marginal integrity can be influenced by the type of material used. With regard to the success score, the greatest performance, stated as no gaps presence (score 0) was demonstrated for Single Bond (100%) and Vitremer (90%). Even though these are different types of materials, their performance in preventing gaps in marginal sealants was similar. In this context, the success rate of the sealing procedure depends on the enamel treatment and the composition of the sealer material in order to resist the high physical and mechanical stress. It has been demonstrated that the filler contents, elastic modulus, photo-initiators, resin matrix, viscosity, and wettability greatly affect gap formation and the material adaptation into the fissure.[10,22,28]

Considering the adhesive systems used, the success rate of Single Bond was statistically superior to Clearfill Protect Bond. The results observed in this study demonstrated that the success rate of the sealing procedures seems to be dependent on not only the composition of sealer material, but also the enamel treatment, since adhesive systems usually present different adaptation performances on the enamel surface. The phosphoric acid etching used with Single Bond removes surface contaminants and creates an irregular, microporous enamel surface that is infiltrated by the Bis-GMA sealing material.[19] The enamel surface is changed from a low energy, weakly reactive, and a hydrophobic state to a high-energy, strongly reactive hydrophilic substrate, into which the sealant is attracted to flow.[29] Thereby, the prismatic enamel structure is exposed providing sufficient microretentive bonding of the fissure sealant.[30,31] In addition, the presence of HEMA, ethanol and water reduces the viscosity and generates a hydrophilic effect, allowing high wettability of Single Bond.

The above-mentioned characteristics allow easy penetration and adaptation of the uncured material into the fissures, enabling the complete filling of the fissure during the sealant application. Percinoto et al.[32] and Irinoda et al.[10] mentioned that sealants with a low viscosity had a greater potential to penetrate into the fissures and the microporosities produced in the enamel by etching with phosphoric acid. After polymerization of the sealing material, a durable bonding to the enamel surface is achieved by micromechanical retention via rheological and geometrical effects.[19] Single Bond, in this study, demonstrated a high performance with no gaps present in the hybrid area, even after thermo and chemical stress (Fig 2). A full adaptation/penetration of sealants into the etched enamel might improve long-term retention, prevent microleakage, impede the substrate to reach entrapped microorganisms or a (re-) colonization of fissures, and avoid subsurface porosity that might increase caries susceptibility in the event of sealant loss.[10]

The performance of resin-modified glass ionomer cements, Vitremer and Fuji II-LC were quite similar to Single Bond regarding gap formation under SEM evaluation. These materials, especially Vitremer primer, had acidic monomers that were able to etch the substrate and enhance the micromechanical retention.[33] Additionally, chemical adhesion plays a factor; the polyacrylate ions react with the apatite structure or bond directly to the calcium in the apatite. Clinical studies have confirmed the good retention rate of Vitremer.[34-36] These authors hypothesized that when the Vitremer's primer was applied to the fissure system, the wettability produced in the enamel surfaces improved material adaptation. This mechanism might increase the successful rate of sealant application. In the present study, Vitremer showed no gap formation on the interfacial zone after thermal and ph-cycling. This finding can be attributed to the good adhesion of this material to enamel.[37] However, the high viscosity and the presence of large filler particles can make the penetration and adaptation of this material into deep and sharp fissures difficult.

Interestingly, resinous sealants and glass ionomer cements demonstrated similar performance regarding gap formation. FluroShield and Helioseal Clear Chroma resin sealants demonstrate a low percentage of success regarding gap formation. The success rate of these materials was 30 and 20%, respectively. However, the sealing procedure used for these materials is quite similar to the one applied to the Single Bond sealing procedure; the enamel was etched with phosphoric acid, allowing the same pattern of substrate to bond. The presence of a higher quantity of filler particles makes the elastic modulus of FluroShield higher than that observed with Single Bond, giving them a higher viscosity, and increasing the probability of gap formation. In addition, the absence of solvent in their composition, leads to poorer wettability than that observed with Single Bond.

The glass-ionomer sealants also showed low results of success rate regarding gap formation. A recent clinical study[34] indicated a retention rate of 1.6% of glass ionomer sealants after 5 years. However, even with this low retention rate, the sealing with glass-ionomer cements was demonstrated to be effective in preventing caries. Other studies have observed that GICs provide some protection against a carious attack even after visible loss of material.[38,39] Thus, the fluoride release, besides the gap formation and retention must be considered for the good performance of GIC sealants, specifically in clinical studies. Further studies should be conducted in order to observe the ability of these materials to provide caries protection on occlusal surfaces even under chemical and physical stress.

The Clearfil Protect Bond showed the worst results, with no samples considered a success, although these results were comparable with those of resin sealants. Its primer contains phenyl-hydrogen-phosphate along with carbonic acid, but the concentration of acid esters is around 25% to 30%, at pH 2.[40] This primer produces mild morphological changes on the enamel with superficial resin layer penetration at the resin-enamel interface.[40] In contrast to phosphoric acid etching, conducted in the Single Bond group, the treatment with Clearfil Protect Bond primer does not etched properly enamel surface, preventing the permeation of the self-etching prime, leaving some areas partially unetched and more suitable to pH challenges.[29] In addition, a chemical reaction of calcium from hidroxiapatite and MDP take place on the enamel surface, when Clearfil Protect Bond is used. However, in this study, this chemical reaction was not enough to prevent the gap formations.

Furthermore, the etching pattern produced by the self etching primer of Clearfil Protect Bond is relatively shallower than that observed in the substrate etched with phosphoric acid, where thick tag-like extensions penetrate into etched enamel. This finding is supported by previous investigations that compared self-etching primers with total-etch systems.[41,42] These differences in etching capability can be attributed to differences in the composition of the self-etching primers, and could be the reason for the significantly lower success rate percentages for gap formation and sealant adaptation into the fissure, making them more susceptible to thermal and pH challenges.

This present study is in agreement with previous reports[19,20,30,31,43,44] that used dye penetration and SEM to evaluate the microleakage and internal seal of fissure sealants. These were replaced by the use of self-etching priming agents instead of phosphoric acid etching of enamel. It has been observed that the performance of self-etching bonding systems is inferior to that of the conventional acid etching technique, even though they are not submitted to high stress.

With regard to gap formation in the fissure, when sealed teeth were submitted to physical-chemical challenges, the best performances were achieved for Single Bond and Vitremer. The performance of these materials should be evaluated for secondary caries into occlusal fissures, since this condition is clinically observed in high caries risk children.

Conclusion

Within the limits of this study, the Single Bond and Vitremer sealant materials were able to support the stress conditions produced by this in vitro model. They proved to be effective in preserving the marginal adaptation in the enamel occlusal fissure.

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References

1. Vinnet D, Tandon S. Comparative evaluation of marginal integrity of two new fissure sealants using invasive and non-invasive techniques: a SEM study. *The Journal of Clinical Peaditric Dentistry* 2000; **24**:291-8.
2. Kaczmarek E, Matthews-Brzozowska T, Miskowiak B. Digital image analysis in dental research applied for treatment of fissures on occlusal surfaces of premolars. *Annals of Biomediacal Engineering* 2003; **31**:931-6.
3. Salama FS, Al-Hammad NS. Marginal seal of sealant and compomer materials with and without enameloplasty. *International Journal of Paediatric Dentistry* 2002; **12**:39-46.
4. Brown LJ, Kaste LM, Selwitz RH, Furman LJ. Dental caries and sealant usage in U.S. children, 1988-1991: selected findings from the Third National Health and Nutrition Examination Survey. *Journal of the American Dental Association* 1996; **127**:335-43.
5. Kaste LM, Selwitz RH, Oldakowski RJ, Brunelle JA, Winn DM, Brown LJ. Coronal caries in the primary and permanent dentition of children and adolescents 1-17 years of age:United States, 1988-1991. *Journal of Dental Research* 1996; **75**:631-41.
6. Cueto EI, Buonocore MG. Sealing of pits and fissures with an adhesive resin. Its use in caries prevention. *Journal of the American Dental Association* 1967; **75**:121-8.
7. Kunzel W, Fischer T, Lorenz R, Bruhmann S. Decline of caries prevalence after the cessation of water fluoridation in the former East Germany. *Community Dentistry and Oral Epidemiology* 2000; **28**:382-9.
8. Feigal RJ. The use of pit and fissure sealants. *Pediatric Dentistry* 2002; **24**:415-21.
9. Barrie AM, Stephen KW, Key EJ. Fissure sealants retention: A comparison of 3 sealants types under field conditions. *Community Dent Health* 1990; **7**:273-7.
10. Irinoda Y, Matsumura Y, Kito H, Nakano T, Toyama T, Nakagaki H, Tsuchiga T. Effect of sealant viscosity on the penetration of resin into etched human enamel. *Operative Dentisty* 2000; **25**:274-82.

11. Council on Dental Material and Devices, Council on Dental Therapeutics. Pit and fissure sealants. *Journal of the American Dental Association* 1971; **82**:1101-3.
12. Parkhouse RC, Winter GB. A fissure sealant containing methyl-2-cyanoacrylate as a caries preventive agent: A clinical evaluation. *British Dental Journal* 1971; **130**:16-9.
13. Powell KR, Craig RG. An in vitro investigation of the penetrating efficacy of Bis-GMA resin pit and fissure coating. *Journal of Dental Research* 1978; **57**:691-5.
14. Bowen RL. Adhesive bonding of various materials to hard tooth tissues II. Bonding to dentin promoted by a surface-active comonomer. *Journal of Dental Research* 1965; **44**:895-902.
15. Mc Lean JW, Wilson AD. Fissure sealing and filling with adhesive glass-ionomer cement. *British Dental Journal* 1974; **136**:269-76.
16. Grande RHM, Lima ACP, Rodrigues Filho LE, Witzel MF. Clinical evaluation of an adhesive used as a fissure sealant. *American Journal of Dentistry* 2000; **13**:167-70.
17. Pinar A, Sepet E, Aren G, Bolukbasi N, Ulukapi H, Turan N. Clinical performance of sealants with and without a bonding agent. *Quintessence International* 2005; **36**:355-60.
18. Buonocore MG. Simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *Journal of Dental Research* 1955; **34**:849-53.
19. Hannig M, Grafe A, Atalay S, Bott B. Microleakage and SEM evaluation of fissure sealants placed by use of self-etching priming agents. *Journal of Dentistry* 2004; **32**:75-81.
20. Perry AO, Rueggeberg F. The effect of acid primer or conventional acid etching on microleakage in a photoactivated sealant. *Pediatric Dentistry* 2003; **25**:127-31.
21. Pascon FM, Kantovitz KR, Caldo-Teixeira AS, Borges AFS, Silva TN, Garcia-Godoy F, Puppin-Rontani RM. Clinical evaluation of composite and compomer restorations in primary teeth: 24-month results *Journal of Dentistry* 2005; **17**:[epub ahead of print].
22. Kubo S, Yokota H, Yokota H, Hayashi Y. The effect of light-curing modes on the

microleakage of cervical resin composite restorations. *Journal of Dentistry* 2004; **32**:247-54.

23. Stavridakis MM, Favez V, Campos EA, Krejci I. Marginal integrity of pit and fissure sealants. Qualitative and quantitative evaluation of the marginal adaptation before and after in vitro thermal and mechanical stressing. *Operative Dentistry* 2003; **28**:403-14.
24. Featherstone JDB, O'Reilly NA, Shariati M, Brugler S. Enhancement of remineralization In vitro and in vivo. In: S.A. Leach, editors. Factors relating to demineralization and remineralization of the teeth. Proceedings of a Workshop 1985. IRL Press. Oxford: Elseviers; 1986. p.23-34.
25. Waggoner WF, Siegal M. Pit and fissure sealant application: updating the technique. *Journal of the American Dental Association* 1996; **127**:351-61.
26. Pérez-Lajarín L, Cortés-Lillo O, Garcia-Ballesta C, Hidalgo-Cózar A. Marginal microleakage of two fissure sealants: A comparative study. *ASDC Journal of Dentistry for Children* 2003; **70**:24-8.
27. Weintraud JA. Pit and fissure sealants in high-caries-risk individuals. *Journal of Dental Education* 2001; **65**:1084-9.
28. Sahafi A, Peutzfeldt A, Asmussen E. Soft-start polymerization and marginal gap formation in vitro. *American Journal of Dentistry* 2001; **14**:145-7.
29. Papacchini F, Goracci C, Sadek FT, Monticelli F, Garcia-Godoy F, Ferrari M. Microtensile bond strength to ground enamel by glass-ionomers, resin-modified glass ionomers, and resin composites used as pit and fissures sealants. *Journal of Dentistry* 2005; **33**:459-67.
30. Kanemura N, Sano H, Tagami J. Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. *Journal of Dentistry* 1999; **27**:532-30.
31. Hanning M, Bock H, Holth-Hanning W. Inter-crystallite nanoretention of self-etching adhesives at enamel imaged by transmission electron microscopy. *European Journal of Oral Sciences* 2002; **110**:464-70.
32. Percinoto C, Cunha RF, Delbem AC, Aragones A. Penetration of light-cured glass-ionomer and a resin sealant into occlusal fissures and etched enamel. *American*

Journal of Dentistry 1995; **8**:20-2.

