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KELLY MARIA SILVA MOREIRA

SELANTES DE FÓSSULAS E FISSURAS: EFEITO DE MATERIAIS, TÉCNICAS E ESTÁGIO DE IRRUPÇÃO DENTÁRIA - METANÁLISE E ESTUDO CLÍNICO RANDOMIZADO

PIT AND FISSURE SEALANTS: EFFECT OF MATERIALS, TECHNIQUES, AND DENTAL ERUPTION STAGE - METANALYSIS AND RANDOMIZED CLINICAL TRIAL

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Orientadora: Profa. Dra. Regina Maria Puppin Rontani

Coorientadora: Profa. Dra. Kamila Rosamilia Kantovitz

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RESUMO

As superfícies oclusais dos dentes posteriores são os locais mais susceptívies às lesões cariosas. Nesse sentido, a aplicação do selante vem sendo utilizada com vistas à prevenção adicional à cárie dentária na superfície oclusal. Esta Dissertação possui dois artigos, estando de acordo com o formato alternativo aprovado pela CCPG. Artigo 1: teve como objetivo discutir, por meio de Revisão Sistemática e Metanálise, a evidência clínica da eficácia de selantes e técnicas de selamento na prevenção de cárie em dentes permanentes. Foram consultadas as bases de dados PubMed, Embase e Scielo, de janeiro-1984 a outubro-2015, com os descritores "clinical trial", "sealants" e "permanente tooth", totalizando 179 artigos. Após triagem e eligibilidade, vinte e dois estudos foram incluídos e classificados em níveis de evidência (1A, 7B e 14C). Dez artigos com parâmetros comparáveis foram submetidos à metanálise pelo software Review Manager 5.3 ($p \leq 0,05$). A utilização de selante promoveu a prevenção da cárie quando comparado a não realização de tratamento. Os selantes resinosos (SR) apresentaram maior retenção, quando comparados aos selantes de cimentos de ionômero de vidro (CIV), aos 24 meses. A técnica de selamento com utilização de camada intermediária previamente ao SR favoreceu a retenção, após 24 meses de análise. Em relação à prevenção da cárie dentária, não houve diferença estatística significante entre os SR e CIV, após 12 meses. Enquanto aos 24 meses, os CIV favoreceram a prevenção da cárie dentária. Artigo 2: teve como objetivo avaliar o impacto em longo prazo dos Estágios de Irrupcão dentária (EI) na retenção de selantes em superfícies oclusais, aplicando previamente camada intermediária adesiva, e determinar o nível prevenção de cárie em estudo clínico randomizado (ECR) cego e boca-dividida. Foram selecionadas 65 crianças (6-10 anos), com quatro primeiros molares permanentes livres de cárie em diferentes EI (OP- Opérculo Presente; CM- Crista Marginal; CI- Completamente Irrompido). Os dentes foram randomizados: F- Fluroshield; H- Helioseal Clear Chroma; SF- Single Bond + F; EH- Excite + H. Os dados foram submetidos ao modelo Cox Proportional Hazard (análise de sobrevivência) e ao teste da razão de verossimilhança (correlação) por meio do softwer SAS 9.3, $p \leq 0,05$. Observou-se correlação direta entre a retenção dos selantes e o EI. Entretanto, para todos os EI, não houve diferença significativa entre a técnica de selamento, com e sem camada intermediária, na retenção do material. A prevenção da cárie dentária nas superfícies oclusais foi $\cong$ 99%, não mostrando diferença entre os grupos após 2 anos. Baseado em ambos os artigos, pode-se concluir que selantes são eficazes na prevenção de cárie em pacientes de alto risco. Na metanálise, observou-se que embora os selantes resinosos apresentem maior

retenção, os selantes ionoméricos convencionais mostram maior prevenção da cárie dentária. Em estudos de baixa evidência, a técnica de selamento com camada intermediária previamente ao SR favoreceu a retenção após 24 meses de acompanhamento. Entretanto, no ECR, a camada intermediária não favoreceu a retenção do material selador, independente do estágio de irrupção oclusal. Além disso, independente da técnica de selamento, dentes completamente irrompidos apresentaram maior percentual de retenção dos selantes.

Palavras-chave: Selantes de fóssulas e fissuras. Ensaio clínico radomizado. Odontologia preventiva. Erupção dentária. Sistema adesivo.

ABSTRACT

The occlusal surfaces of posterior teeth are the most susceptible places to develop carious lesions. Thus, the application of sealant has been utilized to additional prevention of dental caries on the occlusal surface. This Dissertation has two articles, which is consistent with the alternative format approved by CCPG. Article 1: aimed to discuss, in Systematic Review and Metanalysis, the clinical evidence of the effectiveness of sealants and sealing techniques in the caries prevention in permanent teeth. The Pubmed, Embase and Scielo databases were consulted, from january-1984 to october-2015, with the keywords "clinical trial", "sealants" and "permanent tooth," totaling 179 articles. After screening and eligibility, twenty-two studies were included and classified into levels of evidence (1A, 7B and 14C). Ten articles with comparable parameter were subjected to metanalysis by the Review Manager 5.3 software ($p \leq 0.05$). Sealant promoted the caries prevention compared to no treatment. The resin sealants (RS) had a higher retention when compared to sealants of glass ionomer ciments (GIC), at 24 months. The sealing technique using an intermediate layer prior to RS favored the retention, after 24 months of analysis. Regarding the prevention of dental caries, was not statistically significant difference between the RS and GIC, after 12 months. However, at 24 months, the GIC favored the prevention of dental caries. Article 2: aimed to assess long-term impact of dental Eruption Stages (ES) on sealant retention on occlusal surfaces previously coated with intermediary bonding layer and to determine the level of caries prevention in randomized clinical trial (RCT) single-blind and split-mouth. Sixty-five school children were selected (aged 6-10 years), with four non-carious permanent first molar in different ES (OP- Operculum Present; ME- Marginal Edge; CE- Completely Erupted). The teeth were randomized: F- Fluroshield; H- Helioseal Clear Chroma; SF- Single Bond + F; EH- Excite + H. Data were submitted to Cox Proportional Hazard model (survival analysis) and Likelihood ratio $\chi 2$ test (correlation) by softwer SAS 9.3, $p \leq 0.05$. There was direct correlation between the retention of the sealants and ES. However, for all ES, there was no significant difference between the sealing technique, with and without intermediate layer, in the retention of the material. The dental caries prevention on tooth occlusal surfaces was $\cong$ 99% and there was not differ between groups at 2 years follow-up. Based on both articles, it can be concluded that sealants are effective in the caries prevention in high risk patients. The metanalysis showed that while the resin sealants exhibit greater retention, conventional glass ionomer sealants show greater prevention of the dental caries. Bass evidence studies showed that the sealing technique with intermediate layer prior to RS favored the retention, after 24 months of follow-up. However, RCT showed that the intermediate layer did not favor the

retention of the sealer material, regardless of occlusal eruption stage. In adittion, regardless of the sealing technique, the highest retention percentage was observed for complete erupted first molars.

Key Words: Pit and fissure sealants. Randomized clinical trial. Preventive dentistry. Tooth eruption. Adhesive system.

