KAMILA ROSAMILIA KANTOVITZ

DESEMPENHO DE MATERIAIS SELADORES E INFILTRANTES SOBRE A LESÃO ARTIFICIAL DE CÁRIE EM ESMALTE. ANÁLISE MECÂNICA E MICRO-MORFOLÓGICA

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para a obtenção do Título de Doutor em Odontologia – Área de Odontopediatria.

Orientadora: Profª Drª Regina Maria Puppin Rontani

Co-Orientadora: Profª Drª Cinthia P. Machado Tabchoury

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RESUMO

Os selantes de fossas e fissuras oclusais vêm sendo propostos na prevenção de cárie em pacientes de alto risco. As lesões iniciais de cárie em esmalte necessitam de atenção preventiva especial e de um diagnóstico preciso. A decisão se o selante deve ser realizado na superfície hígida, com lesão inicial de cárie ou remineralizada pode gerar dúvidas. No intuito de facilitar a apresentação desta Tese, a mesma foi dividida em dois capítulos, como descrito a seguir. **Capítulo 1:** teve como objetivo revisar a literatura a respeito do efeito dos infiltrantes e materiais seladores na inibição da desmineralização do esmalte em lesões de cárie não cavitadas. Dezoito estudos foram selecionados na avaliação crítica realizada. Dois artigos foram classificados como grau A (estudos *in vivo*), nove como grau B (estudos *in vitro*) e sete com grau C (estudos *in vitro*). **Capítulo 2**: teve como objetivo avaliar a resistência de união à micro-tração (μTBS) de materiais seladores (FluroShield® - F e Helioseal Clear Chroma® - H) aplicados em diferentes substratos de esmalte (hígido - H, lesão inicial de cárie - C e lesão inicial de cárie + aplicação tópica de verniz de flúor - CF) após desafio cariogênico (ciclagem de pH). Baseado no estudo experimental, a resistência de união à micro-tração foi influenciada pelos diferentes substratos de esmalte. O grupo do substrato de esmalte com lesão inicial de cárie + aplicação tópica de verniz de flúor mostrou maior valores de resistência da união esmalte/material à micro-tração, seguido do hígido e da lesão inicial de cárie. Além disso, os valores de resistência da união esmalte/material à micro-tração foram dependentes do material selador e da ciclagem de pH. Observou-se que os valores de μTBS foram maiores para FluroShield que Helioseal Clear Chroma associados à ciclagem de pH. A fratura tipo mista foi predominante em todos os grupos. Pôde-se concluir que enquanto o selamento da superfície externa do esmalte das fóssulas e fissuras age como uma barreira à difusão dos ácidos na superfície de lesão de mancha branca, a técnica de infiltração cria uma barreira dentro da lesão inicial de cárie, por meio da substituição do ar dos espaços da área de perda mineral por uma resina fotopolimerizável de baixa viscosidade. Os valores mais altos foram encontrados para o substrato com lesão inicial de cárie associado a aplicação de verniz fluoretado. Quando os materiais seladores foram expostos ao desafio cariogênico (ciclagem

de pH), FluroShield demonstrou os maiores valores de resistência de união ao esmalte. A resistência de união foi substrato dependente.

Descritores: Esmalte dentário, cárie dentária, selantes de fóssulas e fissuras, literatura de revisão como assunto, prevenção primária, desmineralização do dente, resistência à tração.

ABSTRACT

Pit and fissure dental sealants are recognized as an important adjunct approach for caries prevention in high caries risk patients. The caries-like lesions enamel need special preventive action and accurate diagnosis. The decision whether the sealant must been made on the sound, inicial carious enamel or remineralized enamel may lead to uncertainties. In order to facilitate the accomplishment of this Thesis, it was divided into two chapters, as described on the following descriptions. **Chapter 1:** to present a literature review on the effects of infiltrants and sealers on the inhibition of enamel demineralization of non-cavitated enamel lesions. Eighteen studies identified were included in the project critical appraisal. Two papers were classified as grade A (*in vivo* studies), nine as grade B (*in vitro* studies), and seven as grade C (*in vitro* studies). **Chapter 2**: to evaluate the microtensile bond strength (μTBS) of resinous sealant materials (FluroShield e Helioseal Clear Chroma) on different enamel substrates (sound, caries-like lesion, and caries-like lesion + varnish topical fluoride appication) after a cariogenic challenge (pH-cycling). The caries-like lesion + varnish topical fluoride showed the highest values, following sound and caries-like lesion enamel. Additionally, μTBS values were dependent on both materials and pH-cycling. FluroShield presented the highest μTBS values, when the materials were submitted to pH-cycling. Mixed failure was the most frequently observed failure for all groups. It could be concluded that while fissure sealing act as a diffusion barrier on the top of the lesion surface, the infiltration technique creates a barrier inside the lesion, replacing lost mineral with low-viscous light-curing resin. The highest values were found for caries-like lesion + varnish topical fluoride enamel. When sealer materials were submitteted to cariogênico challenge (pH-cycling), FluroShield showed the highest microtensile bond strength values. The sealant/enamel microtensile bond strength (μTBS) was enamel substrate dependent.

Descriptors: Dental enamel, dental pellicle, dental white spots, pit and fissures sealants, review literature as topic, primary prevention, tooth demineralization, tensile strength.

SUMÁRIO

INTRODUÇÃO

Nas últimas décadas, tem-se observado um considerável declínio na experiência de cárie em superfícies dentárias lisas livres em crianças e adolescentes na maioria dos países industrializados e economicamente em desenvolvimento (Narvai *et al.*, 2000; Brown *et al.*, 2002; Mejàre *et al.*, 2004; Tagliaferro *et al*., 2008). Entretanto, o número de lesões nas superfícies oclusais e proximais de pacientes de alto risco à cárie continua elevado (Mejàre *et al.*, 2004). Além disso, observa-se a polarização desta doença, a qual continua a ser de importância clínica considerável (Feigal, 2002).

A suscetibilidade à cárie dentária na superfície oclusal dos molares parece estar relacionada a característica morfológica singular deste sítio podendo ser obstáculo na prevenção de lesões de cárie e no processo de remineralização (Carvalho *et al.*, 1989; Fejerskov & Kidd, 2003). Assim, o complexo anatômico desta superfície dificulta o debridamento mecânico do biofilme acumulado nas fóssulas e fissuras bem como a penetração do flúor nesta região (Tandon *et al.*, 1989; Carvalho *et al.*, 1989). Estes aspectos estão associados à formação de lesões iniciais desta doença por meio do maior acúmulo de biofilme na fossa mesial e central dos molares superiores e inferiores, respectivamente (Carvalho *et al.*, 1989). De fato, a superfície oclusal representa cerca de 55 a 60% de todas as superfícies cariadas na faixa etária de 5 a 17 anos de idade em áreas com ou sem fluoretação das águas de abastecimento (Sthal & Katz, 1994; Hicks *et al.*, 2000).

Em relação as lesões iniciais de cárie de esmalte na superfície proximal, estas regiões são consideradas de difícil acesso para a realização da higiene bucal adequada tornando-se de alto risco à cárie (Sønju Clasen *et al.*, 1997). Embora estas superfícies se caracterizarem pela lenta progressão da doença, 44% das crianças e adolescentes que não possuíam lesões de cárie na superfície proximal após 4 anos de estudo apresentaram este tipo de lesão (Mejàre *et al.*, 2004).

Deve-se considerar que os dados citados acima representam as lesões de cárie que podem ser detectáveis clinicamente. Holst *et al.* (1999) verificaram a ocorrência e a distribuição de cárie em crianças na faixa etária de 6 anos e observaram que 65% da lesões eram de mancha branca indicando que a prevalência destas lesões iniciais de cárie são

subestimadas. Autio-Gold & Tomar (2005) ao estudarem a prevalência de lesões de mancha branca em crianças de 5 anos de idade verificaram que 71% delas apresentavam este tipo de lesão. Em adolescentes, Peressini *et al.* (2004) encontraram 96% da população estudada com uma ou mais lesões de cárie ativa ou inativa.

Durante o desenvolvimento das lesões iniciais de cárie em esmalte, pode se observar em microscopia de luz polarizada a presença de quatro zonas histológicas típicas: 1 – Zona superficial: camada relativamente intacta (20 a 50 µm de espessura), de aspecto normal, com uma perda mineral de aproximadamente 1%, clinicamente a superfície aparece inalterada; 2 - Corpo da lesão: é a zona mais larga e com maior índice de desmineralização, região que apresenta a maior porcentagem de volume de poros, chegando a apresentar perdas de mineral de até 50%; 3 - Zona escura: a qual é passível de visualização quando o meio de ebebição é a quinolina observa-se nesta região gradual diminuição das perdas minerais; 4 – Zona translúcida: sua presença não é comum em todas as lesões, sendo a perda de mineral nesta camada de 1%, representa a frente de avanço da lesão de cárie, sua espessura varia de 5 a 100 µm. Apesar de possuir uma superfície realtivamente intacta, as lesões iniciais de cárie em esmalte possuem uma superfície 10 a 50 vezes mais porosa que o esmalte sadio (Silverstone, 1973; Fejerskov & Kidd, 2003). São caracterizadas pela presença de poros e perda mineral no corpo do esmalte. A dissolução do esmalte se propaga ao longo dos prismas apresentando cristais rombóides e irregulares. Estes poros agem como "atalhos" na difusão dos ácidos e permitem a dissolução do esmalte (ten Cate *et al.*, 2003). Se o desafio cariogênico for constante, haverá gradual dissolução do esmalte superficial, sendo que esta será mais pronunciada no interior da lesão. Se entretanto, o desafio cariogênico variar em consequência de melhora da higiene o padrão de distribuição de mineral no interior da lesão pode apresentar-se muito mais irregulares (Fejerskov & Kidd, 2003).

A aplicação de flúor netas regiões desmineralizadas vem sendo utilizada como tratamento preventivo de escolha para auxiliar no processo de remineralização das lesões iniciais de cárie, se estas se apresentarem confinadas no substrato de esmalte e não estiverem cavitadas. O fluoreto de cálcio (CaF), formado a partir das aplicações tópicas do íon flúor, age como um reservatório de fluoretos na cavidade oral, aumentando a

remineralização e retardando o processo de desmineralização, sendo o responsável pelo efeito preventivo e terapêutico do íon flúor. Além disso, o flúor age na deposição de minerais, na redistribuição do conteúdo mineral dentro da lesão de cárie, proporcionando o aumento da resistência do esmalte a futuras dissoluções (Kidd & Joyston-Bechal, 1986; ten Cate, 2003). Como o processo de remineralização também é dependente da melhora da higiene bucal individual do paciente, aqueles que possuem hábitos de higiene bucal inadequado, visita irregular ao dentista e baixa exposição ao flúor, as estratégias preventivas paciente-dependente (*compliant patient*) que poderiam ser utilizadas acabam sendo limitadas e nestes casos as lesões de esmalte progridem para cavitações (Koulourides & Cameron, 1980; Mejàre *et al.*, 1999; Kielbassa *et al.*, 2006).

No momento de realizar a decisão do tratamento odontológico a suscetibilidade/atividade de cárie do paciente devem ser consideradas. Para pacientes com alto suscetibilidade a cárie os materiais seladores vêm sendo utilizados na superfície oclusal desde 1967 e sua crescente utilização vem se mantendo uniforme ao longo dos anos (Cueto & Buonocore, 1967; Simonsen 2002; Ahovuo-Saloranta, 2008). Isso se deve ao fato de que uma única aplicação do material selador previne as lesões de cárie nas superfícies oclusal, por aproximadamente 10 anos (Simonsen 1987; Romcke *et al.*, 1990). Kantovitz *et al*. (2006) demonstraram que materiais à base de resina fotopolimerizável, ionoméricos ou adesivos agem como barreira física na prevenção e progressão da lesão de cárie de fóssulas e fissuras. Os resultados do trabalho citado acima demonstrou que Single Bond e Vitremer foram efetivos na preservação da interface material selador/superfície oclusal do esmalte, suportando as condições de 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.

Um tratamento operatório alternativo que vem sendo utilizado para prevenir as lesões iniciais de cárie em esmalte nas superfícies proximais é a infiltração/selamento da

lesão de cárie com monômeros de baixa viscosidade e sem partículas de carga como os adesivos dentinários e alguns materiais seladores (Gómez *et al.*, 2008; Martingnon *et al.*, 2006, Meyer-Lueckel *et al.*, 2006). Terapia que tem por objetivo obliterar os poros do esmalte desmineralizado e promover suporte mecânico à esta estrutura fragilizada, prevenindo desta maneira a progressão da lesão inicial de cárie (Meyer-Lueckel *et al.*, 2006).

Assim, as lesões iniciais de cárie em esmalte são locais que necessitam de atenção preventiva especial e diagnóstico preciso. A decisão clínica sobre a infiltração/selamento do esmalte nestas condições pode gerar dúvidas se a superfície desmineralizada necessita ser remineralizada ou não antes do selamento. Lee (1972) e Kochavi (1975) mostraram que a aplicação tópica de flúor, no esmalte sadio, pode interferir no efeito do condicionamento do ácido fosfórico na superfície do esmalte, resultando na redução da resistência da união dente/material. Por outro lado, outros estudos (Brannstrom *et al.*, 1982; Bryant *et al.*,1985; Thornton *et al.*, 1986) verificaram que essa aplicação tópica de flúor antes do condicionamento ácido (H_3PO_4) do esmalte sadio não afeta o padrão do esmalte condicionado, bem como a resistência de união do esmalte/material. Entretanto, são necessários estudos que verifiquem a resistência de união do material resinoso nas lesões iniciais de cárie, após aplicação tópica de flúor, com o objetivo de aumentar a retenção e a eficiência deste procedimento terapêutico.

Diversas têm sido as técnicas empregadas para se avaliar as interações físicas e biológicas entre o elemento dentário e os materiais restauradores/seladores. A resistência de união ao esmalte de materiais seladores tem sido avaliada pelos testes de cisalhamento (el-Kalla & Garcia-Godoy, 1998; Perez-Lajarin *et al.*, 2000; Yamamoto *et al.*, 2003) e pelo tradicional teste de tração (Torii *et al.*, 2002). Entretanto, o teste de micro-tração vem sendo apontado como o de maior precisão para avaliar a resistência de união do material sobre a superfície do elemento dentário (Pashley *et al.*, 1999).

A influência dos diversos materiais seladores na dinâmica do processo de cárie dentária tem sido estudada *in vitro*, em modelos que simulam desafio cariogênico, com o objetivo de desenvolver lesões com características comparáveis às encontradas *in vivo*. (Hicks & Flaitz, 2000; Hicks *et al.*, 2000). Os modelos podem ser químicos: estáticos -

imersão de substratos dentários em soluções ou géis ácidos (Hicks & Flaitz, 2000; Hicks *et al.*, 2000), ou dinâmicos - ciclos de desmineralização e remineralização (Featherstone *et al.*, 1983,1986; van Dorp *et al.*, 1990), ter natureza microbiológica - exposição do substrato a uma ou mais espécies de microrganismos cariogênicos (Gilmour *et al.*, 1990,1997; Lobo *et al.*, 2005; Seemann *et al.*, 2005) ou ainda o modelo *in situ* - o qual submete-se o corpo-de-prova, através de um aparelho removível intra-oral ao ambiente bucal (Tenuta *et al.*, 2005; Lagerweij & ten Cate, 2002).