33. Glasspoole EA, Erickson RL, Davidson CL. Effect of surface treatment on the bond strength of glass ionomers to enamel. *Dental Materials* 2002; **18**:454-62.
34. Pardi V, Pereira AC, Mialhe FL, Meneghim M de C, Ambrosano GM. A 5-year evaluation of two glass-ionomer cements used as fissure sealants. *Community Dentistry and Oral Epidemiology* 2003; **31**:386-91.
35. de Luca-Fraga LR, Pimenta LA. Clinical evaluation of glass-ionomer/resin-based hybrid materials used as pit and fissure sealants. *Quintessence International* 2001; **32**:463-8.
36. Pereira AC, Pardi V, Basting RT, Menighim MC, Pinelli C, Ambrosano GM, Garcia-Godoy F. Clinical evaluation of glass ionomers used as fissure sealants: twenty-four-month results. *Journal of Dentistry for Children* 2001; **68**:168-74.
37. Alonso RCB, Correr GM, Borges AFS, Kantovitz KR, Puppin-Rontani RM. Minimally invasive dentistry: bond strength of different sealant and filling materials to enamel. *Oral Health and Preventive Dentistry* 2005; **3**:87-95.
38. Hicks MJ, Flaitz CM, Silverstone LM: Secondary caries formation in vitro around glass ionomer restorations. *Quintessence International* 1986; **17**:527-32.
39. Seppa L, Forss H. Resistance of occlusal fissures to demineralization after loss of glass ionomer sealants in vitro. *Pediatric Dentistry* 1991; **13**:39-42.
40. Hipólito V, Goes MF, Carrilho MRO, Chan DCN, Daronch M, Sinhoreti MAC. SEM evaluation of contemporary self-etching primers applied to ground and unground enamel. *Journal of Adhesive Dentistry* 2005; **7**:203-11.
41. Torii Y, Itou K, Hikasa R, Iwata S, Nishitani Y. Enamel tensile bond strength and morphology of resin-enamel interface created by acid etching system with or without moisture and self-etching priming system. *Journal of Oral Rehabilitation* 2002; **29**:528-33.
42. Hashimoto M, Ohno H, Yoshida E, Hori M, Sano H, Kaga M et al. Resin-enamel bonds made with self-etching primers on ground enamel. *European Journal of Oral Sciences* 2003; **111**:447-53.
43. Perdigao J, Lopez L, Lambrechts P, Leitao J, Van Meerbeek B, Vanherle G. Effects

of a self-etching primer enamel shear bond strengths and SEM morphology. *American Journal of Dentistry* 1997; **10**:141-6.

44. Pradelle-Plasse N, Nechad S, Tavernier B, Colon P. Effect of dentin adhesives on the enamel-dentin/composite interfacial microleakage. *American Journal of Dentistry* 2001; **14**:344-8.

Table 1

Materials	Types	Composition	Manufacturers and Batch #
FluroShield	Resin sealant	Urethane modified Bis-GMA dimetacrylate; Barium aluminoborosilicate glass (30%), Polymerizable dimetacrylate resin, Bis-GMA, Sodium fluoride, Dipentaerythritol pentaacrylate phosphate, Titanium dioxide, Silica amourphous.	Dentsply, Germany # 317131
Helioseal Clear Chroma	Resin sealant	Bis-GMA, Triethylene glycol dimethacrylate (>99wt.%).Additional contents are stabilizers, catalyts and pigments (<1wt.%)	Ivoclar/Vivadent Schaan Liechtenstein # F54463
Vitremer	Resin-Modified glass-ionomer	Powder: fluoraluminosilicate glass, redox catalyst system, pigments Liquid: aqueous solution of a polycarboxylic acid modified with pedant methacrylate groups, Vitrebond copolymer, water, HEMA, photoinitiators. Primer: Vitrebond copolymer, HEMA, ethanol, photoinitiators.	3M/ESPE St. Paul, MN USA # 20020612
Fuji II – LC	Resin-Modified glass-ionomer	Powder: Alumino-silicate glass Liquid: Polyacrylic acid, 2-Hydroxyethyl methacrylate, proprietary ingredient, and trimethyl hexamethylene dicarbonate.	GC Co Tokyo, Japan # 0405281
Ketac Molar	Glass Ionomer	Powder: Aluminium-calcium-lanthanum-fluorisilicate glass, 5% polycarbonate acid Liquid: Polycarbonic acid and tartaric acid	3M/ESPE St. Paul, MN USA # 159323
Fuji IX	Glass Ionomer	Powder: polyacrylic acid and aluminosilicate glass Liquid: polyacrylic acid and proprietary ingredient.	GC Co Tokyo, Japan # 209271
Single Bond	Adhesive system	BisGMA, HEMA, Dimethacrylates, Ethanol, Water, Photoinitiator system, Methacrylate functional copolymer of polyacrilic and polyitaconic acids.	3M/ESPE St. Paul, MN USA # 4BM
Clearfil Protect Bond	Adhesive system	Primer: MDP, MDPB, HEMA, Hidrophobic methacrylate, water Bond: MDP, Bis-GMA, HEMA, Hidrophobic methacrylate, dI-Camphorquinone, N,N-Diethanol-p-toluidine, silanated colloidal silica, surface treated sodium fluoride	Kuraray Okayama, Japan # 61113

Figure 1

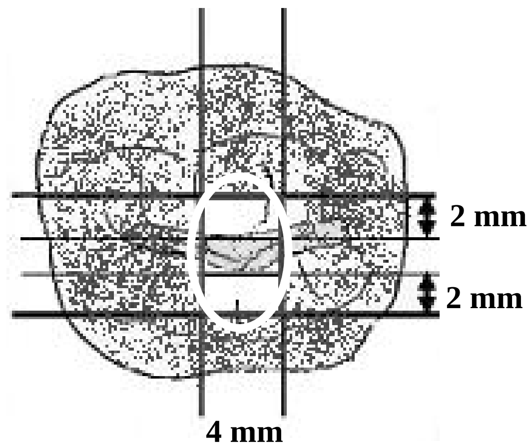
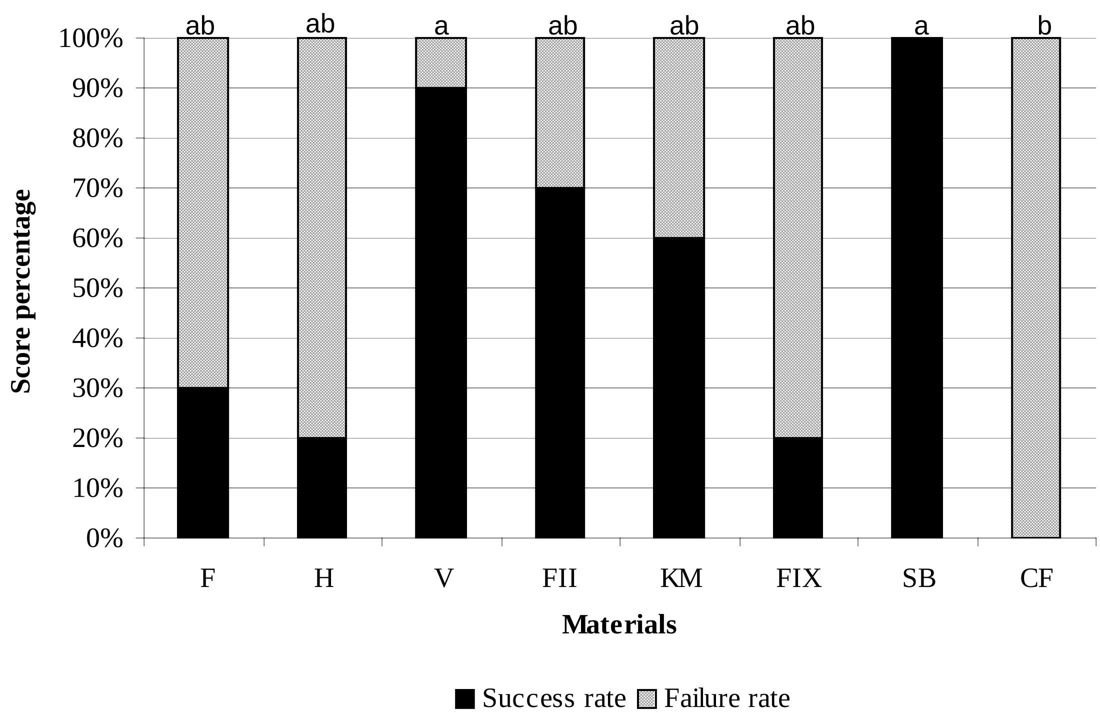
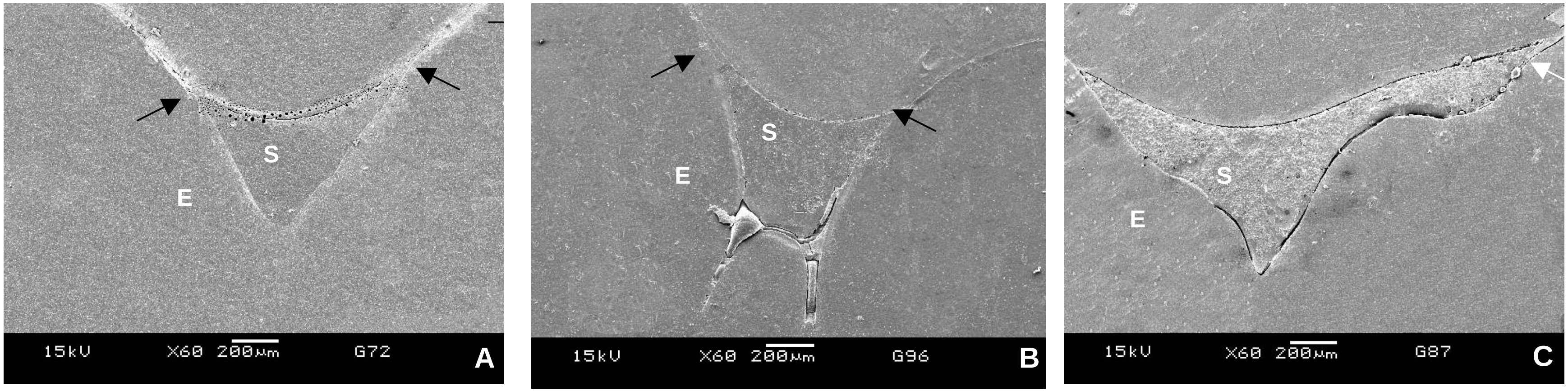


Figure 2



Different small letters represent statistically significant difference for Kruskal-Wallis and post-hoc Bonferroni tests ($p<0.05$).



Black arrows – no marginal gaps; white arrows – marginal gap of enamel/sealant; E – enamel; S – sealant.

Headings for Tables and Legends for Illustrations

Table 1 Brand, composition, batch number of the materials used in present study.

Figure 1 Occlusal enamel exposed area (16 mm^2) inside white circle.

Figure 2 Score percentages of success and failure rate (gap formation in the fissure) in groups.

Figure 3 - SEM photomicrography illustrating score rates for Single Bond (A) – score 0, Vitremer (B) – score 0, and Clearfil Protect Bond (C) – score 1.

CAPÍTULO 2

MINERAL LOSS INHIBITION OF ENAMEL SEALED WITH FLUORIDE- AND NON-FLUORIDE- CONTAINING DENTAL MATERIALS[4]

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Abstract

The aims of this in vitro study were to quantitatively evaluate the enamel mineral loss (EML) effect of fluoride- and non-fluoride-containing materials at different distances from the sealant margin and verify the fluoride releasing capability of these materials. Extracted molars were randomly assigned into 9 groups (n=12): Concise(C), FluroShield(F), Helioseal Clear Chroma(H), Vitremer(V), Fuji II-LC(FII), Ketac Molar(KM), Fuji IX(FIX), Single Bond(SB), and Clearfil Protect Bond(CF). All groups were subjected to thermo cycling and 14 days of pH cycling. EML was evaluated by cross-section microhardness analysis at the distances: -100µm, 0, 100µm, 200µm. The EML data were analyzed by a multi-factor ANOVA with slip-plot design. The results of the fluoride released into the cycling solutions after 7-14th days were submitted to ANOVA and Tukey tests. FIX demonstrated a lower EML than C, F, and H, but did not differ from the SB, CF, V, FII and KM, which also demonstrated no difference among them. C, F, H and V presented the highest EMLs, with no difference among them. V did not differ from the other groups ($p<0.05$). Regarding the different distances from the sealant margin, -100µm presented the lowest EML. FIX showed the highest fluoride release on the 7th as 14th days of evaluation, whilst CF demonstrated high fluoride release only on the 7th day. In conclusion, resin sealant did not prevent EML, in contrary to glass-ionomer cement, which showed the highest capacity for fluoride release. Only the presence of fluoride in a material's composition may not indicate a material's behavior with respect to its capability to interfere with the development of enamel caries-like lesions.

Introduction

Although, a considerable reduction in caries experience has occurred in children and adolescent populations in developed nations [Kaste et al., 1996], as well as in Brazil [Narvai et al., 2000], over the past several decades, subgroups of overall populations continue to experience high incidence of dental caries [Feigal, 2002]. Studies have demonstrated that the occlusal surfaces of first and second permanent molars are the sites most frequently struck by dental caries in 6- to 17-year-old patients [Hicks et al., 2000; Feigal 2002]. More importantly, it has been shown that, in fluoridated communities, over 90% of dental caries are exclusively pit and fissure caries [Brown et al., 1996; Kaste *et al.*, 1996]. This high prevalence of occlusal caries in children is due to the ease accumulation of bacteria and nutrients into the pits and fissures close to the dentin-enamel junction, and to the difficulty or inability of mechanical cleaning of this area [Tandon et al., 1989]. Its has been confirmed by the analyses of detailed mapping of plaque accomplished by Carvalho et al. [1989] that caries and plaque accumulation occur in the central fossa of mandibular and mesial fossa of maxillary molars. Thus, the development of effective preventive measures against pit and fissure caries is necessary.