SUMÁRIO

1 INTRODUÇÃO

As superfícies oclusais são os locais mais vulneráveis à cárie dentária, devido à anatomia acidentada e irregular (Jurić, 2013). Consequentemente, maior incidência de cárie tem sido detectada nessas regiões (AL-Darwish et al., 2014). Assim, estudos epidemiológicos apontam para a importância da prevenção da cárie oclusal, visto que estratégias incluindo a utilização de fluoretos, melhorias na higiene bucal e no desenvolvimento de hábitos alimentares adequados têm eficácia limitada no controle da cárie nessas superfícies, quando comparadas às superfícies lisas (Jodkowska, 2008).

Dentre as técnicas adicionais de prevenção da cárie dentária, o selamento de fissuras é considerado uma importante estratégia (Courson et al., 2011), pois atua como barreira protetora e impede o crescimento/desenvolvimento de bactérias causadoras da cárie no interior da fissura, o que culmina na redução do risco de cárie nessas regiões susceptívies (AAPD, 2014). Vários estudos demonstraram que a presença do selante na superfície oclusal constitui um método seguro e efetivo de prevenção em superfícies de risco à cárie, e quando aplicado como parte de um programa bem estruturado é de grande benefício para a saúde bucal, principalmente comparado a superfícies não seladas (Wendt et al., 2001; Adair, 2003; Tagliaferro et al., 2011; Ahovuo-Saloranta et al., 2013).

Um ponto de destaque quanto à prevenção da cárie na superfície oclusal, relaciona-se ao uso de materiais com fluoretos em sua composição, dentre eles os selantes, podendo reduzir a perda de minerais do esmalte (Kantovitz et al., 2006, 2013). Os fluoretos liberados desses materiais, em condições de alto risco à cárie, poderiam proporcionar proteção adicional à essa superfície (Lobo et al., 2005). Por outro lado, a inclusão do flúor no selante é questionável, dado que a liberação do mesmo do material é dependente da forma como é introduzido, e ainda poderia resultar em porosidades no material, e alterar a longevidade devido à degradação hidrolítica apresentada, diminuindo assim a capacidade de selamento (Morphis et al., 2000; Yildiz et al., 2004).

A literatura apresenta controvérsias quanto a eficácia dos selantes e estudos apontam ser dependente da retenção do material na superfície selada (Puppin-Rontani et al., 2006; Bendinskaite et al., 2010). Dentre os fatores apontados como sendo relacionados à retenção dos selantes, as características e propriedades adesivas são os fatores mais importantes, bem como uma boa técnica clínica. A principal vantagem dos selantes resinosos tem sido atribuída à adesão ao esmalte dentário, proporcionando retenção adequada do material e, portanto, o bloqueio físico do sistema de fissuras (Dhar et al., 2012), que parece ser muito mais importante do que a liberação transitória de fluoretos. Entretanto, mais

estudos clínicos randomizados são necessários com selantes resinosos contendo fluoretos para identificar a eficácia dos mesmos em relação aos selantes sem fluoretos (Simonsen, 2002; Cagetti et al., 2014).

Outro fator importante a ser considerado é o tempo de atuação dos selantes em relação à prevenção da cárie oclusal. Os achados de Benteke et al. (2006) reforçam sugestões anteriores de que selantes aplicados na infância, em primeiros molares permanentes recém-irrompidos, podem ter uma longa duração no efeito preventivo à cárie sobre as superfícies seladas, embora seu efeito em longo prazo sobre a saúde bucal geral podem ser limitados. Por outro lado, os estudos de Messer et al. (1997) e Francis et al. (2008) sugerem que os selantes resinosos continuam a prevenir novas lesões de cárie, mesmo quando eles aparecem parcial ou totalmente perdidos, reduzindo assim a prevalência da cárie dentária oclusal.

Além disso, a eficácia parece estar relacionada ao risco do paciente à cárie dentária, pois o risco inicial, isto é, o risco apresentado durante a aplicação do selante, mostrou-se associado à maior frequência de falhas do selante e incremento da cárie nas superfícies oclusais. Assim, os indivíduos com moderado ou alto risco apresentaram aumento das taxas de insucesso, o que pode ser contornado com aumento da frequência de reavaliação e reaplicação do material nesses indivíduos (Simecek et al., 2005; Veiga et al., 2014). Essa maior iminência de incremento de cárie em pacientes de risco à cárie pode ser atribuída à tendência desses indivíduos apresentarem cuidado insuficiente com a saúde bucal. Dessa forma, Tagliaferro et al. (2011) reforçaram a importância da utilização do selante associada à educação em saúde para essas crianças no controle das lesões de cárie oclusal, já que para as crianças de baixo risco a educação em saúde mostrou-se suficiente. Em concordância, Ahovuo-Saloranta et al. (2013) relataram que os selantes são efetivos em crianças de alto risco, porém para outros níveis de risco as informações são escassas.

A despeito de avaliar o risco de cárie do indivíduo para a indicação do selante, deve-se considerar ainda o risco específico da superfície a ser tratada (Li e Wang, 2002). Durante a irrupção dentária, segundo Carvalho et al. (1989, 2014), a incidência de lesões cariosas em superfícies oclusais é ainda maior, em razão das condições favoráveis para o acúmulo de biofilme dentário. À medida que os dentes atingem a oclusão funcional, o número de lesões ativas diminui. Nesse período, quando a superfície oclusal encontra-se em infra-oclusão, os dentes também estão mais susceptíveis à cárie dentária, pois se encontram nas fases iniciais da maturação pós-eruptiva, necessitando assim de maiores cuidados (Lynch, 2013). Portanto, quando os molares permanentes ainda não estão totalmente

irrompidos, maior dificuldade, principalmente em relação à técnica do efetivo selamento deve ser enfrentada, pois a umidade na superfície oclusal é de difícil controle, notadamente no momento em que a mesma se encontra ao nível da gengiva, ou ainda com a superfície distal parcialmente coberta pelo opérculo gengival. A união do material selador nesses casos, pode ficar comprometida, uma vez que são compostos por monômeros hidrófobos e a presença da umidade, seja por fluido crevicular, saliva ou água diminui a adesão à superfície do esmalte (Hebling e Feigal, 2000).

Em situações clínicas críticas, em que o uso do isolamento absoluto encontra-se impeditivo, especialmente em pacientes jovens, com dentes recém-irrompidos (Eskandarian et al., 2015) pela falta de retenção do lençol de borracha, o isolamento relativo e consequente controle insuficiente da umidade pode comprometer a retenção do material. Como uma forma de atenuar esses problemas, pesquisas foram propostas utilizando-se uma camada intermediária de sistema adesivo, pelo seu caráter mais hidrófilo que o dos selantes e pela menor viscosidade do mesmo, a fim de obter-se melhores resultados na microinfiltração e retenção dos selantes (Feigal et al., 2000; Lygidakis et al., 2009; Meller et al., 2015; McCafferty e O'Connell, 2016). Entretanto, outros estudos mostraram que a colocação de um sistema adesivo sob o selante não afetou significativamente o sucesso clínico (Pinar, 2005; Mascarenhas et al., 2008). Até então, a eficácia relativa das diferentes técnicas e tipos de selantes, com ou sem fluoretos, ainda não foram estabelecidos.