Tendo em vista que, na literatura atual não se encontram estudos que avaliem a resistência de união e a interface esmalte com lesão inicial de cárie/material selador submetidos a um desafio cariogênico, os objetivos desta Tese são: (1) discutir, através da revisão de literatura, os efeitos dos infiltrantes e materiais seladores na inibição da progressão da desmineralização do esmalte; (2) avaliar a resistência de união à micro-tração de materiais seladores aplicados em diferentes substratos de esmalte após desafio cariogênico (ciclagem de pH). Para alcançar esses os objetivos, esta Tese[*] foi dividida em 2 capítulos, correspondentes aos objetivos descritos.

[*] Esta Tese está baseada na resolução da CCPG/002/06, a qual dispõe a respeito do formato das teses de mestrado e doutorado aprovados pela UNICAMP.

CAPÍTULO 1

Review on the effects of infiltrants and sealers on non-cavitated enamel lesion.[*]

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ABSTRACT

Purpose. The aim of this article was to perform a review on the effects of infiltrants and sealers on the inhibition of enamel demineralization. **Materials and Methods.** The authors searched the Cochrane Library, Embase, Pubmed, and Web of Science for papers from 1970-2008. The main search terms were “artificial caries” or “caries treatment” or “caries - like- lesion” or “white spot lesion” or “enamel demineralization” or “natural caries” and “enamel” and “sealant” or “resin infiltration”. The inclusion criteria were studies that produced artificial non-cavitated enamel lesion before sealant application in in vivo or in vitro studies. Studies excluded were those that had not produced artificial non-cavitated enamel lesion before sealant application, had evaluated the demineralization inhibition around restorations, sealants, and orthodontic bracket/bands, had not evaluated the demineralization inhibition after the sealant application, and had not applied sealant materials. Selected papers were given scores, from A to C, according to predetermined criteria. **Results.** 18 studies identified were included in the project critical appraisal. Two papers were classified as grade A, nine as grade B, and seven as grade C. **Conclusion.** It can be concluded that while fissure sealing act as a diffusion barrier on the top of the lesion surface, the infiltration technique creates a barrier inside the lesion, by replacing the mineral lost by a low-viscous light-curing resin.

INTRODUCTION

Although, during recent years, a decline in caries has been detected in most industrialized countries, dental caries remain an important health problem, especially in children (Mejàre et al, 2004) and adolescent populations (Brown et al, 2002) at high dental caries risk. Moreover, the amount of non-cavitated enamel lesion on approximal surfaces is still high (Mejàre et al, 2004; Mejàre et al, 1999). Recently, high numbers of non-cavitated lesions (81%) in five-year-old children have been evaluated (Autio-Gold and Tomar, 2005). In adolescents, it was found that 96% of this population had 1 or more past or active carious lesions (Peressini et al, 2004). Additionally, it was demonstrated an average of 4 caries lesions in adolescents living in a high caries risk area (Moberg Skold et al, 2005). A longitudinal study indicated that the progression rate of approximal caries is higher when the lesion has reached into the dentine than when they are more superficial in the enamel (Mejàre et al, 1999). These findings point out that for high caries risk patients, preventive measures are of prime importance to prevent the caries progression during an early stage.

The early non-cavitated enamel lesion is termed the “white spot lesion” and such lesion has a surface that is 10 to 50 times more porous than sound enamel (Silverstone, 1973). In patients with good oral hygiene and regular fluoride exposure the porous enamel can remineralize and become more resistant to further dissolution than sound enamel (Silverstone, 1973; Kidd and Joyston-Bechal, 1986). However, for noncompliant patients (Paris et al, 2006), these regimens are not really efficient. Thus, clinicians are advised to implement timely preventive measures based on dietary advice, oral hygiene, application of fluoride containing materials (Toumba et al, 2003), and infiltrating/sealing of approximal non-cavitated enamel lesions with low viscous resins such as dental adhesives and sealants (Martingnon et al 2006, Meyer-Lueckel et al, 2006). In addition, the clinicians should be aware with the high rate of cavitation even at early stages of the caries process, which should be closely monitored when a preventive treatment regimen is chosen (Kielbassa et al, 2006). In this cases, it is not enough to implement preventive measurements, since the high mineral loss maybe not be reached only by the infiltrants and sealants. The initiative to arrest caries by infiltration of resins has been followed since the studies of Davila et al (1975) and Robinson et al (1976). The latter

authors reported a filling of caries lesion by infiltration of an organic resin and demonstrated a reduction in pore volume of the lesion following the application of resorcinol-formaldehyde resin.

The white spot lesion is also characterized by a loss of mineral in the bulk of enamel, whereas the surface of the lesions remains relatively intact (ten Cate et al, 2003). The tiny pores within the lesion body act as diffusion pathways for acids and minerals, therefore, allowing the dissolution of enamel at the advancing front of the lesion (ten Cate et al, 2003). The aim of infiltrating regimen is to occlude these pores with low viscous light curing resins by penetration into the lesion body. Moreover, after curing the material, a mechanical support of the fragile enamel framework in the lesion might be achieved (Meyer-Lueckel et al, 2006). Evidence of the effects of infiltrants and sealers in the inhibition of non-cavitated enamel lesions has been reported in the literature. However, a knowledge gap still exists as to what material yields the best results. Thus, the purpose of this article was to carry out a literature review on the effects of infiltrants and sealers on the inhibition of enamel demineralization of non-cavitated enamel lesions to in vivo and in vitro studies.

MATERIALS AND METHODS

Literature searching

The authors searched the Cochrane Library, Embase, Pubmed, and Web of Science (ISI) for papers from January 1970 to September 2008. The search was supplemented by manual searching of reference lists from each relevant paper identified.

The main search terms were “artificial caries” or “caries treatment” or “caries - like- lesion” or “white spot lesion” or “enamel demineralization” or “natural caries” and “enamel” and “sealant” or “resin infiltration”. A total of 221 papers were originally identified. The search was limited to randomized, in vivo and in vitro studies, which were considered relevant papers. Only original papers were considered. Interim reports, abstracts, letters, short communications, reviews, and chapters in textbooks were discarded. Only articles in English were accepted. Only papers related to the subject were selected, which resulted in 60 articles. These were printed as abstracts, or full-text articles, if the

abstract was missing. In a second step, two examiners selected relevant records independently and the papers that were considered of interest for this review were ordered in full-text versions. During the evaluation process, reference lists were searched by hand.

Inclusion and exclusion criteria

After appraisal, papers were included only if they studied non-cavitated enamel lesion before sealant application in *in vivo* and *in vitro* studies. Studies excluded were those that had not produced artificial non-cavitated enamel lesion, had evaluated the demineralization inhibition around restorations, sealants, and orthodontic bracket / bands, had not evaluated the demineralization inhibition after the sealant application, and had not applied sealants materials.

Evaluation of scientific papers and levels of evidence

The papers that met the inclusion criteria were subjected to critical appraisal, carried out independently by at least two authors. Data were extracted using a pilot tested form and each paper was attributed with a score from A to C, according to predetermined criteria for methodology and performance, as defined in Table 1. In the case of disagreement between the examiners, the paper was re-evaluated and discussed by the entire group until consensus was reached. If, for some reason, a selected paper was found to be irrelevant for the research question, the article was excluded. Thus, a total of 18 papers were selected.

Table 1. Criteria for grading the assessed papers

Grade A All criteria stated on the right should be met	Study group representative, inclusion criteria described; *in vivo* studies; Natural human non-cavitated enamel caries lesion presence; Randomization of teeth; The reliability of evaluation methods described; Independent outcome assessment; Statistical analysis.
Grade B All criteria stated on the right should be met	Study group representative, inclusion criteria described; *in vitro* studies; Non-cavitated enamel caries lesion has to be produced before material application; Artificial bovine/human non-cavitated enamel caries lesion produced in *in vitro* studies; Randomization of teeth; The reliability of evaluation methods described; Independent outcome assessment; Statistical analysis.
Grade C One or more of the conditions stated on the right	Artificial non-cavitated enamel caries lesion has to be produced before material application; Artificial bovine/human non-cavitated enamel caries lesion produced in *in vitro* studies; No or unclear randomization; Methodology not completely described; The reliability of evaluation methods not described; No independent outcome assessment; Several non-calibrated examiners.

RESULTS

Eighteen studies identified during the search were included in the project critical appraisal (Table 2). Papers classified as grade A (Martigon et al, 2006; Gomez et al, 2005) described the non-cavitated approximal caries lesion (white spot lesion) naturally stated before material application in *in vivo* studies. In grade B (Meyer-Lueckel and Paris, 2008; Celiberti and Lussi, 2007; Paris et al, 2007; Paris et al, 2006; Meyer-Lueckel et al, 2006; Mueller et al, 2006; Schmidlin et al, 2004; Gray and Shellis, 2002; Robinson et al, 2001), papers verified the artificial and natural non-cavitated enamel lesion (white spot lesion) produced before material application in *in vitro* studies. The articles by Bjarnason et al (2003), Garçía-Godoy et al (1997), Van Dorp and ten Cate (1992), Goepferd and Olberding (1989), van Dorp and ten Cate (1987), Robinson et al (1976) and Davila et al (1975); were included in grade C, since they did not completely describe the methodology used and/or did not carry out statistical analyses. The main reasons for excluding 42 of the articles was that these studies had not produced artificial non-cavitated enamel lesion, had evaluated the inhibition of enamel demineralization around restorations, sealants, and orthodontic bracket / bands, had not evaluated the demineralization inhibition after the sealant application, and had not applied sealants materials (Gomez et al, 2008; Trairatvorakul et al, 2008; Meyer-Lueckel et al, 2007; Paris et al, 2007; Salar et al, 2007; Selecman et al, 2007; Burbridge et al, 2006; Kantovitz et al, 2006; Schmidlin et al, 2006; Soliman et al, 2006; Vatanatham et al, 2006; El-Housseiny and Sharaf, 2005; Hu and Featherstone, 2005; Lobo et al, 2005; Seemann et al, 2005; Schmidlin et al, 2005; Jones et al, 2004; Barnes et al, 2000; Hicks and Flaitz, 2000; Hicks et al, 2000; Tanaka et al, 2000; Smales and Wong, 1999; Hicks and Flaitz, 1998; Bravo et al, 1997; Carlsson et al, 1997; Tandon and Mathew, 1997; Tantbirojn et al, 1997; Frazier et al, 1996; Symons et al, 1996; Percinoto et al, 1995; Banks et al, 1994; Ceen and Gwinnett, 1980; Donly and Ruiz,1992; Hicks and Flaitz, 1992; Arrow and Riordan, 1995; Kuba et al, 1992; Jensen et al, 1990; Thylstrup and Poulsen, 1978; Ohmori et al, 1976; Thylstrup amd Poulsen, 1976; Ulvestad, 1976; Nishino et al, 1974).

Only articles included for evaluating evidence were used as a basis for conclusions.

Table 2. Results of references appraised

First author	Year	Study design[1]	Enamel	Selection Method of Proximal Lesions	Demineralization Solution	pH Temperature	Demineralization Period	Acid Etch (Time)	Material Used[2]	Evaluation Methods	Evidence level
Meyer-Lueckel	2008	*in vitro*	Bovine	NA	6µM methylhydroxydiphosphonate, 3mM $CaCl_2$ $2H_2O$, 3mM KH_2PO_4, 50mM acetic acid (Solution)	5.0 37°C	50 days	37% phosphoric acid gel (5s)	Exerimental resins; Excite	TMR CLSM	B
Celiberti	2007	*in vitro*	Human Molar	NA	1.5 mM $CaCl_2$ $2H_2O$, 0.9mM KH_2PO_4, 50 mM acetic acid (Solution/ pH cycling process)	4.6 37°C	4 weeks	35% phosphoric acid gel (60 or 40s)	Delton	LM	B
Paris	2007	*in vitro*	Human Premolar and Molar	X-rays/ Histological evaluation	NA	NA	NA	37% phosphoric acid gel (120s) 15% hydrochloric acid gel (120s)	Excite	CLSM SEM	B
Martignon	2006	CT	Human Premolar and Molar	Bitewing X-rays	NA	NA	NA	Maleic acid (15s) 35% phosphoric acid gel (20s)	GOB; Concise	X-ray	A
Meyer-Lueckel	2006	*in vitro*	Bovine	NA	6µM methylhydroxydiphosphonate, 3mM $CaCl_2$ $2H_2O$, 3mM KH_2PO_4, 50mM acetic acid (Solution)	5.0 37°C	14 days	20% phosphoric acid gel (5s)	HB; RM; Excite; SBM; APLP; H.	CLSM	B

Continuation…

First author	Year	Study design[1]	Enamel	Selection Method of Proximal Lesions	Demineralization Solution	pH Temperature	Demineralization Period	Acid Etch (Time)	Material Used[2]	Evaluation Methods	Evidence level
Mueller	2006	*in vitro*	Bovine	NA	3mM $CaCl_2$ $2H_2O$, 3mM KH_2PO_4, 50mM acetic acid (Solution)	5.0 37°C	14 day	20% phosphoric acid gel (5s)	HB; RM; Excite; SBM; APLP; H	CLSM	B
Paris	2006	*in vitro*	Bovine	NA	6µM methylhydroxydiphosphonate , 3mM $CaCl_2$ $2H_2O$, 3mM KH_2PO_4, 50mM acetic acid (Solution)	5.0 37°C	14 days	20% phosphoric acid gel (5s)	HB; RM; Excite; SBM; APLP; H.	CLSM	B
Gomez	2005	CT	Human Premolar and Molar	Bitewing X-rays	NA	NA	NA	35% phosphoric acid gel (20s)	Concise	X-ray	A
Schmidlin	2004	*in vitro*	Human Molar	NA	0.1 mol/l acetate in 6% HEC[3], 1.5 mmol/l $CaCl_2$, 0.9 mmol/l KH_2PO_4, 150 mmol/l KCl (Gel)	4.8 5°C	12 weeks	35% phosphoric acid gel (120s)	HB	CLSM	B
Bjarnason	2003	*in vitro*	Human Premolar	NA	0.1 mol/l Na-acetate, 1.5 mmol/l $CaCl_2$, 0.9 mmol/l KH_2PO_4, 150 mmol/l K 6% HEC[3] (Gel)	4.95 Room temperature	6 days	37% phosphoric acid gel (60s)	Concise	PLM SEM	C