Pit and fissure dental sealants are recognized as an important adjunct approach to prevent caries in the oclusal surface. Their safety and effectiveness were demonstrated in more than 30 years of research as a physical barrier formation, which prevents the metabolic exchange between the fissure microorganisms and the oral environment [Buonocore, 1955; McLean and Wilson, 1974; Grande et al., 2000; Weintraub, 2001]. In order to obtain long-term success with sealants, the first and perhaps the most important condition is the maintenance of a satisfactory retention of the material to enamel [Feigal et al., 2000].

In addition to the fact that sealants act by physically protecting vulnerable areas, the introduction of fluoride-releasing sealants has added another dimension to the prevention of pit and fissure caries [Ripa, 1991]. While resin-based sealants (Bis-GMA) act only by isolating enamel from a cariogenic challenge, depending on sealant retention on the occlusal surface, the fluoride-releasing sealants seem to provide another caries inhibition effect, since the fluoride inhibits demineralization and favors the remineralization processes [Featherstone, 1986; Tantibirojn et al., 1997; Hicks et al., 2000].

Different fluoride-releasing materials have been used as fissure sealants, such as glass ionomer cements (GIC) [Pereira et al., 2001], resin-modified GICs (RMGIC), fluoride-releasing composite sealants (FRCS) [Lobo et al., 2005], and adhesive systems [Jensen et al., 1990; Grande et al., 2000]. GICs have provided some protection against a carious attack even after visible loss of material [Hicks et al., 1986; Seppa and Forss, 1991]. The introduction of RMGICs improved retention, adherence, esthetics, manipulation and fluoride release, which could be observed for a prolonged time [Pereira et al., 2001; Poulsen et al., 2001]. However, some concerns have been raised regarding the fluoride release from the resin matrix of RMGICs [Mitra, 1991]. In vitro studies have investigated the effect of fluoride release by dental materials, such as GICs, RMGICs, FRCS, and adhesive systems in caries inhibition [Seppa and Forss, 1991; Hicks et al., 2000; Hicks and Flaitz, 2000]. However, these studies employed qualitative methods to evaluate demineralization on the enamel, since they considered the depth of artificial caries lesions and demineralization zone extension. Moreover, these studies, except those performed by Seppa and Forss [1991], evaluated mineral loss on flat buccal or lingual enamel surfaces, which are less susceptible to enamel demineralization than pit and fissures surfaces.

Consequently, an evaluation located on the pits and fissures that quantitatively measure the mineral loss of the enamel adjacent to the sealant should be considered. Therefore, the aims of this in vitro study were to quantitatively evaluate the effect of fluoride- and non-fluoride-containing occlusal sealants on enamel mineral loss of permanent teeth at different distances from the sealant margin and verify the fluoride releasing capability of these materials. It was first hypothesized that the fluoride-containing materials would allow lower mineral loss than the non-fluoride-containing materials. Additionally, the hypothesis was tested that fluoride-containing materials would release higher fluoride levels and, consequently, would promote a greater caries development inhibition effect.

Materials and Methods

Tooth Selection and Sampling

This study was conducted after approval of the Ethical Committee of Piracicaba Dental School, University of Campinas (protocol #089/2004). One hundred and eight impacted human third molars, extracted for orthodontic reasons and free from apparent caries, macroscopic cracks, abrasions and staining on the occlusal surface, assessed by visual examination, were selected. The teeth were cleaned and stored in 0.5% Chloramine T solution for up to 2 months after extraction. Their roots were sectioned off 1mm below the enamel cement junction using a double-face diamond saw (KG Sorensen, São Paulo, SP, Brazil) and the pulpal chambers of all teeth were filled with resin composite. The occlusal surface was cleaned with pumice/water slurry, and polished with a 5.0 μm alumina paste (Alpha Micropolish, Buehler, Lake Bluff, IL, USA).

Experimental design

Nine materials, indicated for sealing procedures, were tested. Their brand names, type, composition, manufacturers, and batch numbers are listed in Table 1.

Teeth were randomly distributed into nine groups (n=12), according to the sealant materials used as follow: Concise Group, FluroShield Group, Helioseal Clear Chroma Group, Vitremer Group, Fuji II-LC Group, Ketac Molar Group, Fuji IX Group, Clearfil Protect Bond Group and Single Bond Group.

Material placement and grouping

Materials were applied on the total pit and fissure extension following the manufacturer's instructions and are described as follows:

Concise Group - C: The enamel surface was etched using a 35% phosphoric acid (H_3PO_4) gel for 15 s, rinsed for 10 s, and dried; the material was applied to the pit and fissure using a probe; **FluroShield Group - F**: The enamel surface was etched using 37% phosphoric acid (H_3PO_4) gel for 30 s, rinsed for 10 s, and dried. The material was applied and light cured for 40 s; **Helioseal Clear Chroma Group - H**: The enamel surface was etched using 37% phosphoric acid (H_3PO_4) gel for 30 s, rinsed for 10 s, and dried. The material was applied and light cured for 20 s; **Vitremer Group - V**: The enamel surface was treated with Vitremer Primer. The primer was applied using a brush during 30 s, air-dried, and light cured for 20 s. The material was applied, light cured for 40 s, and the surface was protected with Vitremer Finish Gloss and light cured for 20 s; **Fuji II-LC Group - FII**: The enamel surface was treated with GC cavity conditioner for 10 s, rinsed thoroughly with water, and dried. The material was applied and light cured for 20 s; **Ketac Molar Group - KM**: The enamel surface was treated with polyacrylic acid conditioner for 30 s, rinsed for 10 s, dried, and the material was applied. The surface was protected with appropriated varnish; **Fuji IX Group - FIX**: The enamel surface was treated with polyacrylic acid conditioner for 30 s, rinsed for 10 s, dried, and the material was applied. The surface was protected with appropriate varnish; **Single Bond Group - SB**: The enamel surface was etched using 35% phosphoric acid (H_3PO_4) gel for 15 s, rinsed for 10 s, and dried. The material was applied and light cured for 10s; **Clearfil Protect Bond Group - CF**: The enamel surface was etched using primer for 20 s, dried with mild air flow, bond was applied, and light cured for 10 s.

Sealants were applied with a sharp explorer in order to avoid excessive spreading of sealant, and light cured within the recommended time, using a Elipar tri-light unit (ESPE - America Co., Seefeld 82229 - Germany). Light intensity was periodically checked in the unit and was set on 580 mW/cm^2. The specimens were stored for 24 hours at 37^oC and 100% humidity.

Using a digital caliper (Mitutoyo, Suzano, SP, Brazil) and a double layer of acid-resistant nail varnish (Colorama, São Paulo, SP, Brazil), an occlusal area of 4 x 4 mm (16 mm^2), with the main occlusal fissure at the center was delimited on each tooth.

Thermo- and pH-Cycling Regimens

The teeth were subjected to thermo cycling (500 cycles of 5ºC and 55ºC with a dwell time of 30 s) in distilled water. Afterwards, an acid-resistant varnish coating was reapplied to the previously varnished surfaces of the remaining occlusal delimited area. The samples were then subjected to a 14-day pH-cycling model, simulating a high cariogenic challenge according to Featherstone et al. (1986). Each cycle consisted of a 6-hour immersion in demineralizing solution followed by an 18-hour immersion in remineralizing solution. Teeth were individually immersed in 40 mL of a demineralizing solution (2 mM calcium, 2 mM phosphate in 0.075 M acetate buffer, 0.03 μg F/mL, pH 4.3, 37°C). The solution was applied in the proportion of 2.5 mL/mm^2 of exposed enamel area. Teeth were then washed in deionized water for 30 s, dried with absorbent paper and individually immersed in 20 mL of a remineralizing solution (1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl in 0.1 M Tris buffer, 0.05 μg F/mL, pH 7.0, 37°C) applied in the proportion of 1.25 mL/mm^2. Both solutions contained thymol crystals to avoid microbial growth. The solutions (demineralizing and remineralizing) were changed on the 7th day.

Analysis of Fluoride Release

The total of fluoride released by the dental materials during the pH-cycling regimens was analyzed on the 7th and 14th days. Fluoride measurements in demineralizing and remineralizing solutions were made in duplicate, using an ion-specific electrode (Orion 96-09) and an ion-analyzer (Orion EA-940, Orion Research, Boston, MA, USA), which had been previously calibrated in triplicate with fluoride standards (0.015 to 0.5 μg F/mL), in TISAB III.

Cross-Sectional Microhardness Analysis

Each tooth was longitudinally sectioned (Isomet, Buheler, Lake Bluff, IL, USA) in order to obtain a slice of 3 mm wide to include the occlusal-delimited area, perpendicular to the fissure orientation. One side of the slice was randomly selected and embedded in polystyrene resin (Piraglass, Piracicaba, SP, Brazil). The specimens were polished with 400-, 600- and 1200-grit Al_2O_3 paper (Arotec S.A. Ind. and Com., São Paulo, Brazil), and cloth polished with 1.0-μm diamond paste (Buheler Metadi II, Buheler, Lake Buff, IL, USA). Cross-sectional microhardness tests were performed using a Knoop diamond tip under a 25-g load for 5 s [Featherstone et al., 1983] (HMV 2000, Shimadzu, Tokyo, Japan). Four rows (-1, 0, 1 and 2) of twelve indentations each were made at the depths: 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 180 μm. Four rows were made at the sealant margin (row 0), at the distance of 100 μm below the margin (row -1), 100 μm and 200 μm above the margin (rows 1 and 2, respectively) (Fig 1). The Knoop hardness number units data (KHN) at rows –1, 0, 1, and 2 were obtained and converted into volume percentage mineral according to Featherstone et al. [1983], using the equation: volume% mineral = 4.3 $KHN^{1/2}$

+ 11.3. After calculating volume percentage mineral profiles, the mineral loss values (ΔZ) were obtained for all groups [Arends and ten Bosh, 1992].

Mineral profiles were obtained by linear function, passing through the twelve coordinated points of volume percentage mineral (Y axis) and depth under the outer surface (X axis). The area under the curve was calculated by the integral of the third order equation. Enamel mineral loss values (ΔZ) were obtained from the difference between the areas of the mineral profile of the lesion and the mineral profile of sound teeth. Volume percentage mineral was plotted against depth for each specimen and the integrated mineral content of the lesion was calculated. A mean of volume percentage mineral for depths greater than 100 μm was used as a measure of the integrated mineral content of inner sound enamel. To compute ΔZ parameters, the integrated mineral content of the lesion was subtracted from the value obtained for sound enamel [Pecharki et al., 2005].

Statistical Analysis

Originals data from enamel mineral loss means (ΔZ) were transformed (0.6 exponential) before applying ANOVA and Tukey tests, because variance was not homogeneous. A multi-factor ANOVA with slip-plot design was applied to the cross-sectional microhardness data to analyze the interactions between the factors (materials and distance from the sealant margin). In order to assess significant differences within these factors, Tukey test was applied. In addition, a Contrast test was performed to verify the difference among the type of materials. For fluoride release to de-remineralizing solutions on the 7^{th} and 14^{th} days, ANOVA and Tukey tests were applied in order to verify the difference among the dental materials in their fluoride-releasing capability. The software SAS system (version 8.02, SAS Institute Inc., Cary: NC, 1999) was used and the significance limit was set at 5%.

Results

The original averages and 95% confidence interval of the enamel mineral loss (ΔZ) for the nine groups are shown in Figure 2. According to the statistical analysis, multi-factor ANOVA showed no interaction between materials and distance from sealant margin (p=0.0775). There were significant differences among the materials and distance from sealant margin when Tukey test was applied ($p<0.0001$). The enamel mineral loss of FIX was significantly inferior to that of C, F, and H but not from the groups SB, CF, V, FII and KM (Figure 2). Materials such as SB, CF, FII and KM presented ΔZ values with no difference among them. C, F, H and V presented the highest enamel mineral losses, with no difference among them ($p>0.05$). V was not statistically different from any group ($p>0.05$). When types of materials were compared, statistical analysis showed significant differences among resin sealants vs. RMGICs (p=0.002), resin sealants vs. GICs ($p<0.001$), resin sealants vs. adhesive systems ($p<0.001$), and between RMGICs vs. GICs (p=0.01), as shown in Figure 2.

Figure 3 shows the averages and confidence intervals of the enamel mineral loss at different distances from the sealant margin (-1, 0, 1, 2). Statistical analyses showed significant differences among interfaces (-1) and the other distances from the sealant margin (0, 1, 2).

With regard to fluoride release during the pH cycling on the 7th day, FIX and CF were significantly superior to the materials. FII and KM presented intermediate levels of fluoride release and superior to C, F, H, V, and SB ($p<0.01$). Additionally, these materials presented no difference among each other ($p>0.05$). On the 14th day, there was also no significant difference between C, F, H, V, and SB. However, only FIX showed the highest fluoride release level. FII, KM, and CF presented intermediate levels of fluoride release (Figure 4).

Discussion

A cariostatic effect would be expected to occur as a function of integrating retention and fluoride-releasing properties in sealant materials. While the obliteration of pit and fissures by sealants provides the greatest degree of protection against pit and fissure caries, the addition of fluoride-releasing capabilities to sealants provides another method for further caries prevention [Ripa, 1991]. Fluoride is a worldwide recognized anticariogenic substance [Forss and Seppa, 1990; Benelli et al., 1993], and its release from a dental material may be effectively estimated in simulated caries procedures [Hellwig and Lussi, 2001]. In the present study, a de-remineralization cycling was used to reproduce a dynamic situation that is a direct function of conditions that maintain a critical pH in the mouth [Lobo et al., 2005]. The mineral loss model used establishes a correlation with the development of in vivo enamel mineral loss in situations of high cariogenic challenge [Featherstone, 1986].