De maneira geral, o selamento oclusal tem sido considerado um tratamento adequado à prevenção de cárie em fóssulas e fissuras. Entretanto, devido às dificuldades inerentes ao ambiente oral, como a contaminação produzida pela umidade quando da presença de primeiros molares permanentes com diferentes estágios de irrupção em pacientes com risco à cárie dentária, e a baixa adesão de materiais resinosos nesses substratos, estudos clínicos randomizados bem delineados precisam ser realizados. Portanto, os objetivos desta dissertação são: (1) discutir, por meio de uma Revisão Sistemática de Literatura e Metanálise, a evidência clínica da eficácia de selantes, segundo diferentes materiais e técnicas de selamento na prevenção de cárie em dentes permanentes; (2) verificar em um estudo clínico randomizado a retenção e a eficácia de duas técnicas de selamento oclusal (com e sem camada intermediária) de primeiros molares permanentes, após 2 anos da aplicação, por meio da análise em banco de dados obtidos da avaliação clínica direta (visual), relacionando-as aos materiais e técnicas utilizados e nível de irrupção oclusal à época do selamento. Com a finalidade de alcançar esses objetivos, esta dissertação possui 2

artigos, correspondentes aos objetivos propostos, seguindo a resolução para normatização de Dissertações e Teses.

2 ARTIGOS

2.1 ARTIGO: Pit and fissure sealing materials and their application techniques in permanent teeth: A systematic review and meta-analysis*

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Abstract

Objectives: To evaluate the clinical evidence of the effectiveness of sealants and their application techniques on the occlusal pits and fissures, related to retention and caries prevention in permanent teeth.

Data/Sources: The databases PubMed, Embase, and SciELO were accessed.

Study selection: Randomized clinical trials (RCTs) on caries prevention and/or sealant retention on sound surfaces and/or initial carious lesions in enamel, and at least 12 months of follow-up were included. Out of 179 papers identified, 22 were included and classified in levels of evidence (A-high=1, B-moderate=7 and C-bass=14) and 10 equivalent studies were submitted to meta-analysis (Review Manager 5.3 software, $\alpha \leq 0.05$). Sealant prevented caries compared to non- sealant use. Resin sealant (RS) had a higher retention rate over time than glass ionomer cements (GIC). Using of an adhesive intermediate layer prior to RS application increased retention, after 24 months. Regarding the caries prevention, both RS and GIC was effective, however, the GIC showed positive advantage, at 24 months.

Conclusions: Sealants are effective in the caries prevention. Although RS exhibit greater retention, GIC sealants are more effective for caries prevention. Bass evidence studies showed that incorporating adhesive intermediate layer prior to RS improved sealant retention, but it did not affect caries prevention.

Clinical Significance: Sealants are considered an important approach in the occlusal caries prevention in high-risk patients. The clinical evidence of the effectiveness of different types of sealants and their application techniques, related to retention and caries prevention, is essential to determine and improve the performance of this preventive method.

Keywords: sealants; permanent tooth; clinical trial

Introduction

Industrialized countries, over the past several decades, has been experienced a gradual reduction in the prevalence of dental caries on smooth surfaces of children's and adolescent's teeth [1,2]. However, the prevalence of lesions on occlusal and proximal surfaces remains high [3-5], with approximately 70% to 90% of caries incidence involving the occlusal surfaces [4,6]. It should be noted that these data represent only the cavitated carious lesions. Other researchers found that 71% of children under age 5 were affected by early enamel carious lesions on occlusal surfaces [7]. Study involving adolescentes indicated that 96% of the population had one or more active/inactive incipient enamel carious lesions [8]. These reports on carious disease confirms its clinical importance and supports the need for its further study.

The susceptibility to dental caries on the occlusal surface appears to be related to macromorphology presented by the uniqueness of the pits and fissures of this region. These can form niches that promote bacterial biofilm retention and hamper the effectiveness of conventional hygiene proceduress, as well as the action of fluoride agents [9]. Moreover, the newly-erupted tooth may also favor biofilm accumulation and the demineralization process [5].

Therefore, various strategies have been directed at prevent the dental caries. Among these were programs designed to promote greater awareness and education about oral health, the importance of good eating habits, proper home oral hygiene practices and the use of fluoride, either by topical application or use of toothpaste and fluoridated water intake. Epidemiological studies report that these methods are less effective on occlusal surfaces that on smooth ones, and therefore point to the importance of intensifying efforts at the prevention of occlusal caries [10,11].

In this regard, the sealing of pit and fissure is considered an important approach, because it acts as a protective barrier that prevents the development of caries lesions into pits and fissures in patients at risk for dental caries [5,12-14]. The effectiveness of this procedure seems to be closely related to the properties of sealer materials, such as biocompatibility, retentive capacity, resistance to abrasion and wear, bond strength enamel/material, surface tension, viscosity, marginal adaptation and penetration [15-17]. Among these features, the materials' retention capacity on

sealed surfaces stands out, which is related to its physical, chemical and adhesive properties, as well as its application technique [18,19].

Although the first sealer materials were introduced almost 50 years ago, it is still not clear which type of fissure sealant has the superior combination of retentiveness and caries prevention effectiveness [20]. Various materials have been used as sealants, and glass ionomer and resin sealants are the most common. In several studies, resin sealants had better retention capacity than glass-ionomer cements [21-23]. However, other researchers reported better retention [24] and preventive performance to the glass ionomer [25]. On the other hand, Chen et al. [26] found no difference between these types of sealants. Until now, the relative effectiveness of different application techniques of the sealant has also not been established.

Thus, the purpose of this systematic review and meta-analysis was to evaluate the clinical evidence of the effectiveness of (1) different types of sealer materials and (2) different sealant application techniques of the occlusal pits and fissures, as related to long-term retention and caries prevention in permanent teeth.

Materials and Methods

This study was recorded in International Prospective Register of Systematic Review (PROSPERO) – CRD42016032725.

Literature searching

The research question to be responding with this study was:

How effective is occlusal sealing, using different materials and application techniques?

The question followed the *PICO* strategy: *P*atient: permanent teeth; *I*ntervention: dental sealing; *C*omparison: sealants; *O*utcomes: material retention and prevention of caries.

The basis of this systematic review and meta-analysis was a search in the PubMed, Embase and SciELO databases, selecting studies during the period from January 1984 to October 2015. The search was supplemented by individual searching of reference lists from each identified relevant publication. The main search terms were “Clinical trial’’; “Sealants” and “Permanent Tooth”. Only original papers were considered. Reports, abstracts, letters, communications, literature reviews and textbooks chapters were discarded. The analysis was limited to clinical

studies with sealants in the occlusal surface of permanent teeth. Articles in English, Portuguese and Spanish language were accepted.

Inclusion and exclusion criteria

After initial appraisal, studies were included only if they evaluted the sealant retention and/or prevention of occlusal pit and fissure caries and sealing techniques in sound and hypomineralized surfaces and/or initial caries lesions confined to enamel of permanent teeth and had at least 12 months of follow-up. Studies excluded were those regarding the fluoride release capacity of sealer materials; the use of patient's favorite sealing application technique and sealing in dentin lesions on the occlusal surface; had evaluated invasive sealing including minimally invasive techniques, restorations with flow resin and composite; and sealer materials used to correct marginal defects in class I and II restorations.