…to be continued

Continuation…

First author	Year	Study design[1]	Enamel	Selection Method of Proximal Lesions	Demineralization Solution	pH Temperature	Demineralization Period	Acid Etch (Time)	Material Used[2]	Evaluation Methods	Evidence level
Gray	2002	*in vitro*	Human Premolar	NA	15% lactic acid (Gel)	4.5	6 weeks	36% buffered ortho-phosphoric acid gel (5s/10s)	SB; S&P	SEM	B
Robinson	2001	*in vitro*	Human Premolar and Molar	NA	5% lactic acid (Gel)	4.5	6 weeks	10% phosphoric acid gel/maleic acid (15s)	SBMP; G2000; AB2; AP; n-butyl-cyanocrilate.	LM/Clornaphthalene imhibition technique	B
Garcia-Godoy	1997	*in vitro*	Human Molar	NA	15% lactic acid (Gel)	4.2	5 weeks	37% phosphoric acid gel (20s)	PUB2	PLM	C
Van Dorp	1992	*in vitro*	Human Molar	NA	Demineralization solution	---	9 weeks	37% phosphoric acid gel (60s)	Delton	LM X-ray	C
Goepferd	1989	*in vitro*	Human Molar	NA	15% lactic acid (Gel)	4.0	12 weeks	37% phosphoric acid gel (60s)	Resin Sealant	PLM	C
van Dorp	1987	*in vitro*	Bovine	NA	20% w/v 0.1 N lactic acid (Gel)	4.5 37°C	4, 10, 28 or 49 days	36% phosphoric acid gel (60s)	Delton	SEM / LM KH	C

…to be continued

Continuation…

First author	Year	Study design[1]	Enamel	Selection Method of Proximal Lesions	Demineralization Solution	pH Temperature	Demineralization Period	Acid Etch (Time)	Material Used[2]	Evaluation Methods	Evidence level
Robinson	1976	*in vitro* animal study	Human premolar ---	NA	White or brown spot lesions 10% w/v 1 M lactic acid (Gel) Cariogenic diet	NA 4.5 / 16°C NA	NA 2 to 4 weeks NA	1 N HCl (5 to 10s)	Teepol	LM	C
Davila	1975	*in vitro*	Human incisor Human Molar or premolar	NA	0.1 M acetate buffer Natural white spot lesion	5.0 NA	33 or 66 hours NA	50% phosphoric acid (60s or 30s) 50% phosphoric acid (30s)	Nuva Seal	SEM / PLM	C

1 CT – Clinical trial study; NA – Not applicable; HEC - hydroxyethylcellulose.

2 GOB- Gluma One Bond (Heraeus Kulzer); HB-Heliobond (Vivadent); RM-Resulcin Monobond (Merz Dental); Excite (Vivadent); SBM-Solobond M (Voco); APLP-Adper Prompt L-Pop (3M/ESPE); H-Helioseal (Vivadent); Concise (3M/ESPE); SB-Scotchbond (3M/ESPE); S&P-Seal and Protect (Dentsply); SBMP-Scotchbond Multipurpose (3M/ESPE); G2000-Gluma 2000 (Bayer Dental); AB2-All-Bond 2 (Bisco); AP-Almagambond plus (Prakell Bio-materials); PUB2-Prisma Universal Bond 2 (Dentsply); Delton (Dentsply); Teepol-1.3 gm of resorcinol in 2ml 40% formaldehyde solution, 0.1 ml concentrated detergent (Shell Chemical UK ltda); Nuva Seal (L.D.Caulk.Co)

3 CLSM-Cofocal Laser Scanning Microscope; TMR-Transversal microradiodraphy; PLM-Polarized Light Microscopy; SEM-Scanning Electron Microcopy; LM-Light Microscop1ic Photographs; KH–Knoop Hardnes.

DISCUSSION

The present paper reviewed the substantial literature about the effects of infiltrants and sealers on the inhibition the enamel demineralization of non-cavitated enamel lesion. This review may contribute to the development of new methodologies for conducting studies on the effects of dental materials on decreasing enamel lesion progression or enhancing enamel remineralization. Since the early non-invasive intervention has the benefit of being suitable for all patients, operative intervention should not be a management option for the non-cavitated lesion. Thus, the sealing procedure may provide a strategy to arrest initial enamel lesion even in non-compliant patients.

In general, two types of models were used as tools to assess the questions regarding the effect of sealing or infiltrating white spot lesion on enamel surface: *in vivo* and *in vitro* studies. There are some important differences to be considered when these different models are used. While *in vitro* studies have limitations, such as small sample and a possibly lower clinical relevance, in contrast these studies have a high level of scientific control and low variation (Bowen, 1983). In *in vivo* situations, the subjects' dietary habits should be considered as well as the presence of physiologically secreted saliva, biofilm of varying composition and thickness, and a pellicle-coated tooth surface (Bowen, 1983).

In this literature review, the authors found two *in vivo* studies in the databases that assessed the clinical ability of sealant to arrest the progression of non-cavitated approximal posterior carious lesions (Martigon et al, 2006; Gomez et al, 2005). Although the results should be interpreted with caution, they suggest that sealants have the potential to act as a noninvasive treatment for arresting non-cavitated approximal lesions. The authors observed that 93% (Gomez et al, 2005) and 66% (Martigon et al, 2006) of the sealed surfaces do not showed lesion progression after two years and 18 months, respectively. However, these studies did not provide information about caries risk assessment in the approximal sites. In addition, in clinical situations, it is more difficult to examine the sealant retention on the approximal posterior surface without tooth separation. Placing orthodontic separators and soliciting the patient's return is time-consuming and relative costly in public health settings. Another aspect to be considered is the layer of sealants and their retention. This implies that if the sealant was only applied once, its

retention could not be completed during the experimental period, which could lead to lesion progression. Despite of this inconvenience, Martingnon et al (2006) showed that the sealing technique was superior to instructing patients to floss, since the compliance concerning flossing was poor (15%).

In *in vitro* studies, the outcomes of Meyer-Lueckel and Paris (2008), Meyer-Lueckel et al (2006) and Paris et al (2006) led to similar conclusions. They showed that the adhesives (Heliobond, Resulcin Monobond, and Excite), experimental low-viscosity light curing resins, and fissure sealant (Helioseal) were capable of penetrating into initial surface bovine enamel lesion after a short period of etching time (5 s). This could be particularly important for determining whether the infiltrating (low-vistosity light curing resins) and sealing (sealants) of the lesion should be accomplished by occlusion of the pores in the lesion body or rather by coating resin over the lesion substrate. The occlusion of the pores within the lesion body (infiltrant) is capable of further inhibiting demineralization of non-cavitates enamel lesion (Mueller et al, 2006). Therefore, only a resin coat on top of the lesion surface (sealant) does not seem to be sufficient to prevent lesions progression in vitro (Paris et al, 2006). Moreover, Paris et al (2006) suggest that the excessive material could be disadvantageous clinically, since sealant margins and excess of resin material could provide retention sites for biofilm and new sites for caries. In a similar study, Mueller et al (2006) evaluated the progression of infiltrated and sealed initial enamel lesions by Helioseal, Heliobond, Resulcin Monobond, Excite, Solobond M, and Adper Prompt L-Pop, when applied once or twice (90 s and 180 s of penetration time). The authors verified that, except for Solobond M and Adper Prompt L-Pop, the other materials were able to inhibit lesion progression. In addition, there were no differences between lesion depths after the first or second application, with the exception of Solobond M, which decreased lesion progression after the second application. It could be concluded that a resin layer on top of the lesion is not required if the lesion body is homogeneously infiltrated with a resin. However, these studies did not quantify the enamel mineral loss or gain nor provided any evidence that a deeper penetration of the sealers had any effect on the initial enamel demineralization decrease or enhancement of its remineralization. In contrast, Meyer-Lueckel and Paris (2008) used transversal microradiography as gold standard to determine mineral loss and

lesions depth of enamel caries lesion and verify that the higher penetration coefficient of the infiltrants, the more effective it is in inhibition of lesion progress.

The Gray and Shellis (2002) in vitro study investigated methods of achieving resin infiltration into non-cavitated lesion in enamel. They found that two layers of resin applied for 5 s (Scotchbond 1 and Seal and Protect) had the ability to infiltrate the pores of the non-cavitated enamel lesion. It was possible that the resin would coat the surface of the crystallites of hydroxyapatite, protecting them from further dissolution in acid conditions. However, no methodology was employed by the authors to evaluate how effective these materials could be in preventing lesion progression in situations of high cariogenic challenge. Additionally, these authors also suggested that the Seal and Protect resin containing triclosan and fluoride would have a sustained release that could inhibit the accumulation of biofilm in the local area (Gray and Shellis, 2002). However, this study did not perform analyses of triclosan and fluoride release into demineralization solution nor their incorporation into enamel surface.

The Garçía-Godoy et al (1997) and Goepferd and Olberding (1989) studies evaluated the effectiveness of placing a resin sealant over non-cavitated enamel lesion to arrest its progression. The results of these studies indicate the effectiveness of the resin tags in preventing acid demineralization of the sealed white spot lesion. However, these studies and the report by Gray and Shellis (2002) did not assess whether the seal occurred by infiltration and occlusion of the pores in the lesion body or rather by a covering resin coat (sealing). Moreover, these studies employed qualitative methods (Polarized Light Microscopy and Scanning Electron Microscopy) to evaluate enamel demineralization, since they have only considered the depth of artificial caries lesion. It is known that quantitative methods as microradiography (Featherstone et al, 1983), cross-sectional microhardness analysis (Featherstone et al, 1983), and polarization-sensitive optical coherence tomography (PS-OCT) (Baumgartner et al, 2000) would be more appropriate to quantitatively evaluate the effect of sealing white spot lesion on enamel mineral loss or gain.

Robinson et al (2001) demonstrated quantitatively and qualitatively that the bonding agents can be used in infiltrating non-cavitated caries lesions and reduce further

acid demineralization. The infiltration resulted in quantitatively measurable relative reductions in accessible lesion pore volumes in all cases. The authors explain these results due to the presence of methacrylate associated with a cyanoacrylate in bonding agents and concluded that methacrylates seem to be also suitable for resin infiltration, similar to an earlier study that used resorcinol formaldehyde (Robinson et al, 1976). This previous study demonstrated that the treatment of porous enamel reduced the rate of demineralization in vitro and that the resin was retained in the treated teeth of monkeys. However, this resin was unsuitable for clinical use due to its toxic nature. In addition, the most serious objection to this material is the damaging effect of resin on vital dentin or pulp (Robinson et al, 1976). The authors of this review agree with Robinson et al (2001) that before conclusions are drawn, concerning mode of action and potential of therapeutic agent, the visually and quantitatively penetration of dental material in non-cavitates enamel lesion should be assessed.

The *in vitro* studies above cited (Gray and Shellis, 2002; Robinson et al 2001) and that by Davila et al (1975) showed that 60% or more of the pore volume of artificial non-cavitated enamel lesions were occluded following infiltration with unfilled resins. However, these studies focused chiefly on demineralization of a smooth surface. With current concepts in preventive dentistry, some remineralization of initial carious lesions is likely to occur. In this context, Schmidlin et al (2004) assessed the penetration of an unfilled resin into artificial enamel lesions before and after remineralization. They concluded that the penetration of the resin was influenced by the degree of dental hard tissue mineralization. Resin penetration was the deepest in demineralized enamel with a mean tag length of 68±22 µm. However, the remineralized enamel also allowed penetration of the bonding agent (tag length of 49±17µm). In addition, fluoride application did not have a significant impact on tag length in remineralized areas (47±14µm). This finding may be explained by a reduction in the enamel solubility rate due to fluoride-rich inorganic products, which may have been derived from the reaction of fluoride with loosely bond calcium and phosphate (Hicks, 1986).

In spite of those considerations it could not be neglected the nature of the infiltrants. They should be low viscosity monomers in order to infiltrate the caries lesion in

depth. However, if the infiltrant shows very low viscosity at the point of penetrating inside the lesion, it could not be able to form a thick film to offer great resistant to degradation outside the lesion. In addition, the monomer viscosity is inversely proportional to the oxygen inhibition of polymerizing monomers, which is also dependent of the monomer type and activation system. The unpolymerized layer is about 7 to 84 μm depending on the activation system. Self-curing amine/peroxide systems have showed thicker unpolymerized layer than light cure systems. Concerning monomers type, resin system containing BisGMA-dicarbonate/HEMA shows reduction on oxygen inhibition layer thickness when compared to corresponding system based on BisGMA/HEMA, most likely due to the presence of the cyclic carbonate moiety (Causton and Sefton, 1989). The high reactivity cyclic carbonate group turns the propagation step of the polymerization more efficient, while slows the termination process. The chain propagation is improved, what allows increase on degree of conversion and decrease on oxygen inhibition layer. Others monomers, like mono- and dimethacrylate resins, have also been described which, by modification with pendant cyclic carbonate moieties, showed an extremely low oxygen inhibition tendency favorable allow better. It must be noted that the oxygen-inhibited film, even at 60-wt% of HEMA in BisGMA, is thin (only 15 pm), probably due to the activity of the initiator system used. Since viscosity decreases with increasing HEMA content, the supply of oxygen to the radicals by diffusion is increased. Oxygen binds up the reactive site of the growing radicals until the rate of radical formation matches or exceeds the oxygen diffusion rate (Causton and Sefton, 1989). A new one monomer, silorane, whose polymerization reaction is based on using ring-opening polymerization of the silorane molecules, instead of free radical polymerization of dimethacrylate monomers. It reveals lower polymerization shrinkage compared to the dimethacrylates (Guggenberger and Weinmann, 2000). The ring-opening polymerization of the silorane molecule is cationic polymerization reaction where no oxygen inhibition layer exists on the surface of the composite after polymerization in air (Eick et al, 2006). The monomers should polymerize well and include crosslinking components to obtain a high degree of polymerization and a high network density. Thus, simply to fill-up the carious lesions with low viscosity infiltrant maybe it is not enough to resist the degradation, and after that, sealing the lesion

with filled and/or low oxygen inhibition monomers is a good point to be considered and the adverse effects of oxygen on the cure of the unfilled bonding resin can be minimized (Rueggeberg and Margeson, 1990, Ruyter, 1981). In this way, infiltrants could show improved performance by balanced combination of low viscosity and oxygen non-inhibition monomers polymerization or to use an infiltrant associated with sealant.

From the clinical point of view, the authors of this review believe that some considerations have to be made regarding the fluoridation of initial caries lesion. According to Schmidlin et al (2004), if preventive sealing is chosen to treat a non-cavitated enamel lesion, it should not be fluoridated prior to sealant application. Therefore, non-cavitated lesion sealing is a non-invasive procedure that requires minimal chair time and little expense, while arresting progression of enamel demineralization. This procedure would be of particular value in those patients with poor oral hygiene, irregular dental visits and low exposure to fluoride compounds and remineralizing solutions (Garçía-Godoy, 1997).

The condition of enamel (sound, artificial caries or natural caries) is of great importance for sealant performance and effectiveness. Celiberti and Lussi (2007) investigated the sealing ability of an unfilled fissure sealant applied over different substrates under 45 or 90% relative humidity condition and 40 or 60 seconds of etching times. They observed that independent of the humidity condition and etching time, natural caries resulted in more microleakage than artificial caries and sound enamel. This could be explained by the structural, chemical, and physical properties of the different substrates. The natural non-cavitated enamel lesions are produced by intermittent and prolonged demineralization periods (Davila et al, 1975), whereas most of the enamel artificial lesions used in in vitro studies are produced by a continuous acid attack (Paris et al, 2006; Meyer-Lueckel et al, 2006; Mueller et al, 2006; Gray and Shellis, 2002; Robinson et al, 2001). In sound enamel, demineralization and remineralization processes are balanced and no lesion is formed. The surface topographies for natural caries are rough and porous; while for sound and artificial caries, enamel appears quite similar, with relatively smooth and intact surfaces. Therefore, the biological behavior of artificial caries may be somewhat different from that of natural enamel caries (Lee et al, 1995).