The hypothesis that the enamel of the occlusal surface sealed with any of the fluoride-containing materials would show lower enamel mineral loss than that sealed with any of the non-fluoride-containing materials, was not accepted. Fluoride-containing materials, including Fuji IX, Ketac Molar, Fuji II LC, and Clearfil Protect Bond, had a significant effect on enamel mineral loss inhibition, in contrast to the other fluoride-containing materials, such as FluroShield and Vitremer. With respect to the effect of material at different distances from the sealant margin on mineral loss, it was observed that the enamel/sealant interface showed enamel mineral loss significantly lower values than those at the other distances. This may be related to the physical barrier properties of the materials used in this study, as shown by the absence of significant interaction between material/distance.

As expected, Concise and Helioseal Clear Chroma, which did not contain fluoride in their composition, did not affect the development of enamel mineral loss on permanent teeth enamel. This finding is in agreement with those of other studies [Mejare and Mjor, 1990; Lobo et al., 2005] that evaluated the effect of sealants on enamel demineralization. Surprisingly, FluroShield presented high enamel mineral loss and low levels of fluoride released during the pH-cycling regimen when compared to the other fluoride-containing materials (Figure 2,4). Other studies also showed low levels of fluoride release for FluroShield after two weeks of analysis [Cooley et al., 1990; Palma et al., 1994; Rock et al., 1996]. These results may be explained by the characteristics of resin sealant matrix, which is much less hydrophilic, making fluoride release more difficult [Preston et al., 2003], and slower after polymerization [Rock et al., 1996].

The resin sealants (Helioseal and FluroShield) showed the highest values of enamel mineral loss, differing from the other materials, except for the composite resin (Concise) and the RMGI (Vitremer). These four materials did not present statistically significant differences regarding the total levels of fluoride release on the 7th- and 14th-days (Figure 4). This finding suggests that the presence of fluoride in the material's composition alone does not indicate the material's behavior regarding its capability to interfere with the development of like-caries lesions in permanent enamel.

In this study, Single Bond and Clearfill Protect Bond showed a similar ability to reduce the enamel mineral loss despite the fact that the Clearfill Protect Bond released significantly more fluoride into the cycling solutions than Single Bond (Figures 2, 4). This may be attributed to the fact that adhesive systems are performed as a physical barrier, which isolates enamel from a cariogenic challenge. Thus, the success of pit and fissure sealant is dependent on maintaining an intact seal and it suggests possibly that the sealing ability of the materials may be more relevant to inhibit mineral loss than the fluoride releasing capacity.

In this study, RMGICs showed intermediate values of enamel mineral loss. Vitremer and Fuji II LC did not differ from each other, but showed a different profile. Interestingly, it was observed that different material brands from the same material classes, such as RMGICs, performed in a different manner. While Vitremer showed similar results for mineral loss inhibition to those of resin sealants, Fuji II-LC performed similar to GICs. It has been suggested that the fluoride release mechanisms of Fuji II-LC are similar to those of the GiCs [Yip and Smales, 2000]. Similar fluoride-releasing capacities were observed for both Fuji II-LC and Ketac Molar in this study. On the other hand, Vitremer showed no fluoride release at all (Figure 4); this may have occurred due to the type and amount of resin used for the light-curing reaction of Vitremer, and due to the finishing gloss used to coated the sealant, as recommended by manufacturer, that might have some influence on the fluoride releasing process [Momoi and McCabe, 1993]. Mathis and Ferracane [1989] assumed that in the set resin materials, fluoride ions might be firmly encapsulated by the resin matrix and consequently its fluoride release rate into an aqueous environment may be smaller and lower than that of conventional GICs.

The GICs (Fuji IX and Ketac Molar) produced the lowest enamel mineral loss values (ΔZ). GICs were able to interfere with the development of artificial caries lesions on the adjacent enamel to sealant because of the action of fluoride release [Forsten, 1990; Vermmersch et al., 2001], and the continual presence of low concentration of fluoride, which appears to inhibit demineralization and enhance remineralization [Featherstone, 1986]. Moreover, the differences in the composition of ionomeric and resinous materials can result in subsequent differences in fluoride-releasing levels [Glasspoole et al., 2001], and in enamel mineral loss. According to Asmussen and Peutzfeldt [2002], diffusion of water into the material is necessary for the formation of hydrogen ions, which attack the fluoride-containing glass particles, releasing fluoride. Ionomeric materials are more permeable to water, and this aspect would be expected to enhance fluoride diffusion and release.

Fluoride released from GICs concentrates on the enamel surface, can reduce enamel solubility [Tveit, 1980] and acid production by bacteria that initiate caries lesions [Maltz and Emilson, 1982]. These results are in line with those of Serra and Cury [1992], who used microhardness in a situation that simulated high caries risk around GIC restorations, and other studies [Hicks et al., 1986; Hattab et al., 1989; Tantbirojn et al., 1997; Hicks and Flaitz, 2000] that observed a significant reduction of lesions on enamel adjacent to the GIC. With regard to fluoride release, Fuji IX released 2.7x more fluoride than Ketac Molar (Figure 4). In spite of different fluoride releasing characteristics, both materials could be recommended for children with high caries risk.

These research data suggest that further evidence of the importance of fluoride release by sealant materials should be supplied by in situ and in vivo studies. These studies might be designed to analyze the effect of fluoride release on biofilm and also determine the minimum level of fluoride release required to obtain an anticariogenic action in the pit and fissure occlusal.

Within the limits of the present study, it can be concluded that resin sealant fluoride-containing or non-fluoride-containing did not prevent enamel mineral loss, suggesting the need to adopt additional preventive measures. On the other hand, glass-ionomer cement sealant demonstrated the lowest values of enamel mineral loss, even in a situation that simulated a high cariogenic challenge. The fluoride release level of the material was able to prevent enamel mineral loss, when Fuji IX was used. Moreover, the presence of fluoride in the material's composition should not be used as an indication of the material's behavior with regard to its capability to interfere with enamel mineral loss from permanent teeth.

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References

Arends J, Ten Bosch JJ: Demineralization evaluation techniques. J Dent Res 1992;71:924-928.

Asmussen E, Peutzfeldt A: Long-term fluoride release from a glass-ionomer cement, a compomer, and from experimental resin composites. Acta Odontol Scand 2002;60:93-97.

Benelli EM, Serra MC, Rodrigues Jr AL, Cury JA: In situ anticariogenic potential of glass ionomer cement. Caries Res 1993;23:280-284.

Brown LJ, Kaste LM, Selwitz RH, Furman LJ: Dental caries and sealant usage in U.S. children, 1988-1991: selected findings from the Third National Health and Nutrition Examination Survey. J Am Dent Assoc 1996;127:335-343.

Buonocore MG: A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res 1955;34:849-853.

Carvalho JC, Ekstrand KR, Thylstrup A: Dental plaque and caries on oclusal surfaces of first permanent molars in relation to stage of eruption. J Dent Res 1989;68:773-779.

Cooley RL, McCourt JW, Huddleston AM, Casmedes HP: Evaluation of a fluoride-containing sealant by SEM, microleakage and fluoride release. Pediatr Dent 1990;12:38-42.

Featherstone JDB, O'Reilly MM, Shariati M, Brugler S: Enhancement of remineralization in vitro and in vivo; in Leach S.A (ed): Factors relating to demineralization and remineralization of the teeth. Proceedings of a Workshop 1985. IRL Press. Oxford, 1986, pp 23-34.

Featherstone JDB, Ten Cate JM, Shariati M, Arens J: Comparision of artificial caries-like lesions by quantitative microradiograpfy and microhardness profiles. Caries Res 1983;17:385-391.

Feigal RJ: The use of pit and fissure sealants. Pediatr Dent 2002;24:415-422.

Feigal RJ, Musherure P, Gillespie B, Levy-Polack M, Quelhas I, Hebling J: Improved sealant retention with bonding agents: a clinical study of two-bottle and single-bottle systems. J Dent Res 2000;79:1850-1856.

Forss H, Seppa L: Prevention of enamel demineralization adjacent to glass-ionomer filling materials. Scand J Dent Res 1990;98:173-178.

Forsten L: Short and long term fluoride containing filling materials in vivo. Scand J Dent Res 1990;98:179-185.

Glasspoole EA, Erickson RL, Davidson CL: Demineralization of enamel in relation to the fluoride release of materials. Am J Dent 2001;14:8-12.

Grande RHM, Lima ACP, Rodrigues Filho LE, Witzel MF: Clinical evaluation of an adhesive used as a fissure sealant. Am J Dent 2000;13:167-170.

Hattab FN, Mok NY, Agnew EC: Artificially formed caries-like lesion around restorative materials. J Am Dent Assoc 1989;118:193-197.

Hellwig E, Lussi A: What is the optimum fluoride concentration needed for the remineralization process? Caries Res 2001;35:57-59.

Hicks MJ, Flaitz CM, Silverstone LM: Secondary caries formation in vitro around glass ionomer restorations. Quintessence Int 1986;17:527-532.

Hicks MJ, Flaitz CM: Resin-modified glass-ionomer restoration and in vitro secondary caries formation in coronal enamel. Dent Mater 2000;31:570-578.

Hicks MJ, Flaitz CM, Garcia-Godoy F: Fluoride-releasing sealant and caries-like enamel lesion formation in vitro. J Clin Pediatr Dent 2000;24:215-219.

Jensen OE, Billings RJ, Featherstone JDB: Clinical evaluation of FluroShield pit and fissure sealant. Clin Prev Dent 1990;12:24-27.

Kaste LM, Selwitz HR, Oldakowski RJ, Brunelle JA, Winn DM, Brown LJ: Coronal caries in primary and permanent dentition of children and adolescents 1-17 years of age: United States, 1988-1991. J Dent Res 1996;75:631-641.

Lobo MM, Pecharki GD, Tengan C, Silva DD, Tagliaferro EPS, Napimoga MH: Fluoride-releasing capacity and cariostatic effect provided by sealants. J Oral Science 2005;47:35-41.

Maltz M, Emilson CG: Susceptibility of oral bacteria to various fluoride salts. J Dent Res 1982;61:786-790.

Mc Lean JW, Wilson AD: Fissure sealing and filling with an adhesive glass-ionomer cement. Brit. Dent J 1974;136: 269-276.

Mejare I, Mjor IA: Glass- ionomer and resin based fissure sealants: A clinical study. Scand J Dent Res 1990;98:345-350.

Mathis RS, Ferracane JL: Properties of a glass-ionomer/resin composite hybrid materials. Dent Materials 1989;5:355-358.

Momoi Y, McCabe JF: Fluoride release from light-activated glass-ionomer restorative cements. Dent Materials 1993;9:151-154.

Mitra SB: In vitro fluoride release from a light-cured glass-ionomer liner/base. J Dent Res 1991;70:75-78.

Narvai PC, Castelhanos RA, Frasão P: The prevalence of caries on permanent teeth from shoolchildren at São Paulo City, 1970-1996 [in Portuguese]. Saúde Pública 2000;34:196-200.

Palma RG, Onishi AL, Tomita NE, Passanezzi S: Clinical evaluation of the levels of fluoride released from a fluoridated sealant [in Portuguese]. Rev Odontol Univ Sao Paulo. 1994;8:181-185.

Pecharki GD, Cury JA, Paes Leme AF, Tabchoury CP, Del Bel Cury AA, Rosalen PL, Bowen WH: Effect of sucrose containing iron (II) on dental biofilm and enamel demineralization in situ. Caries Res 2005;39:123–129.

Pereira AC, Pardi V, Basting RT, Menighim MC, Pinelli C, Ambrosano GM, Garcia-Godoy F : Clinical evaluation of glass-ionomers used as fissure sealants: twenty-four-mounth results. ASDC J Dent Child 2001;68:168-174.

Poulsen S, Beiruti N, Sadat N: A comparison of retention and the effect on caries of fissure sealing with a glass-ionomer and a resin-based sealant. Community Dent Oral Epidemiol 2001;29:298-301.

Preston AJ, Agalamanyi EA, Higham SM, Mair LH: The recharge of esthetic dental restorative materials with fluoride in vitro-two years' results. Dent Mater 2003;19:32-37.

Ripa LW: Dental materials related to prevention – Fluoride incorporation into dental materials: Reaction paper. Adv Dent Res 1991;5:56-59.

Rock WP, Foulkes EE, Perry H, Smith AJ: A comparative study of fluoride-releasing composite resin and glass ionomer materials used as fissure sealants. J Dent 1996;24:275-280.

Seppa L, Forss H: Resistance of occlusal fissures to demineralization after loss of glass ionomer sealants in vitro. Pediatr Dent 1991;13(1):39-42.

Serra MC, Cury JA: The in vitro effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. Quintessence Int 1992;23:143-147.

Tandon S, Kumari R, Udupa S: The effect of etch-time on the bond strength of a sealant and on the etch-pattern in primary and permanent enamel: an evaluation. ASDC J Dent Child 1989;56:186-190.

Tantbirojn D, Douglas WH, Versus A: Inhibitive effect of resin-modified glass ionomer cement on remote enamel artificial caries. Caries Res 1997;31:275-280.

Tveit AB: Fluoride uptake by cavity walls following application of Duraphat around amalgam restoration. J Oral Rehabilitation 1980;7:167-174.

Vermmersch G, Leloup G, Vreven J: Fluoride release from glass-ionmer cements, compomers and resin composites. Journal of Oral Rehabilitation 2001;28:26-32.