Evaluation of scientific papers and evidence levels

The articles that met the inclusion criteria were subjected to critical evaluation, carried out independently by at least two members (KMSM and KRK) of the research group previously trained and calibrated for papers evaluation. The interexaminer agreement (0.99) was calculed using the kappa coefficient. Sorting was performed through the titles and abstracts and the eligibility of studies was conducted with the full versions. Later, the data were extracted to a predefined data sheet and each work was evaluated with score that ranged from A to C, according to predetermined criteria for methodology and performance, as defined in Table 1. In the event of disagreement between the examiners, the selected paper was reviewed and discussed by the entire group until a consensus reassessment was reached. If, for some reason, the selected article was considered irrelevant to the issue of research, it was deleted.

Statistical analyses

The data of studies with comparable parameters (same level of evidence/score; equal follow-up; similar treatment groups) were subjected to meta-analysis by the RewMan (Review Manager, version 5.3 software, Cochrane Collaboration, Copenhagen, The Netherlands), accepting $p \leq 0.05$ and considering the following factors: (1) Sealer materials; (2) Techniques of sealing; (3) Follow-up time; (4) Retention; and (5) Dental caries prevention. Heterogeneity was assessed using the Chi-square test and I^2 statistics.

Results

A total of 179 articles were initially identified. They were screened based on titles and abstracts, and resulted in 33 studies. In the second step - eligibility, the documents of interest for this review were ordered in complete versions and 11 studies were excluded [27-37]. The remaining twenty-two studies identified during the search were included in the critical appraisal (Figure 1). One paper was classified as score A-high, 7B-moderate and 14C-bass evidence (Chart 1).

The score A study conducted by Muller-Bolla et al. [38], assessed the effectiveness of the resin sealant (Delton plus) on sound surface or with initial lesion of caries in enamel. The use of sealant was associated with preventive program according to the individual caries risk. Papers classified as score B [7,21,23,39-42] examined the effectiveness, for at least 12 months, of different sealants and sealing techniques in sound occlusal surface and/or with early caries lesions. However, these studies did not compare results with representative samples of the studied populations (no external validity).

Score C studies [11,15,24,25,43-52] were conducted similarly to those of score B, however, the applied methodology was not completely described. They were not split-mouth and/or did not evaluated intra- and/or inter-examiner concordance indices, featuring confounding factors (biases).

The results of this meta-analysis were based on 10 studies that had one or more comparable evaluation parameters [23,25,40,42,43,45-49].

In C score studies, GIC sealants promoted caries prevention when compared with the control group (no treatment) at 12 and 24 months of follow-up, $p<0.00001$ and $p=0.0004$, respectivily (Figure 2 and 3). The RMGIC sealants also promoted caries prevention when compared with the control group (no treatment) at 12 and 24 months of follow-up ($p<0.00001$).

As regards sealant retention of sealer material, C score studies showed that the use of RS fared better than the GIC, at 12 months and 24 months follow-up evaluation ($p<0.00001$). However, in B score studies the RS favored the complete retention at 12 months ($p=0.005$), but was not significant difference between RS and GIC at 24 mouths, $p=0.13$ (Figure 4 and 5). The addition of intermediary bonding layer also made a difference in complete sealant retention over the long term. This application technique resulted in better retention outcomes after 24 months of

analysis than did its counterpart control method (without adhesive) in C score studies, p=0.05 (Figure 6).

Regarding the issue of caries prevention, there was no statistically significant difference between the use of RS and GIC sealer materials after 12 months in B and C score studies, p=0.79 and p=0.29, respectivily (Figure 7). However, after 24 months there was a difference with the GIC reporting better caries prevention outcomes compared to RS in the B score studies, p=0.002 (Figure 8). In relation to the application technique (with or without adhesive), there was no difference in the prevention of caries after 24 months in C score studies, p=0.83 (Figure 9).

Heterogeneity varied from 0% (C score studies: GIC X No treatment and SA X CS; B score studies: RS X GIC) to 98% (C score studies: RS X GIC).

Discussion

In this systematic review and metanalysis, it was shown that sealants are effective as preventive method to the occlusal pit and fissure susceptible to dental caries in score A study [38], score B study [39] and score C studies [11,25,42,45-47,51], supporting the current literature [14,53]. It was observed reduction in the incidence of caries in first permanent molars of caries risk populations, when this approach was associated to health education programs with multifactorial intervention in factors of dental caries. However, despite the effectiveness of these preventive programs using occlusal sealants, the performance of the sealant is still dependent on its physical characteristics and application technique [19].

Regarding the types of sealants, the RS showed better retention rates compared to the GIC in score B studies [21,23,40,42] and score C studies [25,43]. However, the GICs, even with low retentiveness, achieved the better caries prevention outcomes in score B studies [21,23,39,40,42] and score C studies [11,43]. The retention results may be explained by the physical characteristics of RS, which are mechanically adhered to the occlusal surface through the polymerized monomers within the conditioned enamel pores (resintags) [54], differing from GIC that bind chemically to enamel substrate [55]. On the other hand, the low retentiveness of the ionomeric material may be compensated by fluoride release of the remaining material within the fissure, which would promote a cariostatic effect [56,57] and formation of fluorapatite on enamel. Thus, the substrate becomes more resistant to pH drops in

the oral environment promoting improvement in the prevention of carious lesion and reduction of bacterial biofilm [56-58], since it aids in the remineralization process and can benefit even close areas to the edge of the sealer material [16,59]. Nevertheless, anticariogenic effect may depend not only on the presence and amount of fluoride ions, but also on the longevity of this release [16,39,58]. Another consideration regarding the presence of fluoride in the sealer materials is the form in which it is introduced into these materials. It may result in porosity in the applied layer and thus decrease sealant retention and longevity due to hydrolytic degradation [60].

Moreover, the application technique is an important factor for preventive success. The resin sealant associated with bonding agent has been used in order to improve even more the retention of the resin sealant. Thus, Feigal et al. [61] observed a higher retention rate with this association throughout different stages of dental eruption. Lygidakis et al. [62], who applied sealant on the occlusal surface of hypomineralized/opacity enamel, also suggested positive influence of bonding agents for retention. In addition, Burbridge et al. [63], Burbridge et al. [64], Meller et al. [65] and McCafferty & O'Connell [66] reported improvement of the sealant infiltration/retention and microleakage prevention, when a bonding agent was additionally applied prior to sealing. However, Pinar et al. [67], Mascarenhas et al. [68], Nazar et al. [52] and Nogourani et al. [69] showed that the use of an adhesive system prior to the sealant does not significantly affect the clinical success. In this metanalysis, when comparing the technique with and without adhesive system, the resin sealant with adhesive system was a superior approach in score C studies [48,49], since the intermediary layer of adhesive system is more hydrophilic and less viscous, enabling greater penetration and retention of sealants [52,61-64,68-70]. However, this result is daring to assert because only score C studies were included for this analysis.