With regard to acid etching in different enamel substrates, the sound enamel,

artificial and natural non-cavitated enamel lesion exhibit a similar morphology after etching, although their degree of damage is different (Lee et al, 1995). A previous study showed that artificial non-cavitated enamel lesion was infiltrated by sealant, independently of whether it was previously etched or not (Davila et al, 1975). Another study by van Dorp and ten Cate (1987) demonstrated that the etched artificial non-cavitated enamel lesion allowed resin penetration and was a suitable substrate for fissure sealant adhesion. Regarding natural non-cavitated enamel lesion, it was only impregnated by resin when previously conditioned and this penetration was less than in artificial lesions (Davila et al, 1975). This behavior may be due to the high concentration of organic material present in natural non-cavitated enamel lesion, which may reduce the size of the lesion microspaces, hampering resin impregnation (Davila et al, 1975). Recently, authors confirmed that the surface layer of non-cavitated natural caries lesion is a barrier that significantly hampers the penetration of light-curing resin. Therefore, no substantial resin penetration could be observed without prior acid conditioning (Paris et al, 2007). In this context, since there are considerable structural differences between artificial and natural caries lesions, it is not applicable to transfer findings from artificial to natural lesions.

With regard to the following studies described below, it may be observed that reports did not completely describe the methods used, nor was statistical analysis carried out. In the Bjarnason et al (2003) study, the microleakage of a fissure sealant on sound and demineralized enamel (smooth surface) using thermocycling was evaluated. However, limited description on the material behavior does not allow any consistent conclusion. The other study compared pit and fissure sealants with fluoride treatment and evaluated their effectiveness in the inhibition or reduction of enamel lesion progression (Van Dorp and ten Cate, 1992). These authors concluded that sealants were the best preventive measure for fissures, since the walls of the fissures were covered by the sealant. These findings are in agreement with previous reports (Feigal, 2002; Weintraub, 2001). Thus, the use of pit and fissure sealants should be encouraged as a standard approach in combination with preventive measures based on dietary advice, oral hygiene, and application of fluoride containing materials for high caries risk patients (Toumba et al, 2003).

From this literature review, it can be concluded that while fissure sealing act as

a diffusion barrier on the top of the lesion surface, the infiltration technique creates this barrier inside the lesion, replacing lost mineral with low-viscous light-curing resin. This latter technique could be a promising alternative therapy in non-compliant individuals with approximal non-cavitated enamel lesions. The results of the present review showed that in vivo studies suggest an arrestment of lesion progression when approximal non-cavitated enamel lesions were infiltrated and/or sealed. Therefore, in in vitro studies, the use of sealer materials on non-cavitated enamel lesion showed a tendency to decrease the progression demineralization due to the occlusion of the pores in the lesion body as a consequence of material penetration and probably enhancement of its retention. Further well-designed in vitro and in vivo researches should be performed in order to provide a high-level evidence studies to guide the best clinical decision.

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CAPÍTULO 2

Influence of different enamel substrates on microtensile bond strength of sealants after cariogenic challenge*

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ABSTRACT

Purpose: To evaluate the microtensile bond strength (μTBS) of resin sealer on enamel substrates after cariogenic challenge. **Materials and Methods:** Enamel blocks were obtained from human third molars, and randomly divided into 6 groups (n=10), according to enamel substrates (S - sound, CL - caries-like lesion or CLTF - caries-like lesion + topical fluoride application) and sealant material (F - FluroShield® or H -Helioseal Clear Chroma®). Sealants were placed on enamel surfaces, stored in 100% humidity (24 h - 37°C), and longitudinally sectioned to obtain hourglasses. According to the groups, pH-cycling was applied and the μTBS test was performed, whereas the fracture patterns were assessed by SEM. **Results:** Regarding substrates, the highest μTBS values (MPa) were observed for CLTF enamel (26.0±7.6), followed by S (22.0±7.4) and CL (15.5±4.9). A significant interaction was found between material and pH-cycling (p=0.0395). F (23.9±7.6) showed the highest μTBS values compared to H (18.3±7.5) when submitted to pH-cycling. The majority of samples presented mixed failure. **Conclusions:** Enamel substrate significantly affected μTBS, with the highest values for remineralized caries-like enamel lesions. Additionally, μTBS values were dependent on both materials and pH-cycling.

INTRODUCTION

Initial enamel caries lesions are usually not treated operatively to avoid the sacrifice of sound hard tissues.[11] Thus, preventive action is important to prevent caries development during an early phase. The maintenance of oral hygiene in conjunction with dietary advice, fluoride therapy and prudent use of pit and fissure sealants has been shown to be a reliable preventive strategy in these populations.[29] The noninvasive pit and fissure sealing has proven to be effective for caries prevention in several studies.[25] In order to obtain long-term clinical success, the sealant retention and the integrity of sealant-enamel bond are important criteria for successful prevention of bacteria leakage and oral fluids that initiate pit and fissure caries.[25]

Some factors that can affect the adhesion of sealer materials to enamel are the structure and organization of tooth enamel. With regard to the condition of the substrates, etching sound enamel with phosphoric acid may form porosities on the surface, thus, the resinous sealants can penetrate into microdepressions created by the acid, allowing high enamel bond strengths.[5,19] The white spot lesion is characterized by a loss of mineral in the bulk of enamel, whereas the surface of the lesion remains relatively intact.[27] The tiny pores within the lesion body may act as diffusion pathways for acids and minerals.[27] In this case, the aim of the sealing regimen is to occlude these pores with light curing resins by penetration into the lesion body, preventing the dissolution of enamel at the advancing front of the lesion.[8,17] Moreover, after curing the material, a mechanical support of the fragile enamel framework in the lesion might be achieved.[17] In the remineralized enamel, its crystalline structure is stabilized by the acquisition of fluoride, which competes with and displaces the hydroxyl groups of the hydroxyapatite molecule to form fluoridated hydroxypatite.[6] Moreover, the formation of calcium fluoride has been reported to reduce the bond strength of resin to enamel.[14]

Considering the structure of the different enamel substrates, such as caries-like lesions or remineralized caries-like lesions, no study has hitherto focused on sealant application on to different enamel substrates in an attempt to prevent the development of the initial lesion. In addition, such treated substrates are constantly submitted to cariogenic

challenge, mainly in high caries risk children. A dynamic chemical model, that is pH-cycling, has been used in order to simulate the oral conditions of a high caries risk children in in vitro studies.[2]

As such, the aim of this in vitro study was to evaluate the microtensile bond strength (μTBS) of resinous sealant materials on different enamel substrates after a cariogenic challenge (pH-cycling). The null hypothesis was that there are no statistically significant differences in the bond strength of different resinous sealant materials on different enamel substrates when submitted or not to pH-cycling.

MATERIAL AND METHODS

This study was conducted after approval from the Ethics Committee of the Piracicaba Dental School, University of Campinas (protocol #046/2006).

Experimental design

The factors under study were: enamel conditions (sound, caries-like lesion or caries-like lesion + topical fluoride application), pH-cycling, and sealant materials (FluroShield® and Helioseal Clear Chroma®). The experimental samples were 60 enamel blocks, which were randomly assigned to six treatment groups (n=10). The response variables were bond strength values and type of failure pattern, as assessed by Scanning Electron Microscopy (SEM).

Preparation of enamel blocks

Ninety and seven impacted human third molars, extracted for clinical and orthodontic reasons and free from apparent caries on the buccal, palatal or lingual surfaces were selected. All teeth were examined under 20 X magnification (LEICA MZ6, Germany) to exclude those with any enamel defect. The teeth were cleaned and stored in 0.5% chloramine T solution for up to 2 months after extraction. Their roots were sectioned off 1 mm below the enamel cement junction using a double-face diamond saw and discharged (KG Sorensen, São Paulo, SP, Brazil). Each tooth was longitudinally sectioned on the fissure orientation (KG Sorensen, São Paulo, SP, Brazil) in order to obtain 194 buccal, palatal or lingual enamel surfaces. The enamel surfaces were flattened (4x4 mm) on a

water-cooled mechanical grinding machine, using 400, 600 and 1200 grit Al_2O_3 abrasive paper (Aropol E, Arotec S.A. Ind. and Com., São Paulo, Brazil), and cloth polished with 1.0-μm diamond paste (Buehler Metadi II, Buehler, Lake Bluff, IL, USA) (Figure 1). Care was taken not to expose the underlying dentin. For block selection, surface microhardness (SMH) determination was accomplished using a Future-Tech FM-ARS microhardness tester (Future-Tech Corp., Tokyo, Japan) with a Knoop diamond under a 50-g load for 5 s.[7] Five indentations were made at the center of the enamel surface (Figure 1). One hundred and twenty-three enamel blocks with 341.6 ± 18.0 Knoop Hardness Number units (KNH) were selected for this study. The rejected blocks (71) were those that did not fit into the mean and standard deviation range, which was considered as 10% above or below the means. Twenty sound enamel blocks were kept in artificial saliva until the experiment (10 enamel blocks were used in the FluroShield® group and the remaining 10 enamel blocks used in the Helioseal Clear Chroma®) and 103 blocks were used for caries-like lesion induction.

Artificial caries-like lesion formation

One hundred and twenty-three enamel block surfaces were isolated with double coats of acid-resistant nail varnish (Colorama, São Paulo, Brazil), except for the polished enamel area (4x4 mm) (Figure 1). Artificial caries-like lesions were produced by suspending each enamel block in 32 mL of a solution containing 0.05 M acetate buffer 50% saturated with enamel, pH 5.0, for 16 h at 37°C. To prepare this solution, enamel powder (particles of 74-105 μm) was agitated in 0.05 sodium acetate buffer, pH 5.0, for 96 h at 37°C (0.50 g/L).[18] The solution was used in the proportion of 2.0 mL/mm2 of exposed enamel area. After caries-like lesion induction, sixty-three blocks with known enamel SMH (81.9 ± 22.8 KHN) were selected. The rejected blocks (60) were those that did not fit into the mean and standard deviation range, which was considered as 10% above or below the means. Twenty enamel blocks with artificial caries-like lesions were then kept in artificial saliva until the experiment (10 enamel blocks were used in the FluroShield® group and the remaining 10 enamel blocks used in the Helioseal Clear Chroma®) and 43 blocks were used

for topical fluoride application on caries-like lesion using 5% NaF varnish, simulating a remineralization procedure.

Artificial caries-like lesion remineralization

Following caries-like lesion formation, forty-three enamel blocks were submitted to topical fluoride application. The enamel surfaces of these blocks were coated with 5% NaF varnish (Duraphat®- Colgate-Palmolive, S. Bernardo do Campo, SP, Brazil), using a microbrush. The varnished blocks were individually immersed in 20 mL of artificial saliva (1.5 mM calcium, 0.9 mM phosphate, 150 mM KCl in 0.1 M Tris buffer, 0.05 µg F/mL, pH 7.0) at 37°C for 1 week. The solution was applied in the proportion of 1.25 mL/mm^2 of exposed enamel area.[9] The varnished blocks were then removed from the artificial saliva and rinsed with distilled deionized water (pH 6.0). After topical fluoride application, twenty blocks with known enamel SMH (140.4 ± 36.5 KHN units) were selected. Ten enamel blocks were used in the FluroShield® group and the remaining ten enamel blocks used in the Helioseal Clear Chroma®). The rejected blocks (23) were those that did not fit into the mean and standard deviation range, which was considered as 10% above or below the means.

Experimental groups

Enamel blocks were then divided into six groups (n=10), according to enamel substrates and sealer materials: Sound enamel + FluroShield® - (SF); caries-like lesion + FluroShield® - (CF); caries-like lesion + topical fluoride application + FluroShield® - (CFF); sound enamel + Helioseal Clear Chroma® - (SH); caries-like lesion + Helioseal Clear Chroma® - (CH); caries-like lesion + topical fluoride application + Helioseal Clear Chroma® - (CFH) (Figure 1).

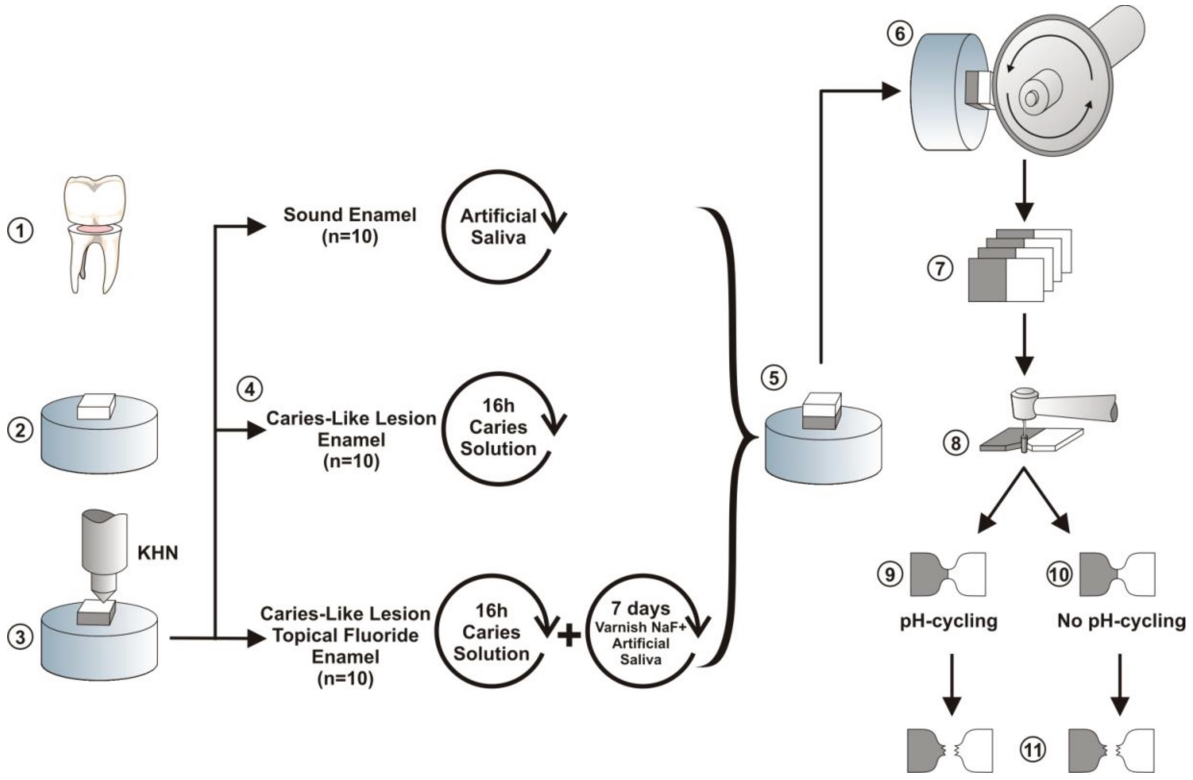

Figure 1. Representative scheme of methodology and experimental design: (1) Root section 1 mm below the enamel cement junction; (2) Enamel blocks preparation (4x4 mm); (3) Surface microhardness determination (SMH); (4) Experimental groups distribution; (5) Sealer material application (4 mm height) (FluroShield® or Helioseal Clear Chroma®); (6) Longitudinal cutting of the sample into a series of 1-mm thick slices; (7) Slices preparation (four slices per enamel block); (8) Hourglass preparation (≅1 mm^2 cross-sectional area); (9,10) Submission or not to pH-cycling; (11) Microtensile bond strength test (μTBS).