Yip HK, Smales RJ: Fluoride release from a polyacid-modified resin composite and 3 resin-modified glass-ionomer materials. Quintessence Int 2000;31:261-266.

Table 1

Materials	Types	Composition	Manufacturers and Batch #
Concise	Resin sealant	Bis-GMA, TEGDMA (78wt.%), Benzoyl peroxide, Tertiary amine of dimethy-paratoluidine, Titaninum dioxide and stable iron oxides	3M/ESPE St. Paul, MN USA # 18094
FluroShield	Resin sealant	Urethane modified Bis-GMA dimetacrylate; Barium aluminoborosilicate glass (30%), Polymerizable dimetacrylate resin, Bis-GMA, Sodium fluoride, Dipentaerythritol pentaacrylate phosphate, Titanium dioxide, Silica amourphous.	Dentsply, Germany # 317131
Helioseal Clear Chroma	Resin sealant	Bis-GMA, Triethylene glycol dimethacrylate (>99wt.%).Additional contents are stabilizers, catalyts and pigments (<1wt.%)	Ivoclar/Vivadent Schaan Liechtenstein # F54463
Vitremer	Resin-Modified glass-ionomer	Powder: fluoraluminosilicate glass, redox catalyst system, pigments Liquid: aqueous solution of a polycarboxylic acid modified with pedant methacrylate groups, Vitrebond copolymer, water, HEMA, photoinitiators. Primer: Vitrebond copolymer, HEMA, ethanol, photoinitiators.	3M/ESPE St. Paul, MN USA # 20020612
Fuji II - LC	Resin-Modified glass-ionomer	Powder: Alumino-silicate glass Liquid: Polyacrylic acid, 2-Hydroxyethyl methacrylate, proprietary ingredient, and trimethyl hexamethylene dicarbonate.	GC Co Tokyo, Japan # 0405281
Ketac Molar	Glass Ionomer	Powder: Aluminium-calcium-lanthanum-fluorisilicate glass, 5% polycarbonate acid Liquid: Polycarbonic acid and tartaric acid	3M/ESPE St. Paul, MN USA # 159323
Fuji IX	Glass Ionomer	Powder: polyacrylic acid and aluminosilicate glass Liquid: polyacrylic acid and proprietary ingredient.	GC Co Tokyo, Japan # 209271
Single Bond	Adhesive system	BisGMA, HEMA, Dimethacrylates, Ethanol, Water, Photoinitiator system, Methacrylate functional copolymer of polyacrilic and polyitaconic acids.	3M/ESPE St. Paul, MN USA # 4BM
Clearfil Protect Bond	Adhesive system	Primer: MDP, MDPB, HEMA, Hidrophobic methacrylate, water Bond: MDP, Bis-GMA, HEMA, Hidrophobic methacrylate, dI-Camphorquinone, N,N-Diethanol-p-toluidine, silanated colloidal silica, surface treated sodium fluoride	Kuraray Okayama, Japan # 61113

Figure 1

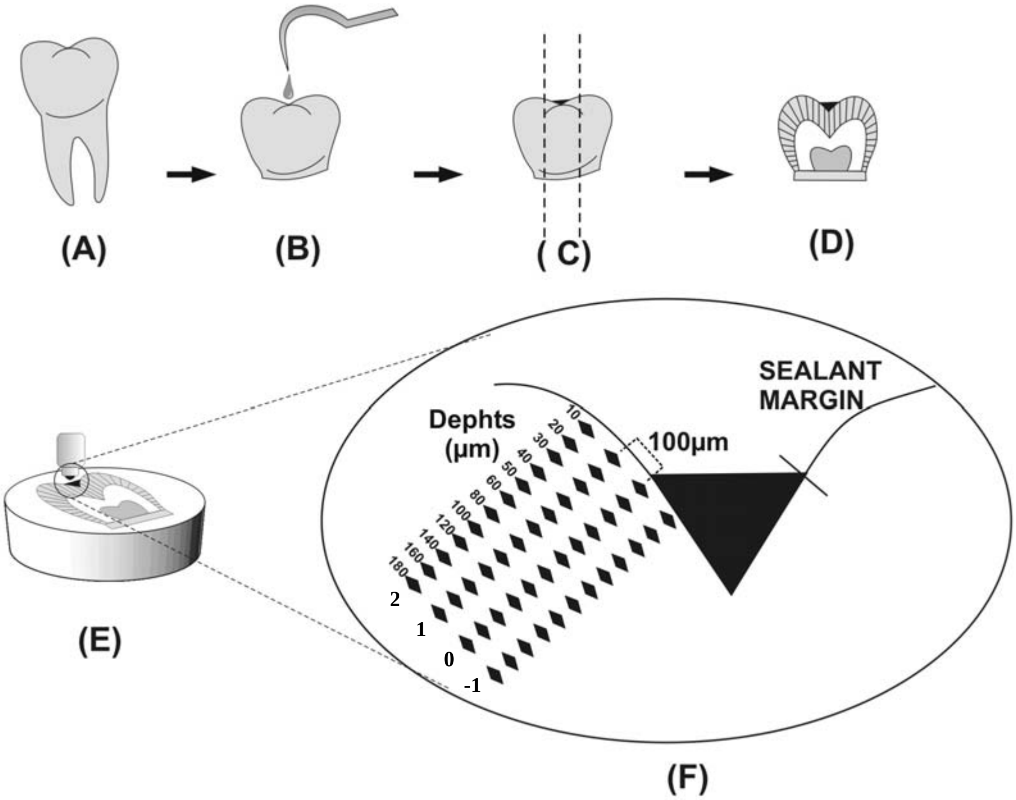
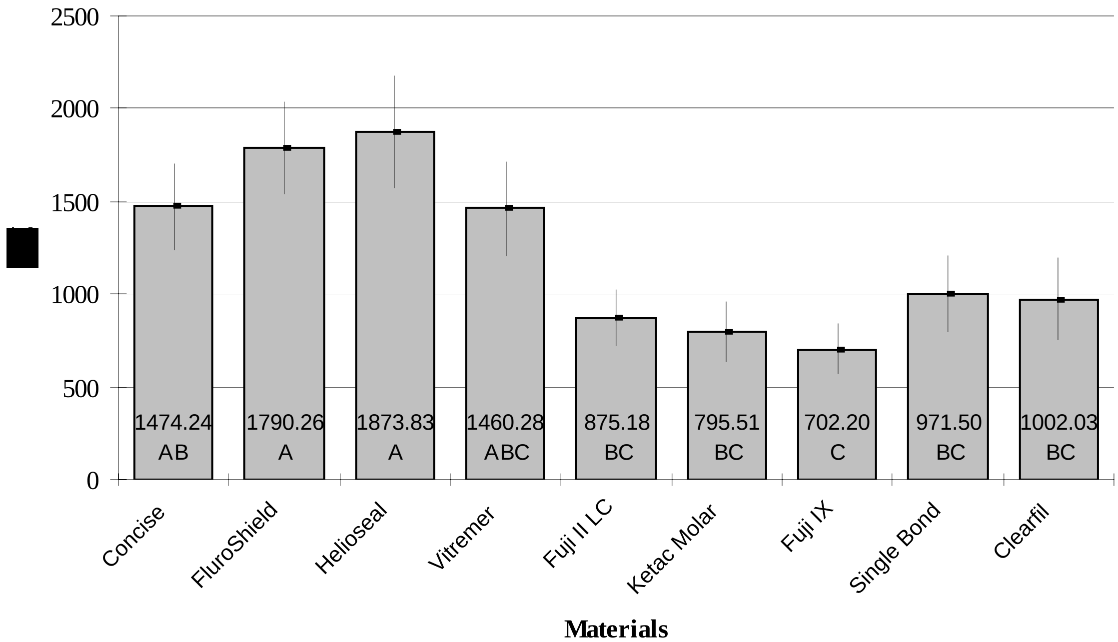
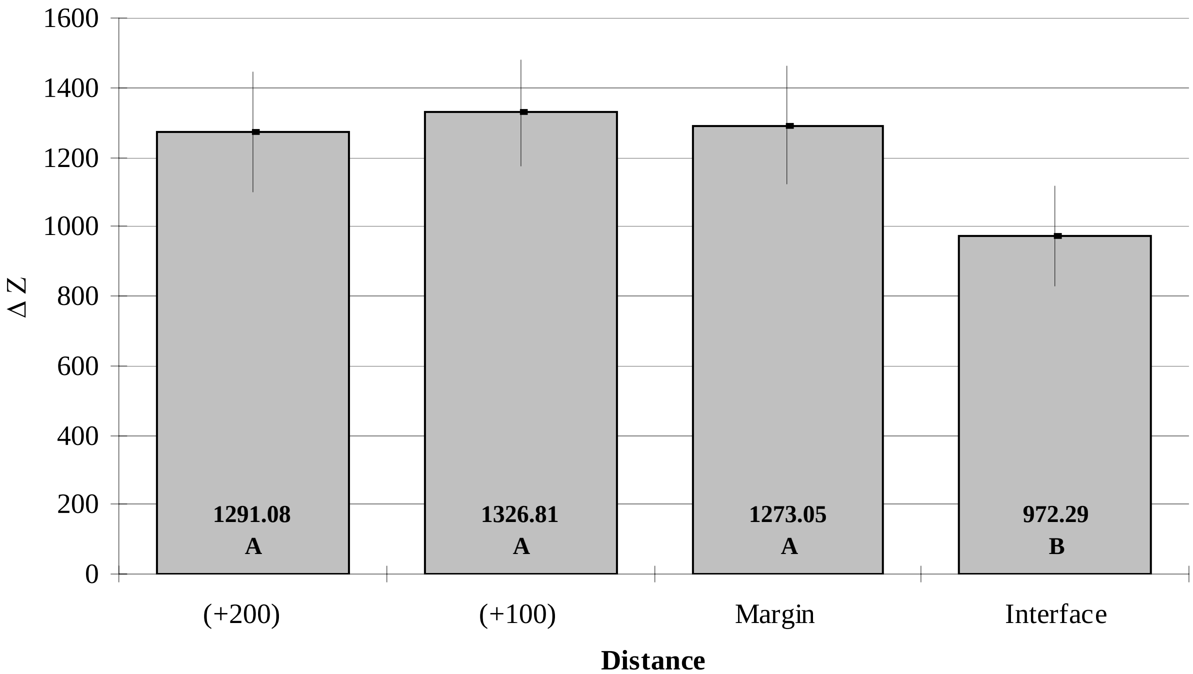

(A)
(B)
(C)
(D)
(E)
Dephts
(µm)
10
20
30
40
50
60
80
100
120
140
160
180
2
1
0
-1
100µm
SEALANT
MARGIN
(F)

Figure 2



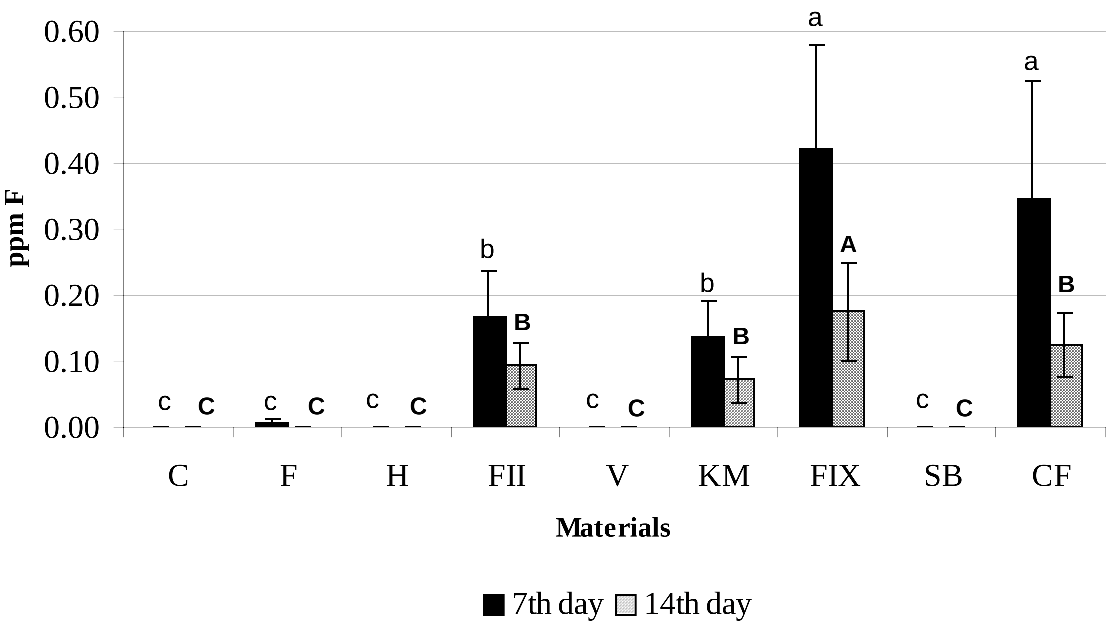
Different capital letters mean statistically significant difference for Tukey test ($p<0.05$) with 95% of confidence interval.

Figure 3



Similar capital letters mean no statistically significant difference for Tukey test ($p<0.05$).

Figure 4



Similar small letters at 7th day mean no significant statistical difference (p>0.05) as ANOVA and Tukey tests.

Similar capital letters at 14th day mean no significant statistical difference (p>0.05) as ANOVA and Tukey tests.

Headings for Tables and Legends for Illustrations

Table 1. Brand, composition, batch number of the materials used in present study.