It is worth mentioning the importance of the reapplication of sealant, since its effectiveness depends on the patient's risk and the tooth surface. In patients at high risk for dental caries, hygiene control is often inadequate which may be a major reason for poor sealant retention. In this metanalysis, even though studies follow-up and monitor patients, with frequent evaluations, few studies reported sealer materials reapplication due to loss between the evaluations [39,42,49,50]. Such efforts would increase the sealant's clinical success and reduce confounding factors. Thus, Jodkowska [11] suggested that the retention of the sealant is directly influenced by

the evaluation time, and accordingly the frequency of material revaluation and reapplication on substrate are crucial.

In relation to scores of the studies, only a A study was found and despite B studies presented control of certain confounding factors as the complete description of the methodology, the sample of the studied populations was not representative, which could be a bias and thus making the data unsuitable for generalization. Moreover, in most of these B studies there was no report the value of Decayed, Missing and Filled Teeth Index (DMFT) of the studied population, and the social group of the sample was not informed. This is a very important factor related to the determination of sealant application, taking into account not only the surface risk, but also the risk of the individual. The main biases found in the score C studies were incomplete description of the methodology, insufficient data of examiner calibration and control groups and/or treatments with confounding factors, which may compromise the accuracy of the results. Similar to score B studies, there were little reports of the sample social group, besides the absence of DMFT values at baseline was common.

In addition, both the score B and C studies had incomplete description of the final outcome, evaluating separately retention of sealant and dental caries prevention, questions that should be simultaneously analyzed to determine the effectiveness of the sealer materials and its application technique. Therefore, more clearly delineated randomized clinical trial evaluating the relationship between retention and prevention are needed to choose the more effective sealing material and technique.

Conclusion

It can be concluded that sealants are effective to prevent caries in high-risk patients. Although the resin sealants exhibit greater retention, conventional glass ionomer sealants provide greater prevention of dental caries. Bass evidence studies showed that the sealing technique with adhesive intermediate layer prior to resin sealant improves its retention, but had no effect on prevention at 24 months. Therefore, it is not exclusively the retention of sealant on occlusal surface that determines its capability to dental caries prevention.

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Table 1. The methodological criteria used in this literature review.

Score A (High evidence) All the criteria present	Description of the inclusion criteria; Stratification by age, gender and social group; Fully described methodology; Randomized and blind studies; Split-mouth studies; Representative sample of the population studied - external and internal validity; Similar control groups and treatments; DMFT* values described at baseline; Evaluation of intra and inter-examiner concordance indices; Statistical analysis with significance level previously considered.
Score B (Moderate evidence) All the criteria present	Description of the inclusion criteria; Stratification by age, gender and social group; Fully described methodology; Randomized studies; Split-mouth studies; No representative sample of the population studied - internal validity; Similar control groups and treatments; Absence DMFT values at baseline; Evaluation methods described; Evaluation of intra and inter-examiner concordance indices; Statistical analysis with significance level previously considered.
Score C (Bass evidence) One or + criteria present	Absence of description of the inclusion criteria; Partial described methodology; Absence of randomization and/or blinding; Control groups and/or treatments with confounding factors (absence of split-mouth design), besides other biases; Absence DMFT values at baseline; Evaluation methods not described; Not calibrated examiners (Intra and inter-examiners); Description of final outcome (clinical success=retention or preventing dental caries.

*Decayed, Missing and Filled Teeth.

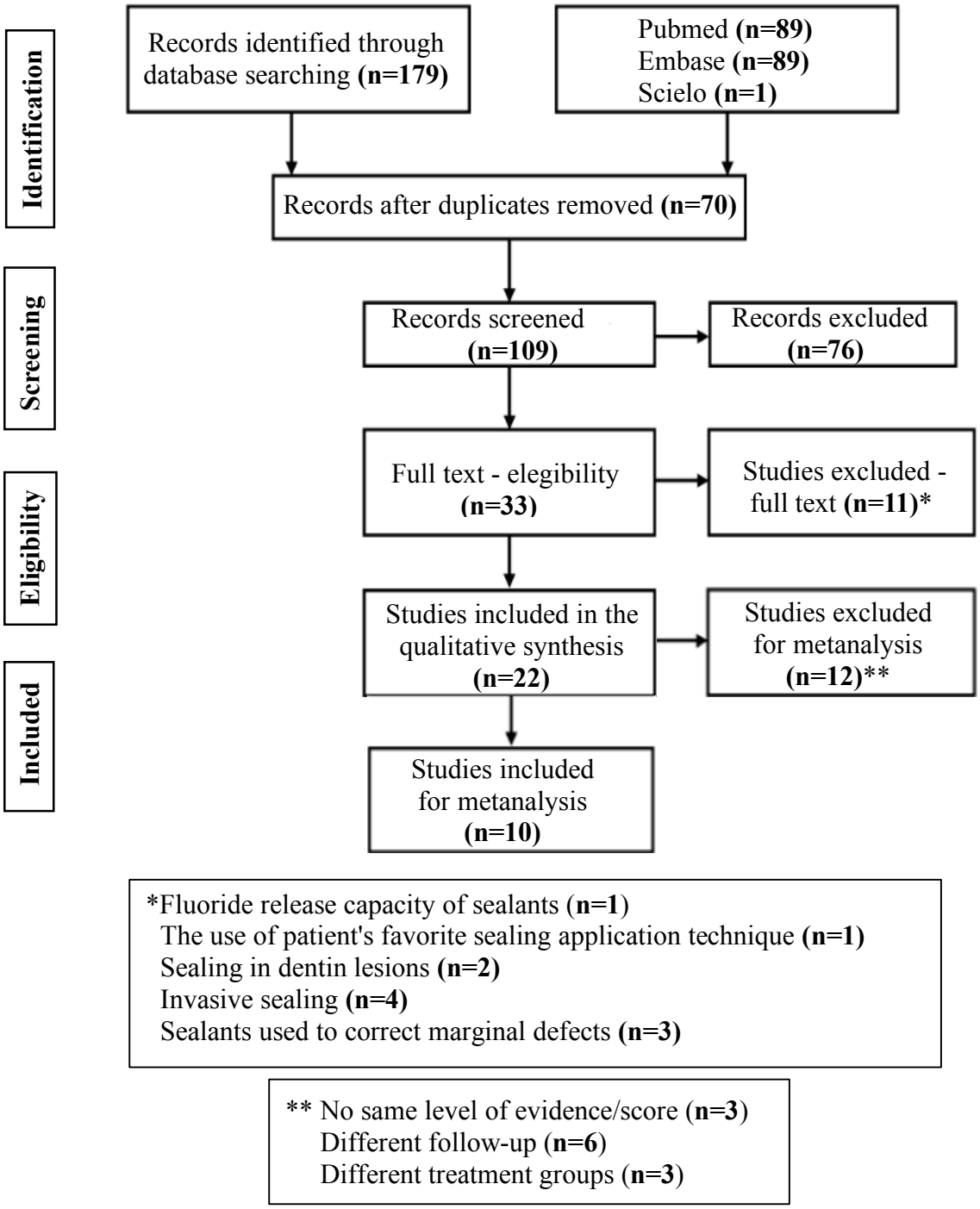

Figure 1. Flowchart of the process for identification and selection of papers**.**

Adapted of the PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement.

Chart 1. Randomized clinical studies used in this systematic review.