Sample preparation

After enamel substrates preparation, the polished enamel surface of the blocks was etched using 37% phosphoric acid gel for 30 s, rinsed for 10 s with water, and dried. The sealer materials were then applied to a flat surface to build up a bonding surface block of about 5 mm in height, with 2 mm-thick increments; samples were then light-cured for 40 s (FluroShield®) or 20 s (Helioseal Clear Chroma®) (Figure 1). The light curing was carried out using the Elipar Tri-light unit (3M ESPE, Seefeld, Germany) with 800 mW/cm^2 light intensity. The sealed blocks were stored for 24 h at 37^{o}C and 100% humidity. The sealants brand names, composition, manufacturers, and batch numbers are listed in Table 1.

Afterwards, each sample was longitudinally cut into a series of 1 mm-thick slices by means of a water-cooled diamond blade (Isomet, Buehler, Lake Bluff, IL, USA) (Figure 1). Four slices were obtained per each block and trimmed to an hourglass shape using a cylinder diamond bur (FG 3097 - KG Sorensen, São Paulo, SP, Brazil) mounted in a high-speed hand piece (Figure 1). Each hourglass was isolated with double coats of acid-resistant nail varnish (Colorama, São Paulo, Brazil), except for the bonding area.

pH-cycling Model

The hourglasses were then submitted or not to a pH-cycling model (Figure 1). Two hourglasses from each sample were subjected to a 7-day pH-cycling model, simulating a cariogenic challenge.[2,9] Each cycle consisted of a 3-hour immersion in demineralizing solution followed by a 21-hour immersion in remineralizing solution. Hourglasses 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) used in the proportion of 2.5 mL/mm^2 of exposed bonding area. Hourglasses 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 KCl in 0.1 M tris buffer, 0.05 µg fluoride/mL, pH 7.0, 37°C) applied in the proportion of 1.25 mL/mm^2.[9] Both solutions contained thymol crystals to avoid microbial growth.

Table 1. Brand, composition, manufacturers, and batch number of the sealer materials.

Materials	Composition	Manufacturers and Batch #
FluroShield	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	Bis-GMA, Triethylene glycol dimethacrylate (>99wt.%). Additional contents are stabilizers, catalyts and pigments (<1wt.%)	Ivoclar/Vivadent Schaan Liechtenstein # F54463

Microtensile bond strength test (μTBS)

The hourglasses were tested individually using cyanoacrylate glue (Super Bonder, São Paulo, Brazil). The μTBS test was performed in a universal testing machine (4411 – Instron, Canton, MA, USA) at a crosshead speed of 0.5 mm/min. The cross-sectional area at the site of fracture was measured with a digital caliper (Mitutoyo, Suzano, Brazil) with an accuracy of 0.01 mm. The load (in Kgf) and the bonding surface area of each specimen were recorded on a worksheet. The microtensile bond strengths were calculated in MPa, using the formula: R=F (Kgf)/A (cm).

Determination of failure pattern

The failure sites were gold-sputter coated (Balzers-SCD 050 Sputter Coater, Liechtenstein) and observed by SEM (JEOL- JSM 5600LV, Tokyo, Japan) at an accelerating voltage of 15 kV, a working distance of 20 mm, and with a magnification of 100X. For each specimen, the failure pattern was defined into four types: mixed failure; adhesive failure; cohesive failure in enamel; or cohesive failure in sealer material. A blind calibrated examiner (K.R.K.) evaluated the failure pattern. The intra-examiner coincidence level of failure pattern was analyzed by Spearman's correlation test and was 95%.

Statistical analysis

Original data from μTBS test were transformed (x0.5) before applying 3-way ANOVA and Tukey test, because variances were not homogeneous. A multi-factor ANOVA was applied to the μTBS data to analyze the interactions among the factors (enamel substrates, materials and pH-cycling). In order to assess significant differences within these factors, Tukey test was applied. The software SAS system software (version 8.02, SAS Institute Inc., Cary: NC, 1999) was used and the significance limit was set at 5% ($p<0.05$).

RESULTS

According to 3-way ANOVA statistical analysis, there were no interactions among: enamel substrates and materials ($p=0.1347$); enamel substrates and pH-cycling ($p=0.8126$); and enamel substrates, materials and pH-cycling ($p=0.0949$). A significant

difference was found among the enamel substrates factors examined ($p<0.001$). The mean values for enamel substrates in the μTBS test (MPa values) and their 95% confidence intervals are shown in Figure 2. The data of sealer material groups were combined. Significant differences were observed between the materials and an effect of pH-cycling when Tukey test was applied ($p=0.0395$). The data of substrate enamel groups were combined (Figure 3). FluroShield (23.9±7.6) presented the highest μTBS values compared to Helioseal Clear Chroma (18.3±7.5), when the materials were submitted to pH-cycling. Sealant materials showed no difference in μTBS when not submitted to pH-cycling as shown in Figure 3.

The percentage of failure pattern for all groups is presented in Figure 4. Mixed failure (cohesive in the sealant and cohesive in the enamel, Figure 5) was the most frequently observed failure for all groups, with the exceptions of SF and CFF when not submitted to pH-cycling.

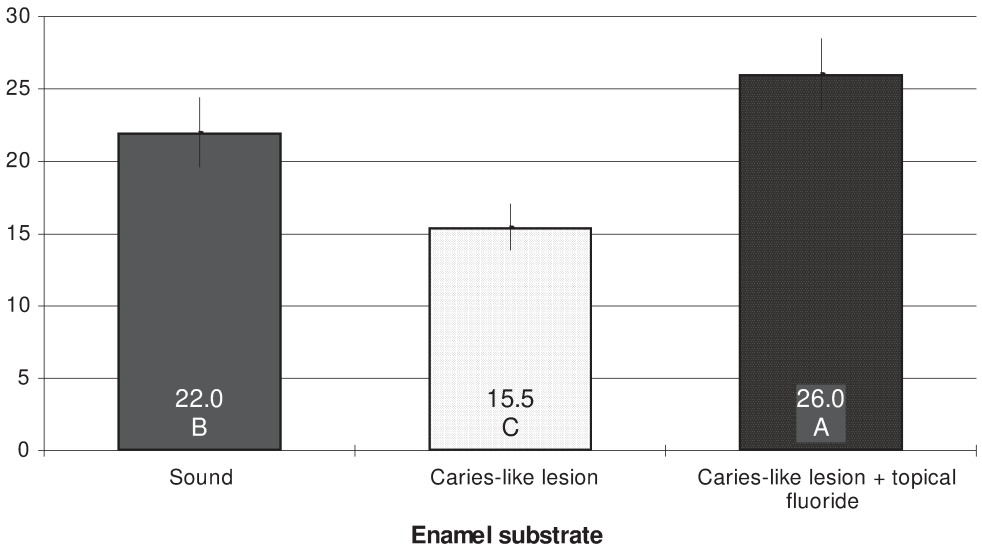

Figure 2. μTBS mean (MPa) and 95% confidence intervals for different enamel substrates.

Different letters represent statistically significant difference by Tukey test ($p<0.05$). The data of sealer material groups were combined.

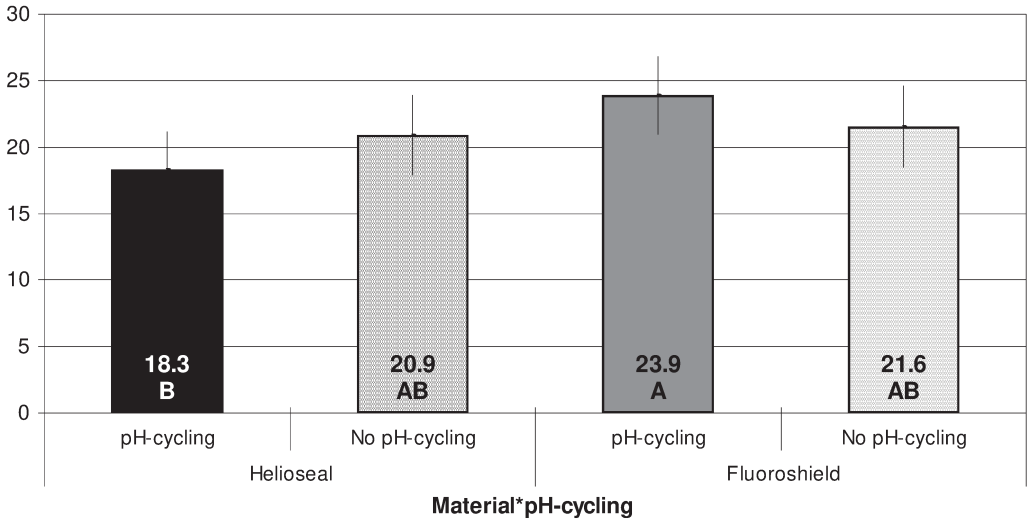

Figure 3. Means (MPa) and 95% confidence intervals of the μTBS measured.

Different letters represent statistically significant difference by Tukey test ($p<0.05$). The data of enamel substrate groups were combined.

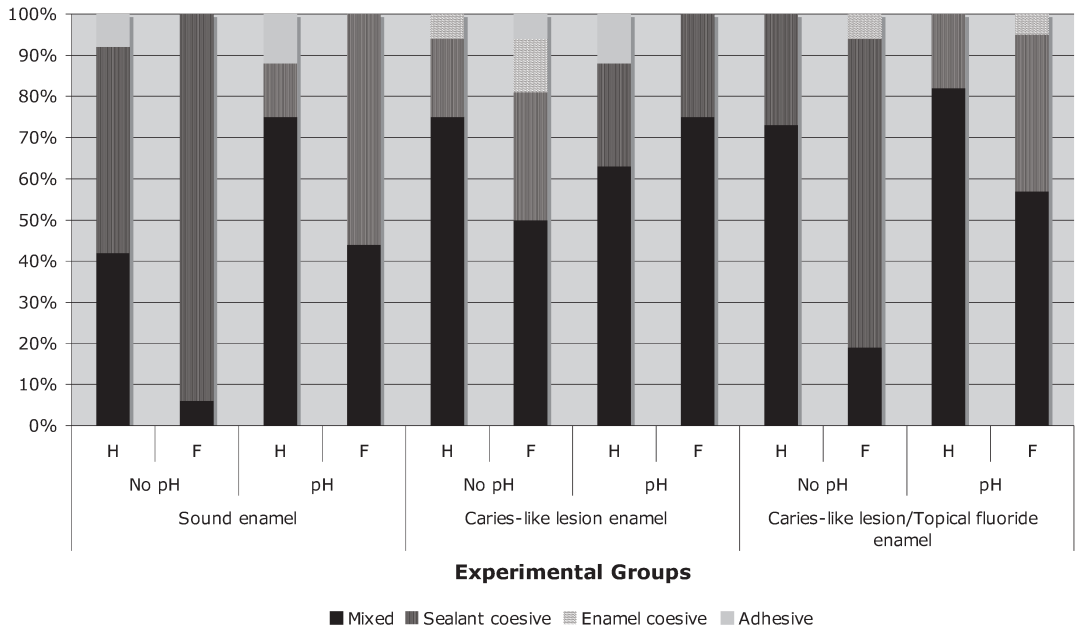

Figure 4. Percentage of failure patterns in the experimental groups after μTBS test.

H – Helioseal Clear Chroma; F-FluroShield; No pH – No pH-cycling; pH – pH-cycling.

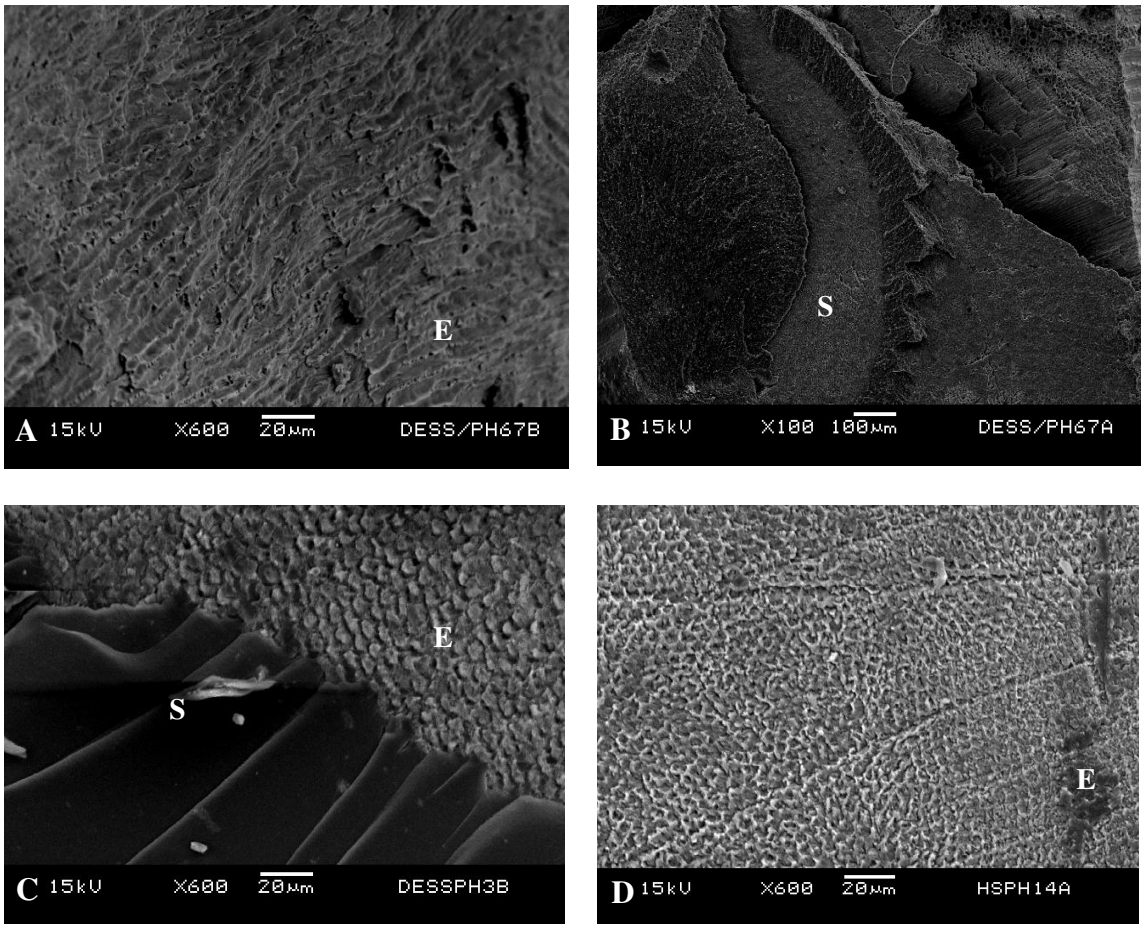

Figure 5. SEM photomicrography (600X and 100X) illustrating the failure pattern for experimental groups: **(A)** Enamel cohesive failure; **(B)** Sealant cohesive failure**; (C)** Mixed failure; **(D)** Adhesive failure. E – enamel; S – sealant.