Figure 1. Diagrammatic representation of cross-sectional micro-hardness assay. (A) Impacted human third molars. (B) Sealant application. (C) Tooth longitudinally sectioned to obtain a slice with 3 mm wide. (D) Obtained slice – mesial view. (E) Knoop-hardness indenter on the embedded slice. (F) Scheme of cross-sectional microhardness measurements. Four rows (-1, 0, 1 and 2) of twelve indentations each at depths 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 180 μm from surface enamel.

Figure 2. Original data of enamel mineral loss (ΔZ) mean and confidence intervals of ΔZ for comparations among materials.

Figure 3. Original data of enamel mineral loss (ΔZ) mean and confidence intervals of ΔZ for distance from sealant margin (n=12).

Figure 4. Total fluoride released during pH cycling (ppm F) according to type of materials, Means (SD; n=12).

CONSIDERAÇÕES GERAIS

O selamento oclusal tem sido utilizado há muitas décadas e tem-se relatado sucesso com a utilização desta técnica, contribuindo para o controle e prevenção das lesões de cárie no esmalte da superfície oclusal (Cueto & Buonocore, 1967).

Embora eficaz, deve-se ressaltar a indicação do selamento oclusal de molares permanentes em crianças de alto risco, para que se consiga otimizar a relação custo/benefício (Weintraub, 2001). Nesses pacientes, a utilização de materiais que liberam flúor e ainda que apresentem adesão à estrutura dentária tem sido recomendada. A eficácia da adesão de um material à estrutura do esmalte tem sido exaustivamente examinada, podendo-se chegar a materiais com altos valores de resistência de união aliada a baixas taxas de infiltração marginal (Alonso *et al.*, 2005) Entretanto, tem se observado a ausência de estudos que avaliem a eficácia da união entre o material selador e a estrutura do esmalte sob alto desafio cariogênico.

Neste estudo pôde-se observar que o Single Bond e o Vitremer, utilizados como selantes de fóssulas e fissuras, sob estresse térmico e químico, apresentaram as maiores taxas de sucesso quanto à adaptação marginal e da interface esmalte/material. A retenção, o embricamento micro-mecânico, na superfície do esmalte, e ainda a penetração do material na fissura são aspectos de relevância para sucesso deste tratamento preventivo, pois se encontram relacionados às altas taxas de prevenção de cárie oclusal (Weintraub, 2001). Deve-se levar em consideração que a retenção micro-mecânica aliada às características físicas dos materiais utilizados para o selamento oclusal, como a baixa viscosidade e, portanto a alta capacidade de molhamento como a apresentada pelo Single Bond, aplicado com o auxílio do condicionamento ácido do esmalte, permitiu adequado embricamento micro-mecânico, protegendo a superfície do esmalte do desafio químico. O Vitremer por sua vez, utilizado com o primer à base de HEMA, permitiu a penetração no esmalte garantindo o embricamento mecânico. Além disso, a ausência de *gaps* considerada como um importante parâmetro indicativo de sucesso clínico do material selador, principalmente nas margens, foi mantida. Selantes sem adaptação marginal podem levar a alteração de cor marginal, à falta de isolamento da superfície oclusal frente aos microrganismos e fluidos orais, e conseqüentemente à lesão de cárie (Kubo *et al.*, 2004). Assim, o Single Bond e o Vitremer provaram ser efetivos na preservação da interface material selador/superfície oclusal do esmalte e da adaptação marginal (Capítulo 1).

Entretanto, o que se questiona é que o efeito do material selador, na prevenção da lesão cariosa em fissuras oclusais, não apenas dependerá da capacidade retentiva do material ao esmalte dentário, mas também da capacidade de interferir no desenvolvimento de cárie oclusal. Uma das formas de se intensificar o efeito da retenção mecânica dos materiais seladores sobre a capacidade preventiva foi à inclusão de algumas formas de fluoretos na composição dos materiais, como o NaF, fluorsilicato de bário, etc. Alguns estudos investigaram o efeito da liberação de flúor desses materiais odontológicos na inibição do desenvolvimento de cárie (Hicks, 1998; Hicks & Flaitz, 2000), embora usando métodos qualitativos para avaliar a desmineralização do esmalte das superfícies vestibular ou lingual.

O presente trabalho avaliou ainda o impacto de materiais seladores, que contêm ou não flúor na composição, na perda mineral do esmalte (Capítulo 2), em superfícies oclusais. Um dado interessante observado foi a proteção oferecida pelos materiais na região subdjacente aos mesmos, isto é, imediatamente sob o material. Ainda, pode-se observar que os selantes resinosos, independente de apresentarem flúor na composição, apresentaram a menor capacidade de proteção do esmalte oclusal, em qualquer distância do material. Os resultados demonstraram que, surpreendentemente, um dos materiais que apresentaram a melhor adaptação marginal, não foram capazes de interferir na perda mineral do esmalte quando exposto ao desafio cariogênico. Pôde-se verificar que esses materiais não exibiram liberação de flúor durante o período do experimento. Entretanto, considerando-se o material selador Fuji IX (selante ionomérico), embora com insatisfatória adaptação à superfície do esmalte, a quantidade de flúor liberada foi capaz de prevenir a perda mineral do esmalte oclusal exposto às mesmas situações anteriormente mencionadas. Os selantes ionoméricos revelaram os menores valores de perda mineral do esmalte e as piores adaptações da interface esmalte/material. Assim, os resultados deste estudo foram conclusivos em demonstrar que apenas a presença de fluoretos na composição dos selantes de fóssulas e fissuras oclusais não pode predizer a capacidade do material de interferir na perda mineral do esmalte de dentes permanentes exposto a este modelo *in vitro*.

A indicação clínica de um determinado material selador deve ser cautelosa. Se por um lado materiais que podem ser usados como selantes oclusais, como Vitremer, apesar de possuírem características físicas que favorecem a adaptação nas fóssulas e fissuras, agindo como barreira física, como discutido no capítulo 1, pode não ser capazes de interferir na perda mineral de esmalte (Capítulo 2). Em adição, materiais como Fuji IX, Ketac Molar, Fuji II LC e Clearfil Protect Bond (selante com flúor na composição) os quais apresentaram significante efeito na inibição da perda mineral do esmalte demonstraram baixas taxas de sucesso na adaptação do material à superfície de esmalte oclusal.

Neste sentido, sugere-se a necessidade de associação de outros métodos preventivos quando do uso de selantes resinosos, como o uso de dentifrícios fluoretados, a supervisão do controle do biofilme bacteriano, bem como a supervisão e controle do dente selado. Por outro lado, sabe-se que a liberação de flúor de um material, mesmo sendo um cimento de ionômero de vidro, não é longa e devido à falta de adaptação destes materiais à superfície do esmalte, bem como a lixiviação que este material permite devido à alta solubilidade que esses cimentos apresentam, eles devem ser utilizados com cautela como materiais seladores, e da mesma forma, acompanhados através de exames clínicos e associados a outras medidas preventivas.

CONCLUSÃO GERAL

Diante dos objetivos do presente estudo, concluiu-se que:

1. Os materiais Single Bond (sistema adesivo) e Vitremer (cimentos de ionômero de vidro modificados por resina) utilizados como selantes de fóssulas e fissuras oclusais, foram capazes de suportar as condições de estresse físico (termociclagem) e químico (ciclagem de pH) oferecidos pelo modelo *in vitro* proposto. Estes materiais provaram ser efetivos na preservação da interface material selador/superfície oclusal do esmalte e da adaptação marginal,

2. Selantes ionoméricos revelaram os menores valores de perda mineral de esmalte mesmo em situação de desafio cariogênico, quando comparados aos selantes resinosos. Considerando o material selador Fuji IX, a quantidade de flúor liberado foi capaz de produzir menor perda mineral do esmalte oclusal, em comparação aos selantes resinosos.

3. Apenas a presença de fluoretos na composição dos materiais utilizados como selantes de fóssulas e fissuras oclusais não pode predizer a capacidade dos mesmos em interferir na perda mineral do esmalte de dentes permanentes exposto ao modelo *in vitro* utilizado neste estudo.

REFERÊNCIAS BIBLIOGRÁFICAS[5]

1. Almuammar MF, Schulman A, Salama FS. Shear bond strength of six restorative materials. ***J Clin Pediatr Dent.*** 2001; 25(3): 221-5.
2. Alonso RCB, Correr GM, Borges AFS, Kantovitz KR, Puppin-Rontani RM. Minimally invasive dentistry: bond strength of different sealant and filling materials to enamel. ***Oral Health Prev Dent. .*** 2005; 3(2): 87-95.
3. Barrie AM, Stephen KW, Key EJ. Fissure sealants retention: A comparison of 3 sealants types under field conditions. ***Community Dent Health.*** 1990; 7(3): 273-7.
4. Bowen RL. Adhesive bonding of various materials to hard tooth tissues II. Bonding to dentin promoted by a surface-active co monomer. ***J Dent Res.*** 1965; 44(5): 895-902.
5. Carvalho JC, Ekstrand KR, Thylstrup A. Dental plaque and caries on oclusal surfaces of first permanent molars in relation to stage of eruption. ***J Dent Res.*** 1989; 68(5): 773-9.
6. Cueto EI, Buonocore MG. Sealing of pits and fissures with an adhesive resin. Its use in caries prevention. ***J Am Dent Assoc.*** 1967; 75(1): 121-8.
7. Featherstone JDB, O'Reilly NA, Shariati M, Brugler S. Enhancement of remineralization in vitro and in vivo. In: Factores relating to demineralization and remineralization of the teeth. Oxford; S. A. Leach, ED; 1986.
8. Feigal RJ, Hiit J, Splieth C. Retaining sealant on salivary contaminated enamel. ***J Am Dent Assoc.*** 1993; 124(3): 88-97.
9. Forss H. & Halme E. Retention of glass ionomer cement and resin based fissure sealant and effect on carious outcome after 7 years. ***Community Dent Oral Epidemiol.*** 1998; 26(1): 21-5.
10. Grande RH, Lima ACP, Rodrigues Filho LE, Witzel MF. Clinical evaluation of an adhesive used as a fissure sealant. ***Am J Dent.*** 2000; 13(4): 167-70.
11. Hannig M, Grafe A, Atalay S, Bott B. Microleakage and SEM evaluation of fissure sealants placed by use of self-etching priming agents. ***J Dent.*** 2004; 32(1): 75-81.

[5] De acordo com a norma da FOP/ UNICAMP, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o MEDLINE.

12. Hicks J, Garcia-Godoy F, Milano M, Flaitz C. Compomer materials and secondary caries formation. ***Am J Dent.*** 2000; 13(5): 231-4.
13. Hicks MJ. Caries formation in vitro around a fluoride-releasing pit and fissure sealant in primary teeth. ***ASDC J Dent Child.*** 1998; 65(3): 161-8.
14. Hicks MJ, Flaitz CM. Resin-modified glass-ionomer restoration and in vitro secondary caries formation in coronal enamel. ***Dent Mater.*** 2000; 31(8): 570-8.
15. Hitt J, Feigal RJ. Use of bonding agent to reduce sealant sensibility to moisture contamination: an in vitro study. ***Pediatr Dent.*** 1992; 14(1): 41-6.
16. Johnson LM, Duke ES, Camm J, Hermesch CB, Buikema DJ. Examination of a resin-modified glass-ionomer material as a pit and fissure sealant. ***Quintessence Int***. 1995; 26(12): 879-83.
17. Kilpatrick NM, Murray JJ, Mc Cabe JF. A clinical comparison of a light glass ionomer sealant restoration with a composite sealant restoration. ***J Dent.*** 1996; 24(6): 399-405.
18. Kubo S, Yokota H, Yokota H, Hayashi Y. The effect of light-curing modes on the microleakage of cervical resin composite restorations. ***J Dent.*** 2004; 32(3): 247-54.
19. Mejare I, Stenlund H, Zelezny-Holmund C. Caries Incidence and lesion progression from adolescence to young adulthood: A prospective 15-year cohort study in Sweden. ***Caries Res.*** 2004; 38(2): 130-41.
20. McLean JW**,** Wilson AD**.** The clinical development of the glass-ionomer cement. II. Some clinical applications. ***Aust Dent J.*** 1977; 22(2):120-7.
21. Meneghim MC et al. Importância do primeiro molar permanente na determinação do índice CPOD. ***J Bras Odontopediatr Odontol Bebê*** 1999; 2(5): 37-41.
22. Nikiforuk G. Understanding Dental Caries In: Prevention, Basic and Clinical Aspects. New York: Karger;1985 p.145-173.
23. Pereira AC, Castellanos RA, da Silva SR, Watanabe MG, Queluz DP, Meneghim MC. Oral health and periodontal status in Brazilian elderly. ***Braz Dent J.*** 1996; 7(2): 97-102.
24. Poulsen S, Beiruti N, Sadat N. A comparison of retention and the effect on caries of fissure sealing with a glass-ionomer and a resin-based sealant. ***Community Dent.***

Oral Epidemiol. 2001; 29(4): 298-301.

25. Ripa LW. Dental materials related to prevention – Fluoride incorporation into dental materials: Reaction paper. ***Adv Dent Res.*** 1991; 5: 56-9.
26. Seppa L, Forss H. Resistance of occlusal fissures to demineralization after loss of glass ionomer sealants in vitro. ***Pediatr Dent.*** 1991; 13:39-42.
27. Tandon S, Kumari R, Udupa S: The effect of etch-time on the bond strength of a sealant and on the etch-pattern in primary and permanent enamel: an evaluation. ***ASDC J Dent Child***. 1989; 56(3): 186-90.
28. Torii Y, Itou K, Hikasa R, Iwata S, Nishitani Y. Enamel tensile bond strength and morphology of resin-enamel interface created by acid etching system with or without moisture and self-etching priming system. ***J Oral Rehabil.*** 2002; 29(6): 528-33.

28. Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ. Minimal intervention dentistry--a review. FDI Commission Project 1-97. ***Int Dent J.*** 2000; 50(1): 1-12.

29. Weintraub JA. Pit and fissure sealants in high-caries-risck individuals. ***J Dent Educ.*** 2001; 65(10): 1084-90.
30. Winkler MM, Deschepper EJ, Dean JA, Moore BK, Cochran MA, Ewoldsen N. Using a resin-modified glass ionomer as an occlusal sealant: a one-year clinical study. ***J Am Dent Assoc.*** 1996; 127(10): 1508-14.

ANEXOS

COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

UNICAMP

CERTIFICADO

Certificamos que o Projeto de Pesquisa "*Inibição de cárie produzida por materiais resinosos e ionoméricos em fissuras oclusais – Estudo da microdureza e infiltração marginal*", Protocolo CEP nº ***089/2004***, dos Pesquisadores ***Kamila Rosamiglia Kantovitz, Marines Nobre dos Santos Uchoa*** e ***Regina Maria Puppin Rontani***, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde - MS e foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Odontologia - UNICAMP.

We certify that the research project "Caries inhibition produced by resin and ionomer materials in occlusal fissures – microhardness and marginal infiltration study", Register Number ***089/2004*** of ***Kamila Rosamiglia Kantovitz, Marines Nobre dos Santos Uchoa*** and ***Regina Maria Puppin Rontani***, is in agreement with the recommendations of 196/96 Resolution of the National Health Committee - Brazilian Health Department and was approved by the Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas - UNICAMP.

Piracicaba - SP, Brazil, August 18 2004

Cinthia Machado Tabchoury

Profa. Dra. Cinthia Pereira Machado Tabchoury

Secretaria

CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Júnior

Coordenador

CEP/FOP/UNICAMP

DELIBERAÇÃO CCPG – 001/98

Dispõe a respeito do formato das teses de Mestrado
e de Doutorado aprovadas pela UNICAMP

Tendo em vista a possibilidade, segundo parecer PG Nº 1985/96, das teses de Mestrado e Doutorado terem um formato alternativo àquele já bem estabelecido, a CCPG resolve:

Artigo 1º - Todas as teses de mestrado e de doutorado da UNICAMP terão o seguinte formato padrão:

I) Capa com formato único, dando visibilidade ao nível (mestrado e doutorado), e à Universidade.
II) Primeira folha interna dando visibilidade ao nível (mestrado ou doutorado), à Universidade, à Unidade em foi defendida e à banca examinadora, ressaltando o nome do orientador e co-orientadores. No seu verso deve constar a ficha catalográfica.
III) Segunda folha interna onde conste o resumo em português e o Abstract em inglês.
IV) Introdução Geral.
V) Capítulo.
VI) Conclusão geral.
VII) Referências Bibliográficas.
VIII) Apêndices (se necessários).

Artigo 2º - A critério do orientador, os Capítulos e os Apêndices poderão conter cópias de artigos de autoria ou de co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, escritos no idioma exigido pelo veículo de divulgação.

Parágrafo único – Os veículos de divulgação deverão ser expressamente indicados.

Artigo 3º - A PRPG providenciará o projeto gráfico das capas bem como a impressão de um número de exemplares, da versão final da tese a ser homologada.

Artigo 4º - Fica revogada a resolução CCPG 17/97.

Figura 1: Materiais utilizados neste estudo*.

A: Concise

B: FluroShield

C: Helioseal Clear Chroma

D: Vitremer

E: Fuji II-LC

F: Ketac Molar

G: Fuji IX

H: Single Bond

I: Clearfil Protect Bond

* Informações quanto à composição, fabricantes e lotes dos materiais utilizados estão descrito nas páginas 25 e 46.


A
3M
Concise™
A
6g
3M
Concise™
B
6g
B
Dental Gel
VLC
C
Helioseal® Clear Chroma
1.25 g
2005-09
D
E
GC Fuji II LC
POWDER 15g
A3
F
Ketac Molar
G
GC Fuji IX GP
1-1 PKG.
GP 9
GC Fuji IX GP LIQUID 3g (2.4mL)
GC Fuji IX GP POWDER 5g A2
H
Single Bond
Adhesive
I
CLEARFIL PROTECT BOND
PRIMER 1.2 ml
KURARAY MEDICAL INC.
2005-12
CLEARFIL PROTECT BOND
BOND 1 ml
2005-12

Figure 2: Ilustrações da metodologia – parte 1.

A: Seleção dos dentes

B: Profilaxia

C: Seccionamento da raiz

D: Aplicação do Material

E: Esquema obtenção da área de exposição

F: Ciclagem Térmica

G: Ciclagem de pH

H: Cortadeira utilizada para obtenção dos espécimes

I: Amostra embutida para análise de microdureza

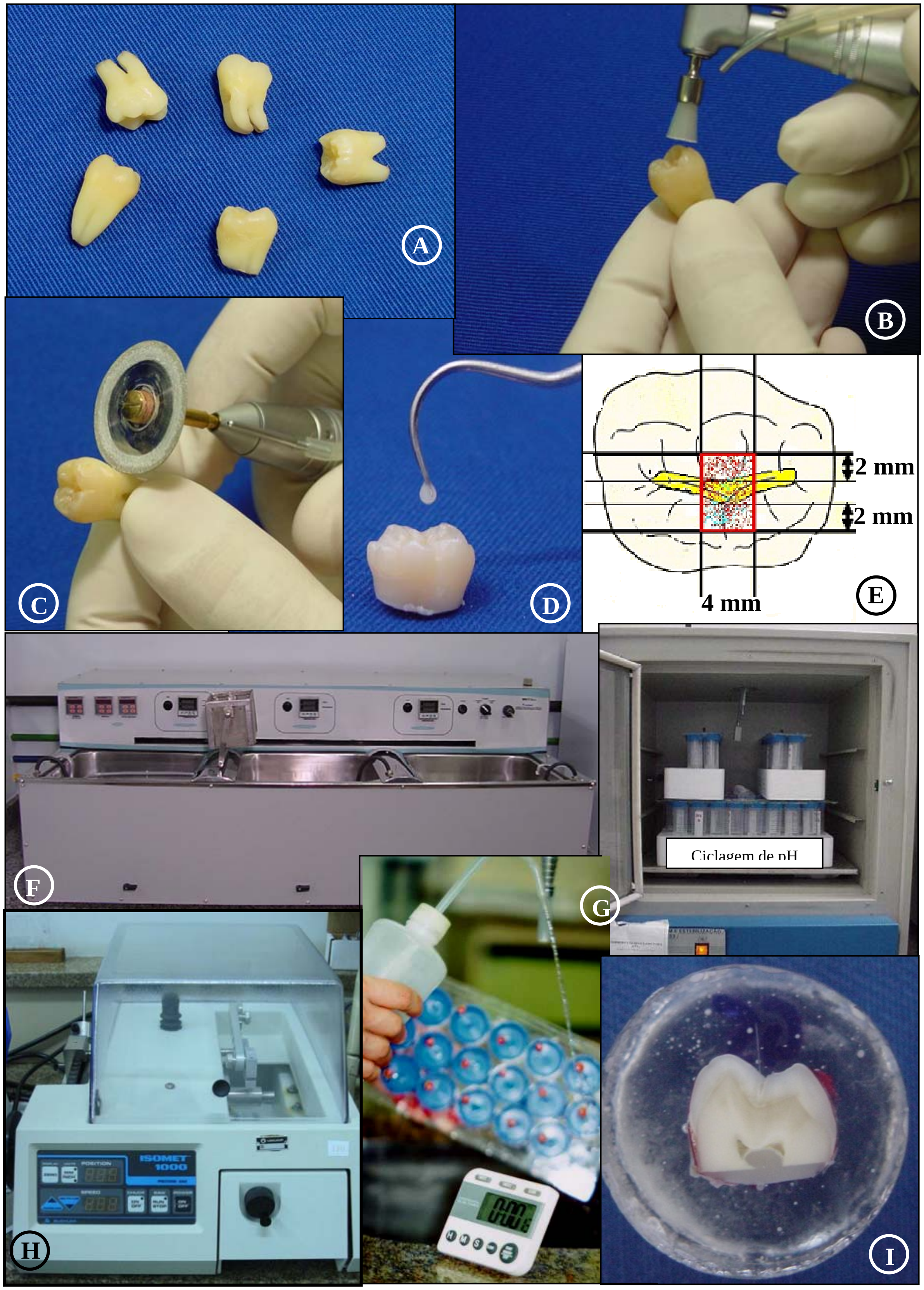

A
B
C
D
2 mm
2 mm
4 mm
E
F
Ciclagem de pH
G
ISOMET 1000
H
I

Figure 3: Ilustrações da metodologia – parte 2.

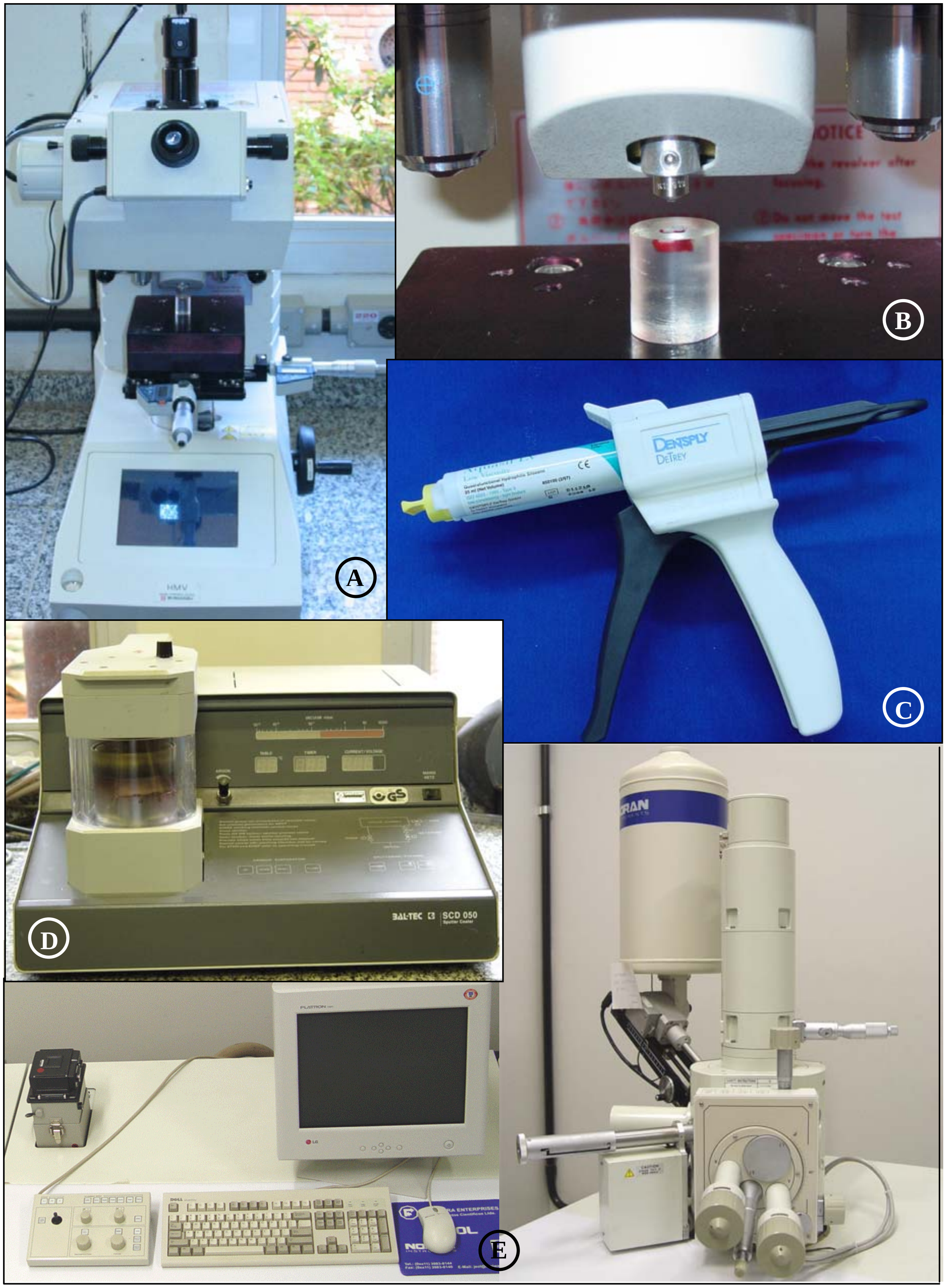
A: Microdurômetro – HMV/2 Shimadzu

B: Penetrador Knoop

C: Aquasil (silicona de adição usada para a confecção das replicas)

D: Metalizadora – Baltec SCD 050

E: Microscópio Eletrônico de Varredura – JEOL – JSM 5600LV, Tókio, Japão.