First author	Year	Techniques*		Sealant type≡ (Commercial Brand)	Sample size (nº)	Patient age (Year)	Follow-up (Months)	Clinical success (%)		Evidence Level
								Full retention	Caries prevention	
Guler	2013	SCGIC	RI	GIC (Fuji VII)	200	7-13	6, 12, 18, 24	13	84	B
		CS		RS (Admira seal)				3	68	
		CS	RI	RS (Delton plus)				52.7	96.9	
Muller-Bolla	2013	C	-	-	552	6-7	12	-	89.3	A
		CS	RI	RS (Delton plus)				52.7	96.9	
Nazar	2013	CS	AI	RS (Delton Plus)	156	6-9	12, 24, 36, 48, 60	19.6	21.3	C
		SA		Adhesive (SMPPS) RS (Delton Plus)				25	26	
Ulusu	2012	CS	RI	RS (Fissurit)	346	7-15	1, 3, 6, 12, 24	16.6	95.2	B
		SCGIC		GIC (Fuji VII)				31.9	96.6	
Dhar	2012	SCRMGIC	AI	RMGIC (GC Fuji VII)	100	6-10	6, 12, 18, 24	12	96	B
		DA		RMGIC (GC Fuji VII)				0	92	
		CS		RS (Clinpro)				34	84	
		DA		RS (Clinpro)				0	88	
Liu	2012	C	-	-	1539	8-9	24	-	83.1	C
		CS	RI	RS (Clinpro)				46	92.6	
		FVA		FV (Duraphat)				-	87.2	
		TFA		SDF (Saforide)				-	87.8	

Chart 1. Continued.

First author	Year	Techniques*		Sealant type≡ (Commercial Brand)	Sample size (nº)	Patient age (Year)	Follow-up (Months)	Clinical success (%)		Evidence Level
								Full Retention	Caries Prevention	
Antonson	2012	SA	RI	Adhesive (VIP) RS (Delton plus)	78	5-9	3, 6, 12, 24	40.7	97.4	B
		SCGIC		GIC (Fuji Triage White)				44.4	100	
Kargul	2009	CS	RI	RS (Delton)	121	6-9	6, 12, 24, 36	30.4	90.7	C
		CS		RS (Helioseal)				10.8	80.4	
Barja-Fidallgo	2009	SCGIC	RI	GIC (Fuji IX)	92	5-8	6, 60	29	90	C
		CS		RS (Delton)				21	75	
Jodkowska	2008	C	-	-	1440	7-8	60, 120,180	-	-	C
		CS	NI	RS (Nuva-Seal)				96.3	74.5	
		CS		RS (CBWSS)				96.9	75.1	
		CS		RS (CEBS)				97.5	99.7	
Dukic	2007	CS	RI	RS (Helioseal Clear Chroma)	100	7-17	12, 24	66.7	93.9	C
		CS		RS (Teethmate F1)				60.6	97	
		CSRF	NI	Adhesive (Exite) RF (Tetric Flow)				76.5	100	
Poulsen	2006	CS	NI	RS (Delton)	728	8-13	Até 56	~ 60-80	95.8	B
		SCGIC		GIC (Fuji III)				< 10	94.8	

Chart 1. Continued.

First author	Year	Techniques*		Sealant type≡ (Commercial Brand)	Sample size (nº)	Patient age (Year)	Follow-up (Months)	Clinical success (%)		Evidence Level
								Full Retention	Caries Prevention	
Güngör	2004	CS	RI	RS (Delton)	192	7-10	3, 6, 12, 24	71.4	85.7	C
		SA		Adhesive (Prime and Bond NT) PMRC (Dyract Seal)				91.1	82.9	
Pereira	2003	C	-	-	832	6-8	6, 12, 24, 36	-	44	C
		SCGIC	NI	GIC (Ketac Bond)				4	56	
		SCRMGIC		RMGIC (Vitremer)				13		
Autio Gold	2002	CS	RI	RS (CuRay-Match)	118	6-11	1, 6, 18	82.2	91	B
		CS		RS (Delton)				66.7	89	
Pereira	2001	C	-	-	832	6-8	6, 12, 24	-	91	C
		SCRMGIC	RI	RMGIC (Vitremer)				11	96.5	
		SCGIC		GIC (Ketac-Bond)				4	98.8	
Lygidakis	1999	CS	RI	RS (Fluorshield)	448	7-8	48	77	91	C
		CS		RS (Delton)				89	90	
Pereira	1999	C	-	-	832	6-8	6, 12	-	77	C
		CS	RI	GIC (Ketac Bond)				15	100	
		SCRMGIC		RMGIC (Vitremer)				36	100	
Carlsson	1997	C	-	-	431	6-7	24	-	59	B
		CS	RI	RS (Helioseal F)				76.6	60	

Chart 1. Continued.

First author	Year	Techniques*		Sealant type[≡] (Commercial Brand)	Sample size (nº)	Patient age (Year)	Follow-up (Months)	Clinical success (%)		Evidence Level
								Full Retention	Caries Prevention	
Raadal	1996	CS	RI	GIC (Vitrebond)	106	5-7	1, 6, 12, 24, 36	9	92.6	C
		CS		RS (CWS)				97	100	
Karlzén-Reuterving	1995	SCGIC	NI	GIC (Fuji III)	148	6-8	36	27.8	98.6	C
		CS		RS (Delton)				79.2	95.8	
Barrie	1990	CS	NI	RS (Prismashield)	134	5-6	6, 12, 24	71	71	C
		CS		RS (Estiseal)				53	53	
		CS		RS (Prismashield)				81	81	
		CS		RS (Concise)				88	88	

* Techniques: CS- Conventional Sealing: dental profilaxius; acid etching; washing; drying and sealant aplication. SA- Sealing with Adhesive: acid etching; washing; drying; adhesive application; resin sealant aplication. SCGIC- Seling with Conventional Glass Ionomer Cement: dental profilaxius; relative isolation and sealant aplication. C- Control/without treatment. FVA- Fluoride Varnish Application. TFA- Topical Fluoride Application. CSRF- Sealing with Resin Flow: dental profilaxius; acid etching; washing; drying; adhesive aplication and flow resin. CSRMGIC- Sealing with Resin-Modified Glass Ionomer Cement: dental profilaxius; acid etching; washing; drying and sealant aplication. DA: Direct Application of sealant, without dental preparation. AI: Absolute Isolation. RI- Relative Isolate.

[≡] Materials: RS- Resin Sealant. GIC- Glass Ionomer Cement. RF- Resin Flow. RMGIC- Resin-Modified Glass Ionomer Cement. FV- Fluoride Varnish. SDF- Silver Diammine Fluoride. PMRC- Polyacid-Modified Resin Composite. CBWSS- Concise Brand White Sealant System. CEBS- Concise Enamel Bond System. CWS- Concise White Sealant. SMPPS- 3M Scotchbond Multi-Purpose Plus System primer and Bond.

º Sample (n): tooth number.

Other acronyms: NI- No informed. RR- Relative Risk.