DISCUSSION

Fissure sealants are currently one of the most effective tools available for protection against caries development on the occlusal surfaces of high dental caries risk children. An important parameter in the evaluation of the clinical success of sealing procedures is the ability of the material to adhere to the enamel surface.[1] The bond strength is an indicator of this ability and the μTBS test was chosen due to its more accurate assessment of the interfacial bond strength of material and dental substrates, since it provides uniform stress distribution over small-sized specimens.[21] Another aspect to be considered is the condition of enamel, which is of great importance for sealant performance and efficiency.

The null hypothesis that there are no differences in the bond strength of the different enamel substrates and resinous sealant materials was rejected. In this study, differences were noted in the μTBS of the sound, caries-like lesion and caries-like lesion with topical fluoride application enamel. The fluoride application of 5% NaF varnish on caries-like lesions of enamel increased the μTBS values, compared to the values of sound and caries-like lesion enamel. This could be explained by the structural,[15,16] chemical, and physical properties of the different substrates and suggests that the mineral status of the underlying dental hard tissue substrates could influence bond strength.

Little is known about μTBS of fissure sealants when applied to different dental substrates. When analyzing remineralized enamel, a study[13] found clinically acceptable tensile bond strength values for sealed pretreated enamel surfaces, in accordance with data from the current study. Conversely, other studies have indicated that topical fluoride application fills the interprismatic spaces occupied by $Ca_5(PO)_3$ and CaF_2 and reduces the bonding capacity of adhesives.[15,16] On the other hand, studies have shown that tensile[28] or shear[10,12] bond strength is not significantly different in groups with and without fluoride pretreatment. In these studies, researchers saw globular structures only on the prism cores of ground enamel surfaces etched with H_3PO_4 containing higher fluoride concentrations; they did not observe adverse effects in the bond strength of bonding resin to etched enamel.

Experimental studies have shown that varnishes supply fluoride more efficiently than other topical agents.[4,24] The use of a varnish as a vehicle for topical fluoride application was chosen in this study due to its prolonged period of contact with the enamel surface, allowing greater uptake of fluoride ions into the enamel and making it more resistant to demineralization.[3,22] Even though that the remineralization procedure used in this in vitro study has virtually no reliance to the in vivo de-remineralization dynamics.

For sound enamel substrates, results demonstrated intermediate μTBS values (22.0 MPa) when compared with topical fluoride application and caries-like lesion enamel. Similar results were found in a report that evaluated the μTBS of different types of materials used as pit and fissure sealants for sound ground enamel.[20]

In caries-like lesion enamel, the surface topographies appear relatively smooth and intact with a slightly larger pore volume than sound enamel.[26] With regard to acid etching of these substrates, it was reported that the surface morphologies of lesions that where acid-etched lesions for 30 s were rough with loss of prisms; this may provide a more reactive surface for fluoride treatment and perhaps enhance the rate of remineralization.[26] In this context, it may be suitable to provide the topical fluoride application on the caries-like lesions before sealant application, instead of applying direct sealant on the early caries lesions, which presented the lowest μTBS values in the present study.

In this study, a pH-cycling model simulated a caries risk situation. Regardless of the substrate, pH-cycling did not influence the μTBS values for either of the materials. However, pH-cycling resulted in higher μTBS values for Fluroshield than Helioseal. Under this condition, Helioseal demonstrated a higher percentage of adhesive failures, showing a weakening of bonding interface. This finding was not observed for Fluroshield, possibly due to its composition. The difference between the sealants tested may occur as a consequence of the presence of fluoride and fillers (30% barium aluminoborosilicate) in the Fluroshield composition, since both materials are resin-based. The presence of fillers in FluoroShield may increase the mechanical resistance of the material and the bond strength of resin-based materials bond strength may depend on the length, shape, and mechanical properties of resinous tags.[23] In addition, fluoride content of Fluroshield seems to protect

the bonding interface from cariogenic challenge, when compared with Helioseal under the same conditions, showing higher percentage of mixed and sealant cohesive failures. Especially the interface below the infiltrated zone, between demineralized, maybe poorly infiltrated enamel and the sealant seem to be the weakest link in bonding.

μTBS may represent an indicator of the sealer material's clinical retention ability. Thus, the results of this in vitro investigation may provide additional information to clinical practice. However, these findings should not be directly extrapolated to clinical conditions. Further in vitro and in situ studies and clinical evaluations are required to assess the long-term bonding performance of these sealer materials and, thus, predict the quality of the adhesion obtained as well as the sealer material degradation in a high caries risk situation, indicating their failures.

CONCLUSION

Within the limitations of this in vitro study, it can be concluded that the μTBS was significantly influenced by different enamel substrates, where remineralized caries-like enamel lesions substrates demonstrated the highest μTBS values. The μTBS values are dependent of both materials and pH-cycling.

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CLINICAL RELEVANCE

Success of sealants is dependent upon material's retention, and in vitro, microtensile bond strength may be an indicator of this clinical ability.

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CONCLUSÃO

Diante dos objetivos e da metodologia empregada no presente estudo, concluiu-se que:

1. Há evidência de que enquanto o selamento de fissuras age como uma barreira de difussão no topo da superfície da lesão inicial de cárie, a técnica de infiltração do material selador/adesivo cria uma barreira dentro da lesão de cárie, repondo a perda de mineral com resina de baixa viscosidade e fotoativada. Esta técnica pode ser uma terapia alternativa em pacientes não cooperadores com as estratégias preventivas utilizadas pelo cirurgião dentista;

2. A resistência de união do material selador foi influenciada pelo tipo de substrato de esmalte. Os valores mais altos foram encontrados para o esmalte com lesão inicial de cárie e aplicação de verniz fluoretado independente do material selador resinoso aplicado. Quando os materiais seladores foram expostos ao desafio cariogênico (ciclagem de pH), FluroShield demonstrou os maiores valores de resistência de união ao esmalte.

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[*] De acordo com a norma da UNICAMP/FOP, baseadas na norma do *International Committee of Medical Journal Editors*-Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o *Medline*.

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APÊNDICE 1. Ilustrações da metodologia empregada no capítulo 2.

Fotografias ilustrativas da sequência da seleção e preparo da amostra.

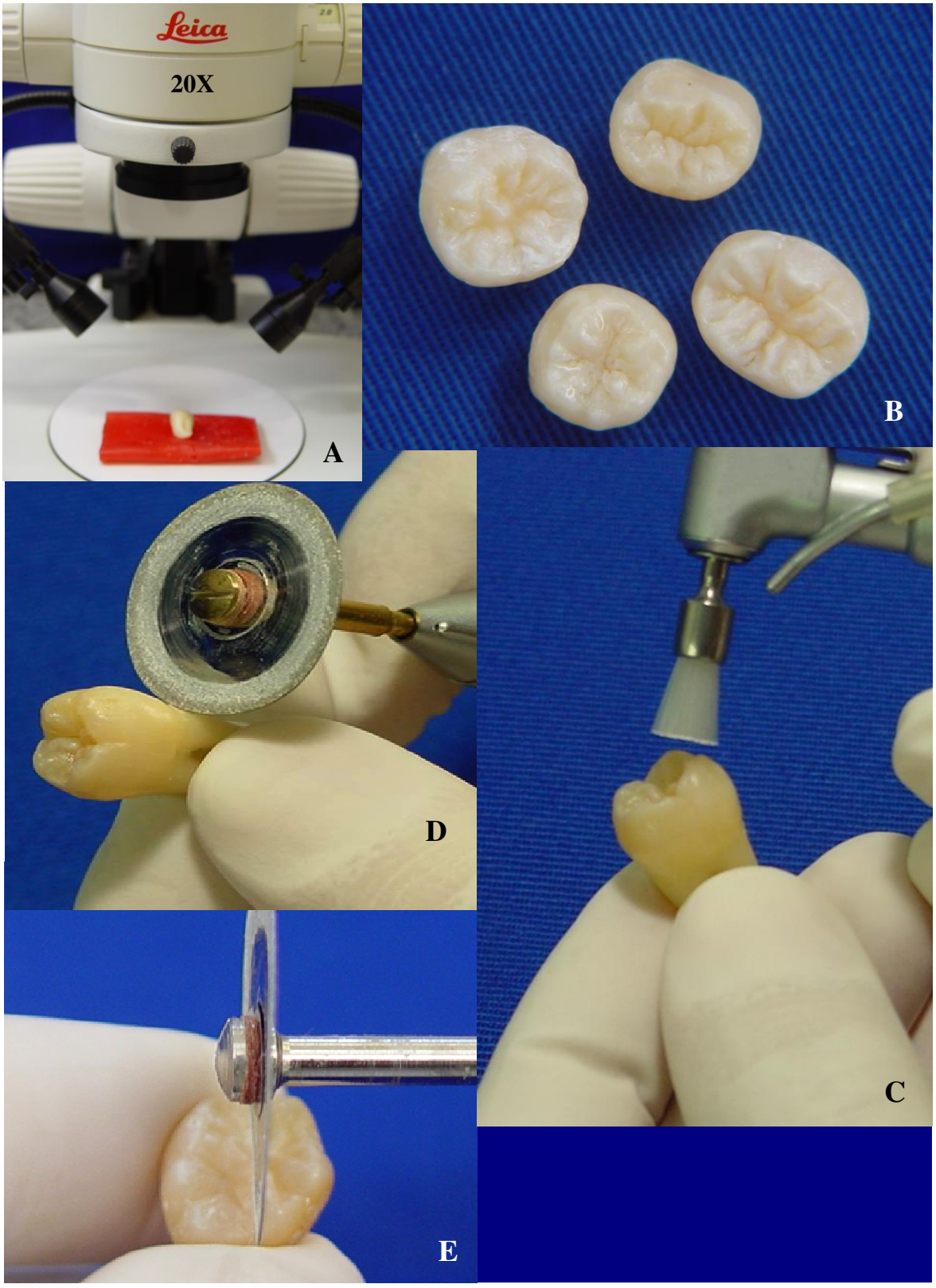
A - Lupa esterioscópica (Leica MZ6, Alemanha);

B - Seleção dos dentes permanentes;

C - Procedimento de profilaxia com pedra pomes e água;

D - Secção transversal das raízes dos dentes humanos permanentes selecionados;

E - Secção longitudinal das coroas dos dentes permanentes (secção sob fissura central do elemento dentário).


Leica
20X
A
B
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APÊNDICE 2. Ilustrações da metodologia empregada no capítulo 2.

Fotografias ilustrativas da sequência do preparo da amostra

A - Politriz (Aropol E, Arotec, Brasil) utilizada para a planificação e polimento das amostras com lixas de óxido de alumínio nos 400, 600, 1200 (Arotec, São Paulo, SP, Brazil) e pasta de diamante de 1μm (Buheler Metadi II, Buheler, Lake Buff, IL, USA);
B - Microdurômetro utilizado para determinação da microdureza Knoop (Future-Tech FM-ARS, Future-Tech Corp., Japan);
C - Amostra impermeabilizada com verniz ácido resistente (esmalte vermelho Colorama®, São Paulo, Brasil) com área de exposição de esmalte de 4x4 mm e a ilustração esquemática da localização e distâncias das 5 impressões realizadas em cada amostra na análise de microdureza superficial (50g / 5s; distância de 200 μm entre elas);
D – Balão de separação do pó de esmalte e do pó de dentina evidenciando as fases de separação: a - pó de dentina; b - solução separadora - acetona + bromofórmio (Reagentes Analíticos Dinâmica, São Paulo, Brasil); c - pó de esmalte (utilizado na solução de cárie).


arotec
arotec
Aropol E
400
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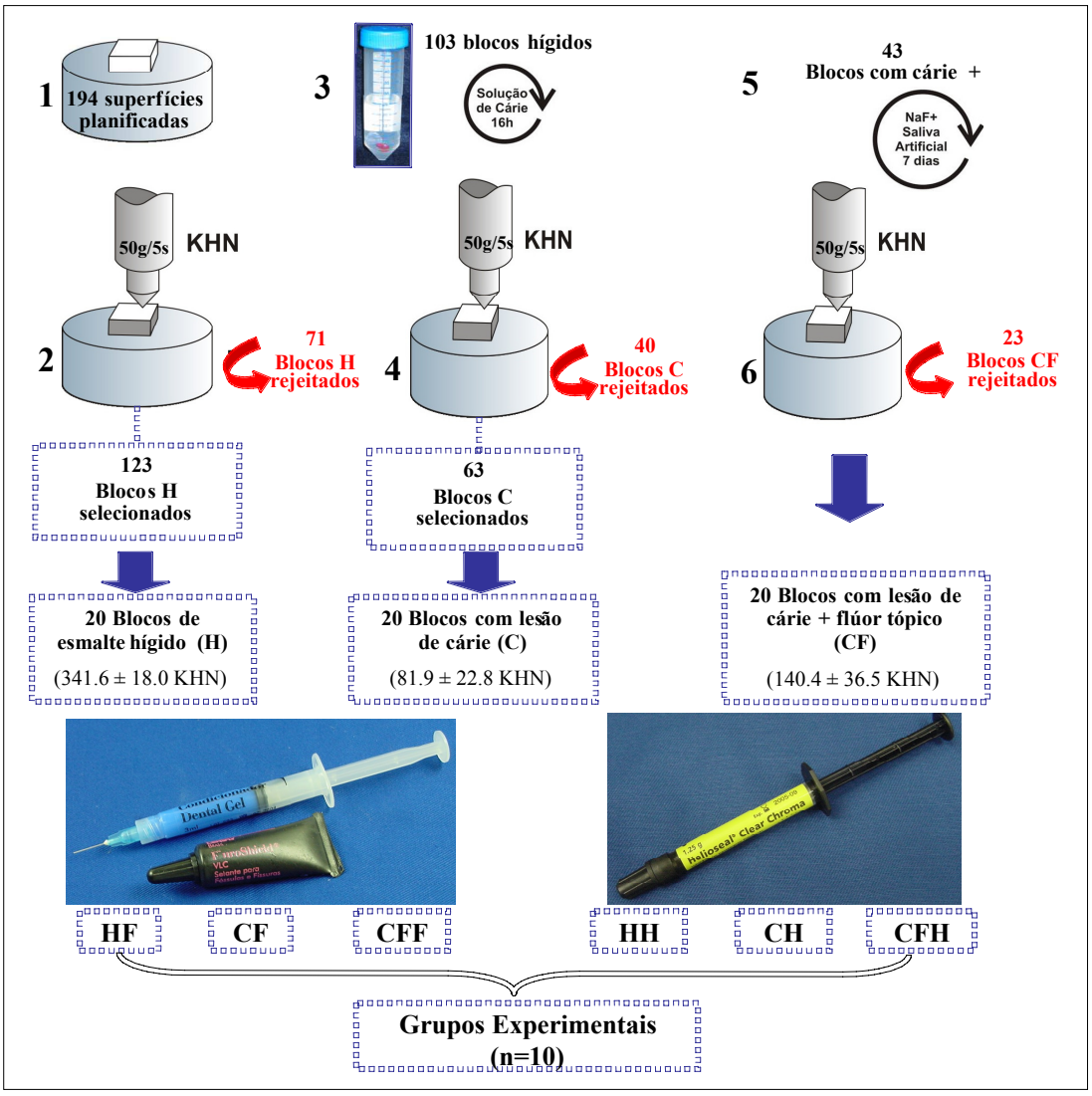
A
HMV-2000
B
200 µm
C
a
b
c
D

APÊNDICE 3. Ilustrações da metodologia empregada no capítulo 2.