A
B
C
DENTSPLY
DeTrey
D
BAL-TEC
SCD 050
E

Figura 4: Fotografias das réplicas (Moda de escores encontrados em cada grupo) no Microscópio Eletrônico de Varredura, ilustrando a adaptação marginal.
* - presença de fendas na margem do material selador; E – esmalte; S – material selador

A: FluroShield

B: Helioseal Clear Chroma

C: Vitremer

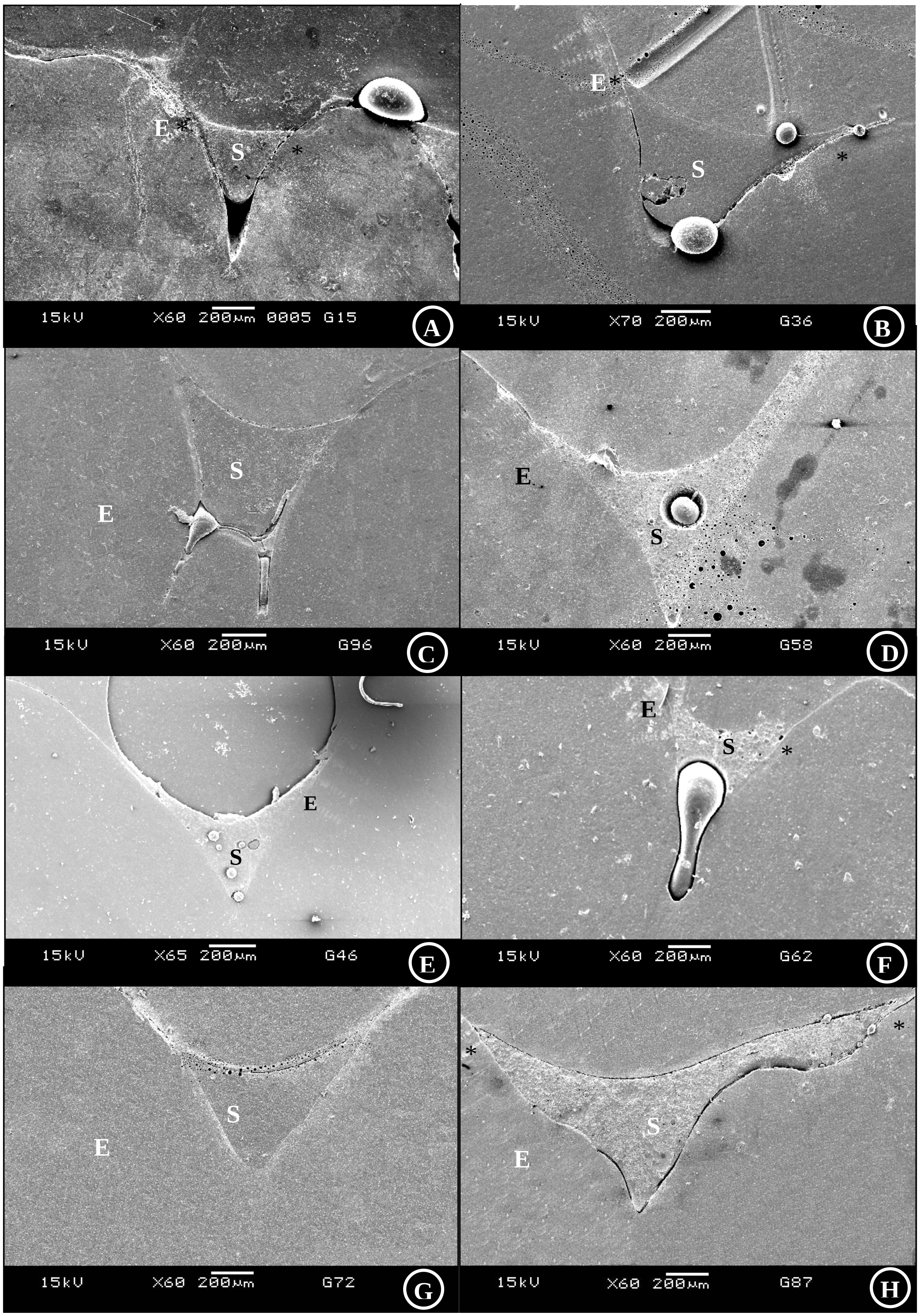
D: Fuji II-LC

E: Ketac Molar

F: Fuji IX

G: Single Bond

H: Clearfil Protect Bond


E
*
S
*
15kV X60 200μm 0005 G15
A
E*
S
*
15kV X70 200μm G36
B
S
E
15kV X60 200μm G96
C
E
S
15kV X60 200μm G58
D
E
S
15kV X65 200μm G46
E
E
S
*
15kV X60 200μm G62
F
S
E
15kV X60 200μm G72
G
*
*
S
E
15kV X60 200μm G87
H

Estatística do Capítulo 1

Analysed with: analyse-it + General 1.73

Test 1 – way between subjects ANOVA

Comparação de Materiais: F, H, V, FII, KM, FIX, SB, CF

Realizado por: Regina Maria Puppin Rontani Data: 3 de Janeiro de 2006

Tabela 1. Estatística descritiva dos diferentes materiais utilizados neste experimento

Materiais	n	Rank sum	Mean rank
F	10	330.0	33.00
H	10	290.0	29.00
V	10	570.0	57.00
FII	10	490.0	49.00
KM	10	450.0	45.00
FIX	10	290.0	29.00
SB	10	610.0	61.00
CF	10	210.0	21.00

Kruskal-Wallis statistic $p < 0.0001$

Tabela 2. Teste de Bonferroni com nível de significância de 5% para comparação dos materiais.

Bonferroni Contrast	Difference	p
F v H	4.000	1.0000
F v V	-24.000	0.5858
F v FII	-16.000	1.0000
F v KM	-12.000	1.0000
F v FIX	4.000	1.0000
F v SB	-28.000	0.1975
F v CF	12.000	1.0000
H v V	-28.000	0.1975
H v FII	-20.000	1.0000
H v KM	-16.000	1.0000
H v FIX	0.000	1.0000
H v SB	-32.000	0.0581
H v CF	8.000	1.0000
V v FII	8.000	1.0000
V v KM	12.000	1.0000
V v FIX	28.000	0.1975
V v SB	-4.000	1.0000
V v CF	36.000	0.0149
FII v KM	4.000	1.0000
FII v FIX	20.000	1.0000
FII v SB	-12.000	1.0000
FII v CF	28.000	0.1975
KM v FIX	16.000	1.0000
KM v SB	-16.000	1.0000
KM v CF	24.000	0.5858
FIX v SB	-32.000	0.0581
FIX v CF	8.000	1.0000
SB v CF	40.000	0.0033

Estatística do Capítulo 2

Analysed with: SAS

Análise de Variância

Tabela 1. Modelo do quadro de análise de variância de acordo com modelo adequado para experimentos inteiramente casualizados com parcelas subdivididas para os dados de Delta-Z.

Causa de Variação	GL
Material	8
Resíduo (A)	99
Distância	3
Material*Distância	24
Resíduo (B)	294
Total	429

Estudo de Suposições

```
D.DADOS
OBSERVATIONS (N=432): all
ANALYSIS: Multiple regression and ANOVA
RESPONSE: t_deltaz
FACTORS:  amostra distancia material
CLASSES:  amostra distancia material
Model:    MATERIAL, AMOSTRA*MATERIAL, DISTANCIA, DISTANCIA*MATERIAL
USER-EXCLUDED OBSERVATIONS: none
ASSUMPTIONS VIOLATED:
   Response scaling
   Outliers
   Constant variance
   Influential observations
INTERPRETATION:
   There is strong statistical evidence that the explanatory variables in
   the model are related to the expected value of t_deltaz. However, some of
   the assumptions underlying the analysis are violated. Please explore the
   assumptions in detail.
```

```
+LAB: Optimal Power Transformation-------------------------------------------+
|  Specify powers:  [ -0.5 TO 1.5 BY 0.1                     ]               |
|                                                       Recalculate          |
|                                                                Power       |
|                                                     Optimal:   0.6         |
|                                                  To be used: [ 0.6     ]   |
+----------------------------------------------------------------------------+
Optimal Power Transformation

The optimal power transformation analysis suggests that the power 0.6
of t_deltaz may be more easily modeled.
```

```
D.DADOS
OBSERVATIONS (N=432): all
ANALYSIS: Multiple regression and ANOVA
RESPONSE: t_deltaz**0.6
FACTORS:  amostra distancia material
CLASSES:  amostra distancia material
Model:    MATERIAL, AMOSTRA*MATERIAL, DISTANCIA, DISTANCIA*MATERIAL
USER-EXCLUDED OBSERVATIONS: none
ASSUMPTIONS VIOLATED:
   Outliers
   Influential observations
INTERPRETATION:
   There is strong statistical evidence that the explanatory variables in
   the model are related to the expected value of t_deltaz**0.6. However,
   some of the assumptions underlying the analysis are violated. Please
   explore the assumptions in detail.
```

```
Potential outlier observations: Prob < 0.05

                                                         Studentized     P-value
                                                           residual        for
Observation                                                without       outlier
   number      t_deltaz    Amostras    Distância       current obs      test

    229         168.586       1        (+200)             4.69766     0.001743
    283         148.542       7        (+200)             4.18296     0.016397
    337          35.851       1        Interfac          -4.44631     0.005344
                                 Outliers

3 observations qualify as outliers by exceeding a studentized
residual value of +/-3.90724 with an overall significance level less
than 0.05. Examine the data for correctness; consider a curvilinear
model, a transformation, or deletion of the observation.

D.DADOS
OBSERVATIONS (N=432): all
   429 observations were used in the analysis
     3 outliers were excluded by the user
ANALYSIS: Multiple regression and ANOVA
RESPONSE: t_deltaz**0.6
FACTORS:  amostra distancia material
CLASSES:  amostra distancia material
Model:    MATERIAL, AMOSTRA*MATERIAL, DISTANCIA, DISTANCIA*MATERIAL
USER-EXCLUDED OBSERVATIONS
   Outliers: #229, #283, #337
ASSUMPTIONS VIOLATED:
   Influential observations
```

```
Potential influential observations: Abs(Dffits) > 2

                                                           Standard
               Original                                    influence
Observation    t_deltaz                                  on predicted
   number      variable    Amostras    Distância            value        Leverage

    231          28.660       3        (+200)            -2.39963        0.31818
    426         143.008       6        Interfac           2.14596        0.31250
    427          14.858       7        Interfac          -2.37396        0.31250
Influential Observations

3 observations qualify as influential by exceeding a DFFITS statistic
value of +/- 2. The results of the analysis may depend too much upon
these observations.
```

Análise de Variância

Tabela 2. Quadro de análise de variâncias adequado para um experimento casualizado em blocos com parcelas subdivididas do fator *materiais* em níveis do fator *distância*

Causa de variação	GL	Σ de quadrados	Quadrados médios	Estatística F	Valor-p
Material	8	52519.23466	6564.90433	6.68	<.0001**
Resíduo (A)	99	97263.85792	982.46321	9.43	
Distância	3	6564.78550	2188.26183		<.0001**
Material*Distância	24	8154.69445	339.77894	1.46	0.0775ns
Residuo (B)	294	68222.3509	232.0488		
Total corrigido	428	232560.4674			
Coeficiente de determinação (R^2): 70,66%			Coeficiente de Variação (CV):15,26		

Tabela 3. Média, desvio padrão e intervalos de confiança de *Delta-Z* calculados com base nos dados originais e teste de Tukey com nível de significância alfa de 5% (p=0,05) para comparações múltiplas de médias de materiais.

Material	Média	Desvio Padrão	Limites do intervalo de confiança da média (95%)		Grupo de Tukey
			Superior	Inferior	
Helioseal	1873.83	1028.35	2172.43	1575.23	A
FluroShield	1790.26	856.07	2038.84	1541.69	A
Concise	1474.24	779.20	1705.63	1242.84	AB
Vitremer	1460.28	860.56	1710.16	1210.40	ABC
Single Bond	971.50	757.32	1191.41	751.60	BC
Clearfill	1002.03	702.21	1205.93	798.13	BC
Fuji II LC	875.18	514.27	1024.51	725.85	BC
Ketac Molar	795.51	548.19	954.69	636.33	BC
Fuji IX	702.20	461.18	837.61	566.79	C

Médias com letras iguais não diferem entre si.

Tabela 4. Médias, desvio padrão e intervalos de confiança de *Delta-Z* calculados com base nos dados originais e teste de Tukey com nível de significância alfa de 5% (p=0,05) para comparações múltiplas de médias de distâncias.

Material	Média	Desvio Padrão	Limites do intervalo de confiança da média (95%)		Grupo de Tukey
			Superior	Inferior	
Interface	972.29	752.42	1116.51	828.08	B
Margem	1291.08	892.14	1461.26	1120.90	A
(+100)	1326.81	806.59	1480.67	1172.95	A
(+200)	1273.05	891.19	1444.68	1101.41	A

Tabela 5. Quadro de análise de variâncias adequado para um experimento casualizado em blocos com parcelas subdivididas do fator *materiais* em níveis do fator *distância*.

Causa de variação	GL	Σ de quadrados	Quadrados médios	Estatística F	Valor-p
Concise vs Outros	1	2432.72028	2432.72028	2.48	0.1188
Resinosos vs Ionoméricos	1	40255.07592	40255.07592	40.97	<.0001
Resin. vs Resin. Mod	1	14654.15626	14654.15626	14.92	0.0002
Resinosos vs Adesivo	1	24241.62646	24241.62646	24.67	<.0001
Resin. Modif. vs Ion	1	6406.12532	6406.12532	6.52	0.0122
Resin. Modif. vs Ade	1	1200.12874	1200.12874	1.22	0.2717
Resíduo (A)	99	97263.85792	982.46321		
Distância	3	6564.78550	2188.26183	9.43	<.0001**
Material*Distância	24	8154.69445	339.77894	1.46	0.0775^{ns}
Residuo (B)	294	68222.3509	232.0488		
Total corrigido	428	232560.4674			
Coeficiente de determinação (R^2): 70,66%			Coeficiente de Variação (CV):15,26		

Comprovante de submissão - artigo do Capítulo 1

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