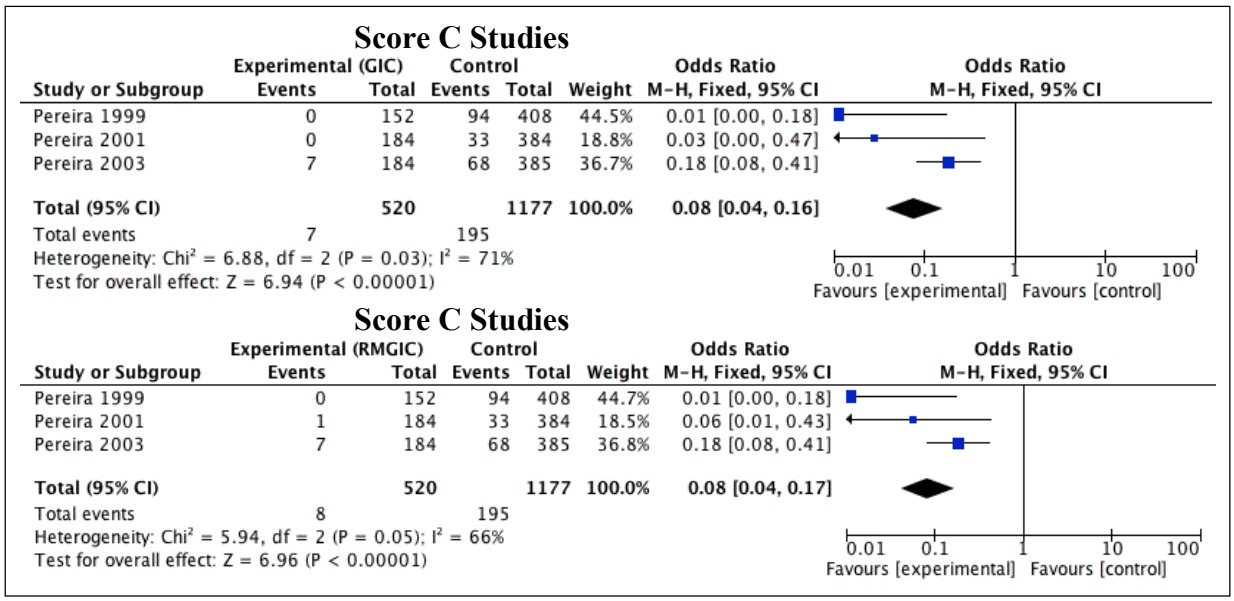

Figure 2. Dental caries prevention evaluation after 12 months: Sealant X Control (No treatment). GIC- Glass Ionomer Cement. RMGIC- Resin-Modified Glass Ionomer Cement.

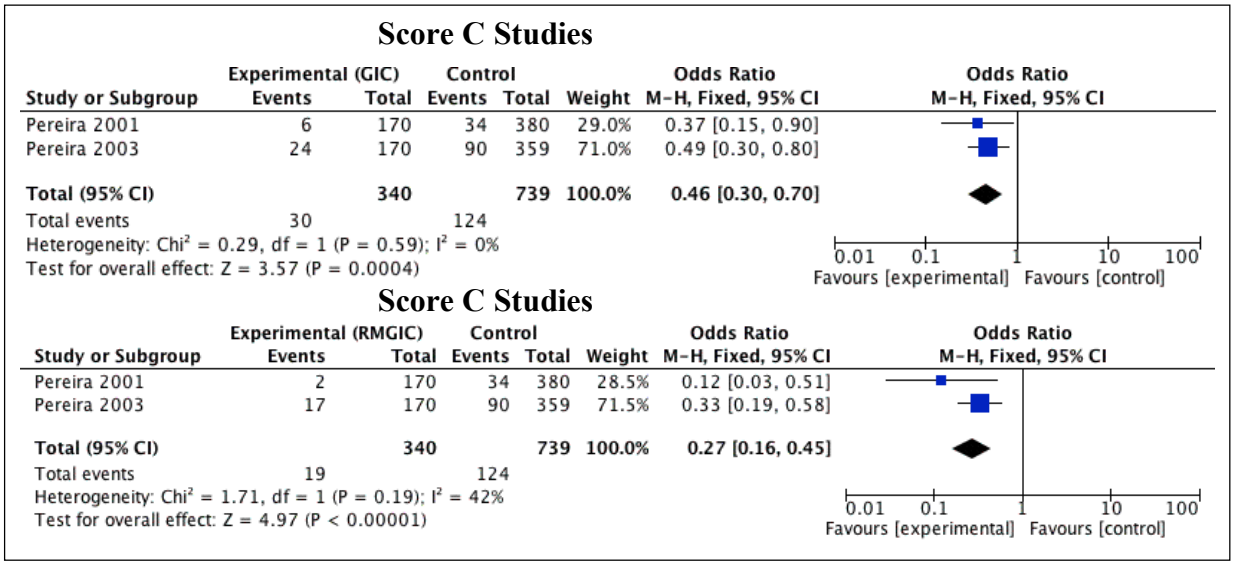

Figure 3. Dental caries prevention evaluation after 24 mouths: Sealant X Control (No treatment), considering score B and C studies. GIC- Glass Ionomer Cement. RMGIC- Resin-Modified Glass Ionomer Cement.

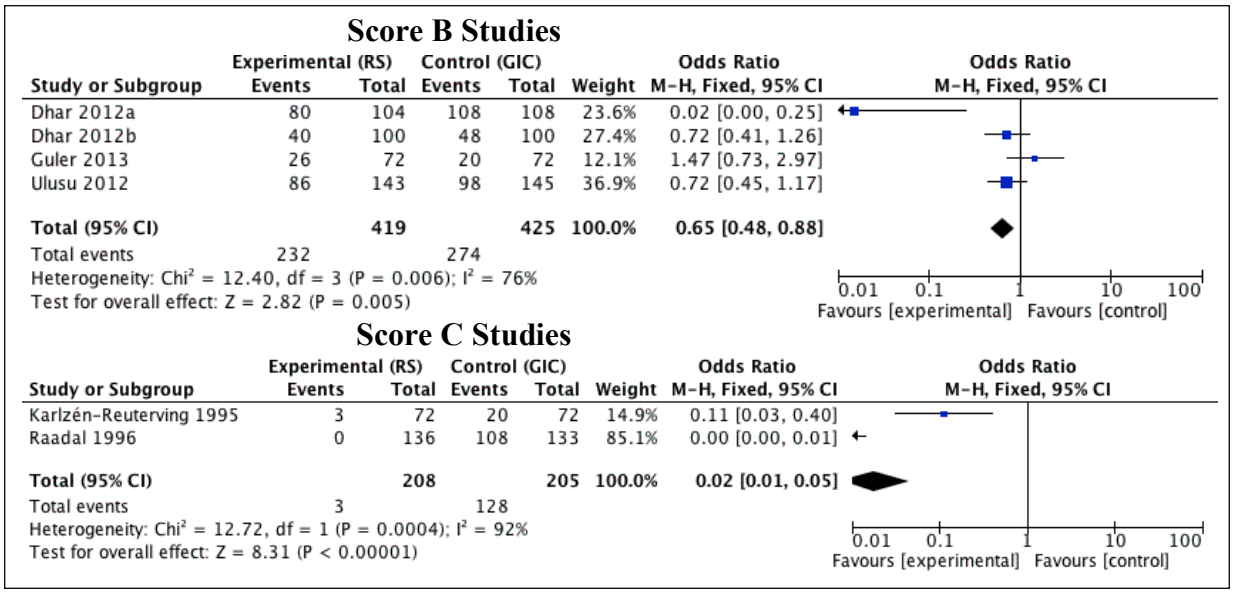

Figure 4. Full retention of different sealants after 12 months: RS X GIC.