Esquema ilustrativo dos passos utilizados para a seleção, preparo e distribuição dos blocos de esmalte nos grupos experimentais

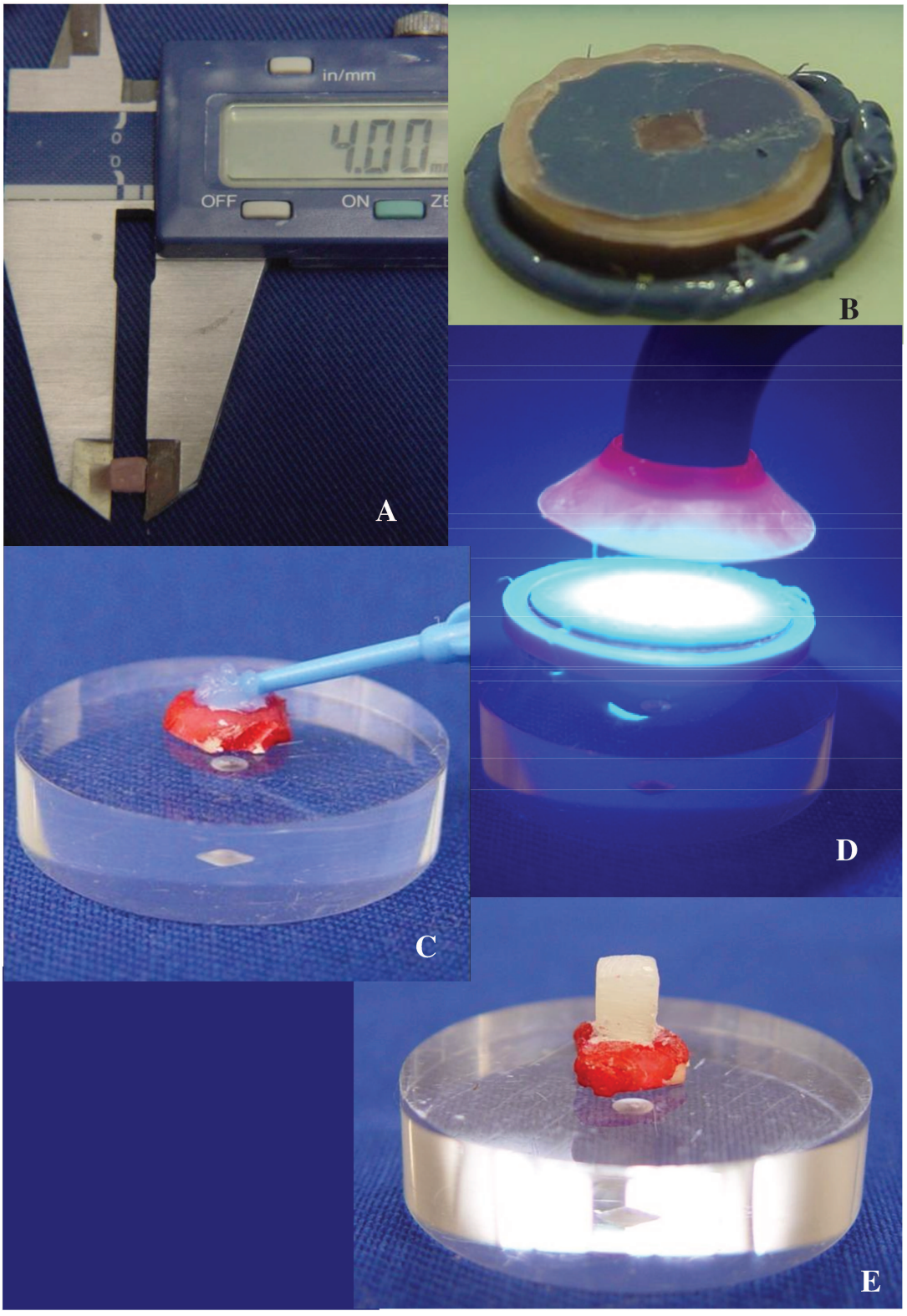
1 – Planificação das superfícies vestibular, ligual ou palatina dos blocos de esmalte;
2 - Obtenção da média e o desvio padrão das 5 impressões realizadas em cada bloco de esmalte hígido; exclusão dos blocos de esmalte com valores de média 10% maior e menor que o valor da média geral (71), seleção de 123 blocos hígidos. Destes, 20 blocos foram aleatoriamente selecionados e mantidos em saliva artificial até o momento do experimento;
3 - 103 blocos de esmalte hígido submetidos a solução de cárie ;
4 - Obtenção da média e o desvio padrão das 5 impressões realizadas em cada bloco de esmalte com lesão inicial de cárie; exclusão dos blocos de esmalte com lesão inicial de cárie (40), seleção de 63 blocos. Destes, 20 blocos foram aleatoriamente selecionados e mantidos em saliva artificial até o momento do experimento;
5 - 43 blocos com lesão inicial de cárie foram submetidos a aplicação tópica de flúor (5% de NaF / 7 dias);
6 - Obtenção da média e o desvio padrão das 5 impressões realizadas em cada bloco de esmalte com lesão inicial de cárie + aplicação tópica de flúor; exclusão de 23 blocos de esmalte, seleção de 20 blocos de esmalte remineralizados.


1
194 superfícies planificadas
3
103 blocos hígidos
Solução de Cárie 16h
5
43
Blocos com cárie +
NaF+ Saliva Artificial 7 dias
50g/5s
KHN
2
71
Blocos H rejeitados
4
40
Blocos C rejeitados
6
23
Blocos CF rejeitados
123
Blocos H selecionados
63
Blocos C selecionados
20 Blocos de esmalte hígido (H)
(341.6 ± 18.0 KHN)
20 Blocos com lesão de cárie (C)
(81.9 ± 22.8 KHN)
20 Blocos com lesão de cárie + flúor tópico (CF)
(140.4 ± 36.5 KHN)
Dental Gel
HF
CF
CFF
HH
CH
CFH
Grupos Experimentais (n=10)

APÊNDICE 4. Ilustrações da metodologia empregada no capítulo 2.

Fotografias ilustrativas da seqüência da técnica de confecção das amostras

A - Confecção do cubo de cera 7 (4x4 mm) com o auxilio de paquimetro digital (Mitutoyo, Mitutoyo Sul Americana Ltda, São Paulo, Brasil);
B - Confecção de matriz de poliéter (Impregum soft, 3M/ESPE, São Paulo, Brasil) com auxílio de tubo de PVC;
C - Condicionamento ácido (ácido fosfórico 37% / 30 s) dos diferentes substratos de esmalte (hígido, lesão inicial de cárie ou lesão inicial de cárie/flúor tópico);
D - Fotoativação do material selador (FluroShield® por 40 s ou Helioseal Clear Chroma® por 20 s) em incrementos de aproximadamente 2 mm de espessura com o auxilio de matriz de poliéster;
E - Confecção da amostra (4x4x5 mm) sobre as superfícies vestibular ou palatina/ligual planificadas nos diferentes substratos de esmalte.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APÊNDICE 5. Ilustrações da metodologia empregada no capítulo 2.

Fotografias ilustrativas da seqüência da técnica de confecção dos corpos-de-prova

A - Cortadeira metalográfica (ISOMET 1000 – Buehler, UK) e do disco diamantado (series 15LC Diamond, Buehler, UK) utilizados para a confecção dos corpos-de-prova;

B - Fatia de 1 mm de espessura formada por material selador (S) e substrato de esmalte (E);

C - Ampulheta confeccionada com o auxílio de broca cilíndrica diamantada (1092-KG Sorensen, São Paulo, Brasil) acoplada a alta rotação (Dabi Atlanti, Ribeirão Preto, SP, Brasil) sob refrigeração;

D - Ampulheta pronta com 1 mm de espessura na interface material selador/esmalte;

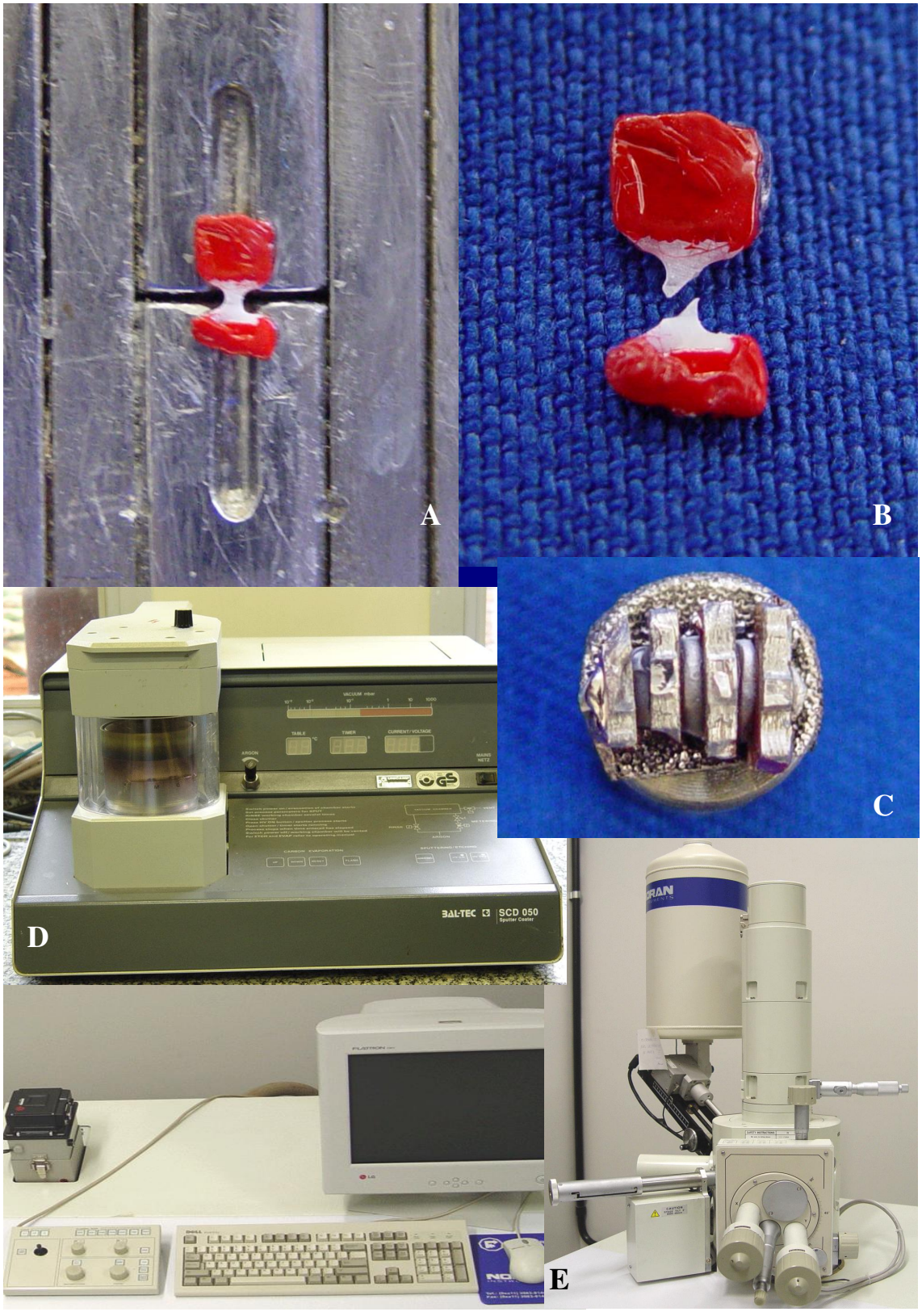
E - Ampulhetas com duas camadas de verniz ácido-resistente, área de exposição de aproximadamente 4x4 mm^2.


ISOMET
1000
POSITION
00.0
SPEED
600
A
S
Área de
união
E
B
C
D
E

APÊNDICE 6. Ilustrações da metodologia empregada no capítulo 2.

Sequência do ensaio de resistência a micro-traçã e análise dos sítios de fratura

A - Ampulheta fixada com cola de cianoacrilato gel (Superbonder gel, Loctite, São Paulo, Brasil) e *spray* acelerador (Loctite 7455 Acivator, Loctite Espana, Madri, Espanha) em dispositivo inserido na máquina de ensaio universal (Instron - Modelo 4411, Canton, USA);
B - Ampulheta após a realização do ensaio mecânico de micro-tração;
C - Corpos-de-prova fixados sobre *stubs* de latão e cobertos com ouro para análise dos sítios de fratura em microscopio eletrônico de varredura (MEV);
D - Metalizadora utilizada em 52 mA por 120 s (Balzers-SCD 050 Sputter Coater, Liechtenstein);
E - Microscópio Eletronico de Varredura (JEOL- JSM 5600LV Tokyo, Japan) regulado para 15 kV, distância de trabalho de 20 mm, spotsize de 35 e aumento de 100X.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ANEXO 1 - Resolução CCPG/002/06 a qual dispõe a respeito do formato das teses de mestrado e doutorado aprovados pela UNICAMP (Parte I)

INFORMAÇÃO CCPG/OO2/06[6]

Tendo em vista a necessidade de revisão da regulamentação das normas sobre o formato e a impressão das dissertações de mestrado e teses de doutorado e com base no entendimento exarado no Parecer PG nº 1985/96, que trata da possibilidade do formato alternativo ao já estabelecido, a CCPG resolve:

Artigo 1º - O formato padrão das dissertações e teses de mestrado e doutorado da UNICAMP deverão obrigatoriamente conter:

I. Capa com formato único ou em formato alternativo que deverá conter informações relativas ao nível (mestrado ou doutorado) e à Unidade de defesa, fazendo referência à Universidade Estadual de Campinas, sendo o projeto gráfico das capas definido pela PRPG.
II. Primeira folha interna dando visibilidade à Universidade, a Unidade de defesa, ao nome do autor, ao título do trabalho, ao número de volumes (quando houver mais de um), ao nível (mestrado ou doutorado), a área de concentração, ao nome do orientador e co-orientador, ao local (cidade) e ao ano de depósito. No seu verso deve constar a ficha catalográfica.
III. Folha de aprovação, dando visibilidade à Comissão Julgadora com as respectivas assinaturas.
IV. Resumo em português e em inglês (ambos com no máximo 500 palavras).
V. Sumário.
VI. Corpo da dissertação ou tese dividido em tópicos estruturados de modo característico à área de conhecimento.
VII. Referências, formatadas segundo normas de referenciamento definidas pela CPG da Unidade ou por critério do orientador.
VIII. Todas as páginas deverão, obrigatoriamente, ser numeradas, inclusive páginas iniciais, divisões de capítulos, encartes, anexos, etc... As páginas iniciais poderão ser numeradas utilizando-se algarismos romanos em sua forma minúscula.
IX. Todas as páginas com numeração "impar" serão impressas como "frente" e todas as páginas com numeração "par" serão impressas como "verso".

§ 1º - A critério do autor e do orientador poderão ser incluídos: dedicatória; agradecimento; epígrafe; lista de: ilustrações, tabelas, abreviaturas e siglas, símbolos; glossário; apêndice; anexos.

§ 2º - A dissertação ou tese deverá ser apresentada na língua portuguesa, com exceção da possibilidade permitida no artigo 2° desta Informação.

§ 3º - As dissertações e teses cujo conteúdo versar sobre pesquisa envolvendo seres humanos, animais ou biossegurança, deverão apresentar anexos os respectivos documentos de aprovação.

Artigo 2º - A critério do orientador e com aprovação da CPG da Unidade, 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.

[6] Disponível em: http://www.prpg.unicamp.br/ccpg_inf002_06.pdf

ANEXO 1 - Resolução CCPG/002/06 a qual dispõe a respeito do formato das teses de mestrado e doutorado aprovados pela UNICAMP (Parte II)

§ único - O orientador e o candidato deverão verificar junto às editoras a possibilidade de inclusão dos artigos na dissertação ou tese, em atendimento à legislação que rege o direito autoral, obtendo, se necessária, a competente autorização, deverão assinar declaração de que não estão infringindo o direito autoral transferido à editora.

Artigo 3º - Dependendo da área do conhecimento, a critério do orientador e com aprovação da CPG da Unidade, a dissertação ou tese poderá ser apresentada em formato alternativo, desde que observados os incisos I, II, III IV, V e VII do artigo 1º.

Artigo 4º - Para impressão, na gráfica da Unicamp, dos exemplares definitivos de dissertações e teses defendidas, deverão ser adotados os seguintes procedimentos:

§ 1º - A solicitação para impressão dos exemplares de dissertações e teses poderá ser encaminhada à gráfica da Unicamp pelas Unidades, que se responsabilizarão pelo pagamento correspondente.

§ 2º - Um original da dissertação ou tese, em versão definitiva, impresso em folha tamanho carta, em uma só face, deve ser encaminhado à gráfica da Unicamp acompanhado do formulário "Requisição de Serviços Gráficos", onde conste o número de exemplares solicitados.

§ 3º - A gráfica da Unicamp imprimirá os exemplares solicitados com capa padrão. Os exemplares solicitados serão encaminhados à Unidade em, no máximo, cinco dias úteis.

§ 4º - No formulário "Requisição de Serviços Gráficos" deverão estar indicadas as páginas cuja reprodução deva ser feita no padrão "cores" ou "foto", ficando entendido que as demais páginas devam ser reproduzidas no padrão preto/branco comum.

§ 5º - As dissertações e teses serão reproduzidas no padrão frente e verso, exceção feita às páginas iniciais e divisões de capítulos; dissertações e teses com até 100 páginas serão reproduzidas no padrão apenas frente, exceção feita à página que contém a ficha catalográfica.

§ 6º - As páginas fornecidas para inserção deverão ser impressas em sua forma definitiva, ou seja, apenas frente ou frente/verso.

§ 7º - O custo, em reais, de cada exemplar produzido pela gráfica será definido pela Administração Superior da Universidade.

Artigo 5º - É obrigatória a entrega de dois exemplares para homologação.

Artigo 6º - Esta Informação entrará em vigor na data de sua publicação, ficando revogadas as disposições em contrário, principalmente as Informações CCPG 001 e 002/98 e CCPG/001/00.

Campinas, 13 de setembro de 2006

Profa. Dra. Teresa Dib Zambon Atvars
Presidente
Comissão Central de Pós-Graduação

ANEXO 2 – Declaração do direito autoral transferido a editora quando a tese for defendida em formato alternativo.

Folha_____
Processo_____
Rubrica_____

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

DECLARAÇÃO

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Tese de Doutorado intitulada "DESEMPENHO DE MATERIAIS SELADORES E INFILTRANTES SOBRE A LESÃO ARTIFICIAL DE CÁRIE EM ESMALTE. ANÁLISE MECÂNICA E MICRO-MORFOLÓGICA", não infringem os dispositivos da Lei nº 9.610/98, nem o direito autoral de qualquer editora.

Piracicaba, 11 de Dezembro de 2009.

KAMILA ROSAMILIA KANTOVITZ
RG: 22575575-0
Autor(a)

REGINA MARIA PUPPIN RONTANI
RG: 10.723.931
Orientador(a)

ANEXO 3 - Certificado do Comitê de Ética em Pesquisa

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Desempenho do selamento de lesões de cárie artificial em esmalte frente ao desafio cariogênico - Estudos in vitro e in situ. análise mecânica, micro-morfológica e bioquímica**", protocolo nº **046/2006**, dos pesquisadores **KAMILA ROSAMILIA KANTOVITZ, REGINA MARIA PUPPIN RONTANI e REGINALDO BRUNO GONÇALVES**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 05/05/2006.

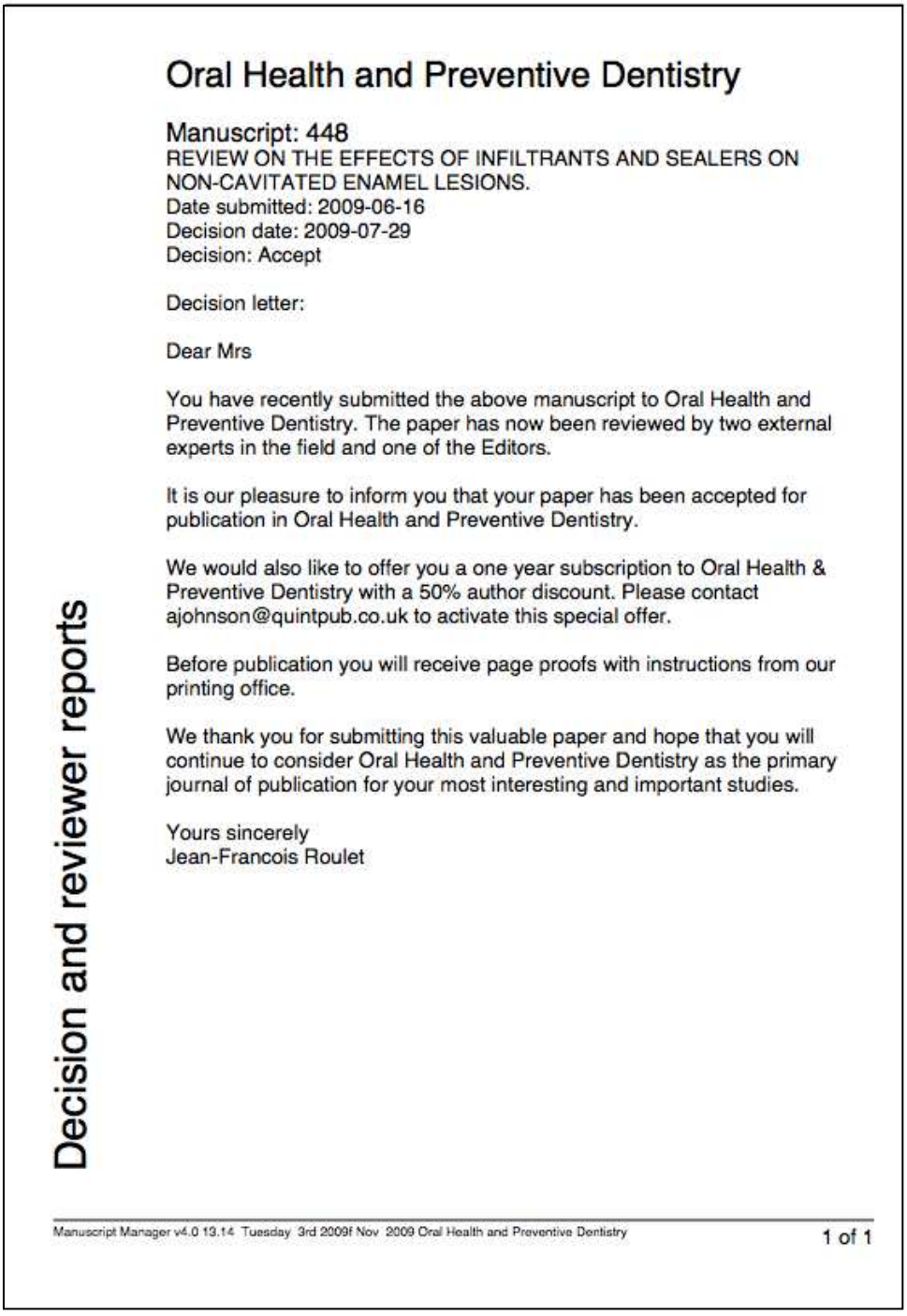
The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Performance of sealant in the enamel white spot lesion under cariogenic challenge - studies in vitro and in situ. Mechanical, micro- morphological and biochemical analysis**", register number **046/2006**, of **KAMILA ROSAMILIA KANTOVITZ, REGINA MARIA PUPPIN RONTANI and REGINALDO BRUNO GONÇALVES**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 05/05/2006.

Profa. Cecília Gatti Guirado
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 4 – Confimação do aceite do primeiro artigo apresentado nesta Tese para o periódico *Oral Health & Preventive Dentistry*



Oral Health and Preventive Dentistry

Manuscript: 448
REVIEW ON THE EFFECTS OF INFILTRANTS AND SEALERS ON NON-CAVITATED ENAMEL LESIONS.
Date submitted: 2009-06-16
Decision date: 2009-07-29
Decision: Accept

Decision letter:

Dear Mrs

You have recently submitted the above manuscript to Oral Health and Preventive Dentistry. The paper has now been reviewed by two external experts in the field and one of the Editors.

It is our pleasure to inform you that your paper has been accepted for publication in Oral Health and Preventive Dentistry.

We would also like to offer you a one year subscription to Oral Health & Preventive Dentistry with a 50% author discount. Please contact ajohnson@quintpub.co.uk to activate this special offer.

Before publication you will receive page proofs with instructions from our printing office.

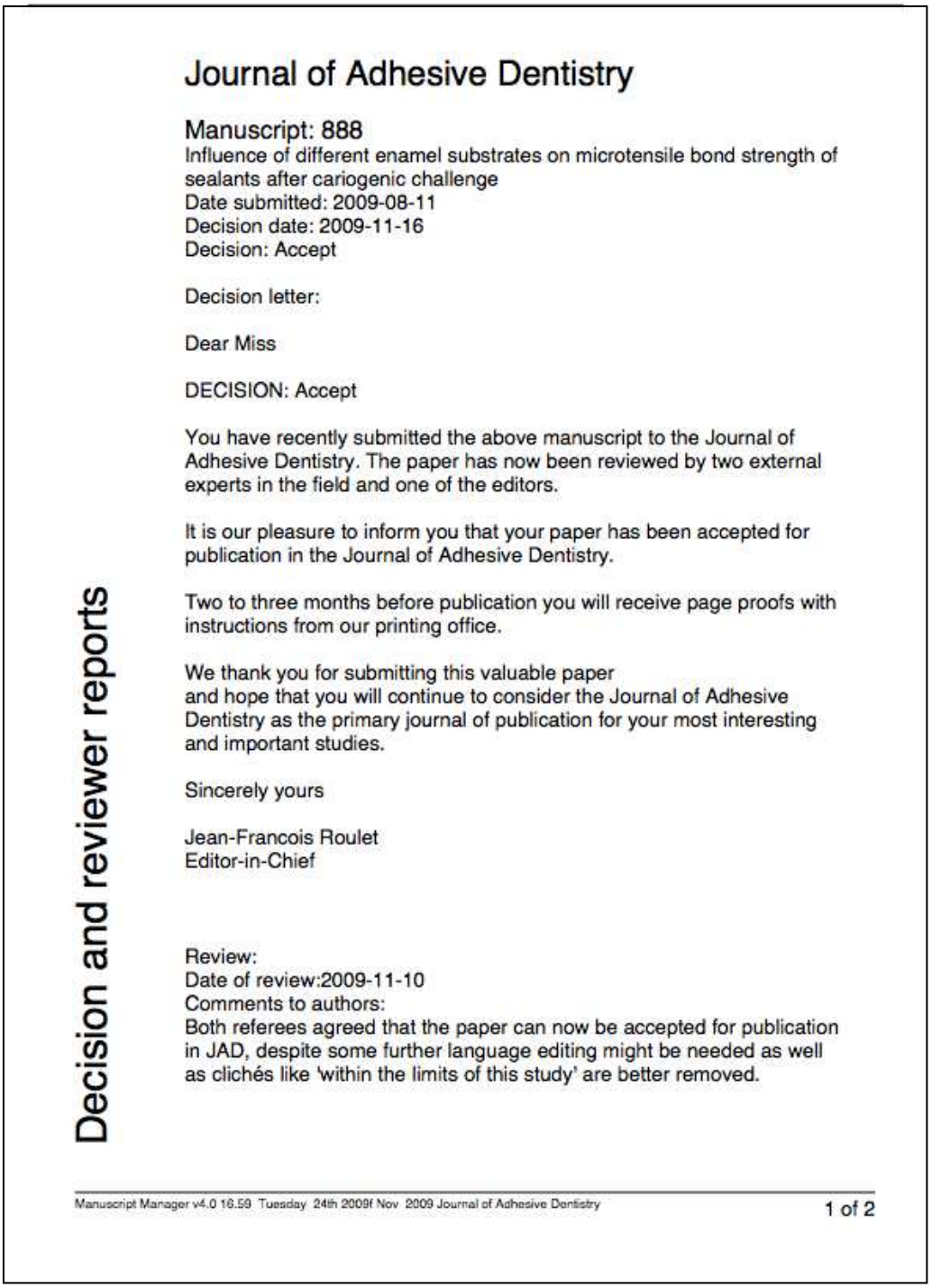
We thank you for submitting this valuable paper and hope that you will continue to consider Oral Health and Preventive Dentistry as the primary journal of publication for your most interesting and important studies.

Yours sincerely
Jean-Francois Roulet

Decision and reviewer reports

Manuscript Manager v4.0 13.14 Tuesday 3rd 2009f Nov 2009 Oral Health and Preventive Dentistry 1 of 1

ANEXO 5 - Confirmação do aceite do segundo artigo apresentado nesta Tese para o periódico *The Journal of Adhesive Dentistry.*



Journal of Adhesive Dentistry

Manuscript: 888
Influence of different enamel substrates on microtensile bond strength of sealants after cariogenic challenge
Date submitted: 2009-08-11
Decision date: 2009-11-16
Decision: Accept

Decision letter:

Dear Miss

DECISION: Accept

You have recently submitted the above manuscript to the Journal of Adhesive Dentistry. The paper has now been reviewed by two external experts in the field and one of the editors.

It is our pleasure to inform you that your paper has been accepted for publication in the Journal of Adhesive Dentistry.

Two to three months before publication you will receive page proofs with instructions from our printing office.

We thank you for submitting this valuable paper
and hope that you will continue to consider the Journal of Adhesive Dentistry as the primary journal of publication for your most interesting and important studies.

Sincerely yours

Jean-Francois Roulet
Editor-in-Chief

Decision and reviewer reports

Review:
Date of review:2009-11-10
Comments to authors:
Both referees agreed that the paper can now be accepted for publication in JAD, despite some further language editing might be needed as well as clichés like 'within the limits of this study' are better removed.

Manuscript Manager v4.0 16.59 Tuesday 24th 2009f Nov 2009 Journal of Adhesive Dentistry 1 of 2