GIC- Glass Ionomer Cement. RS- Resin sealant.

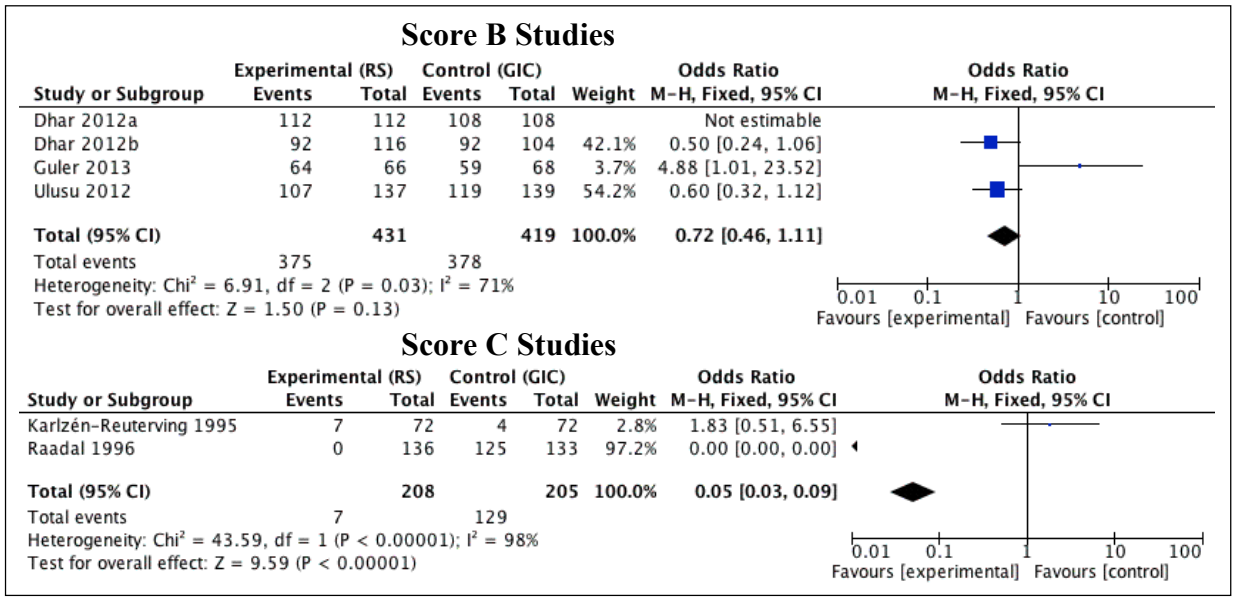

Figure 5. Full retention of different sealants after 24 months: RS X GIC.

GIC- Glass Ionomer Cement. RS- Resin sealant.

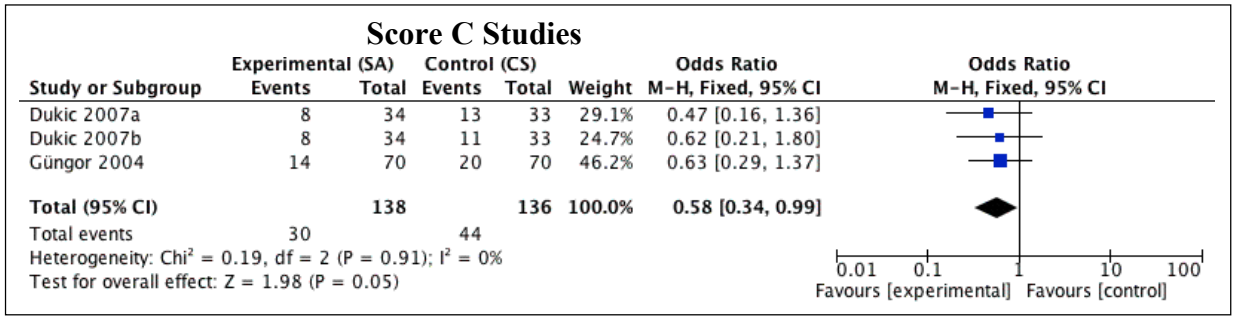

Figure 6. Full retention of different techniques after 24 mouths: CS X SA.

CS- Conventional Sealing. SA- Sealing with Adhesive.

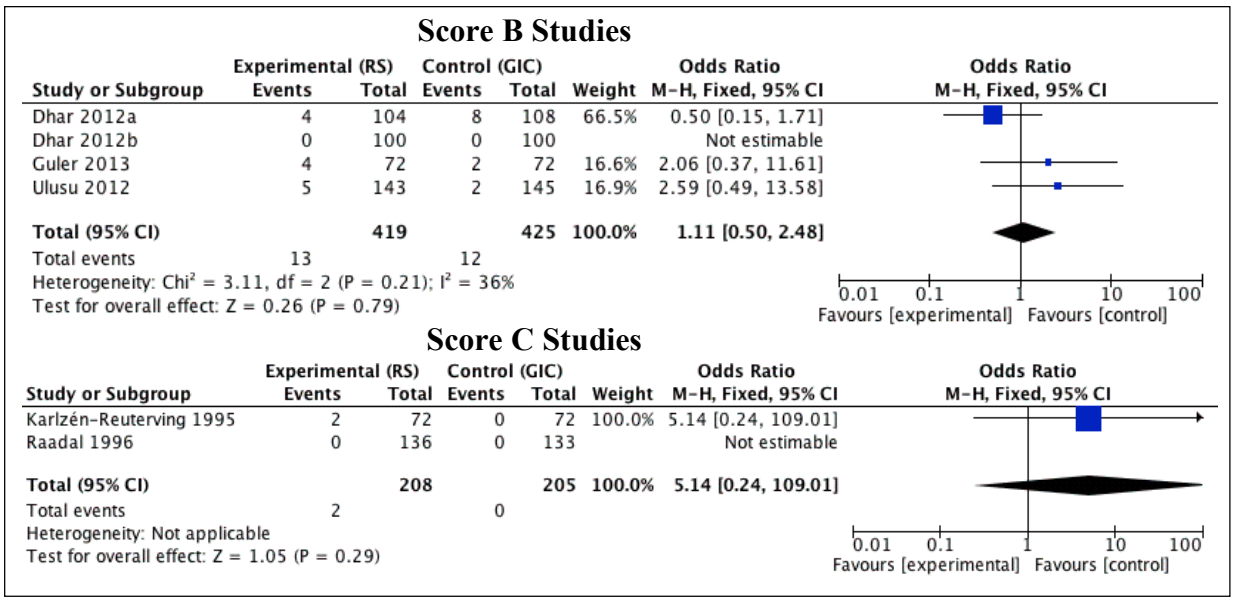

Figure 7. Prevention of dental caries by different sealants after 12 mouths: RS X GIC.

GIC- Glass Ionomer Cement. RS- Resin sealant.

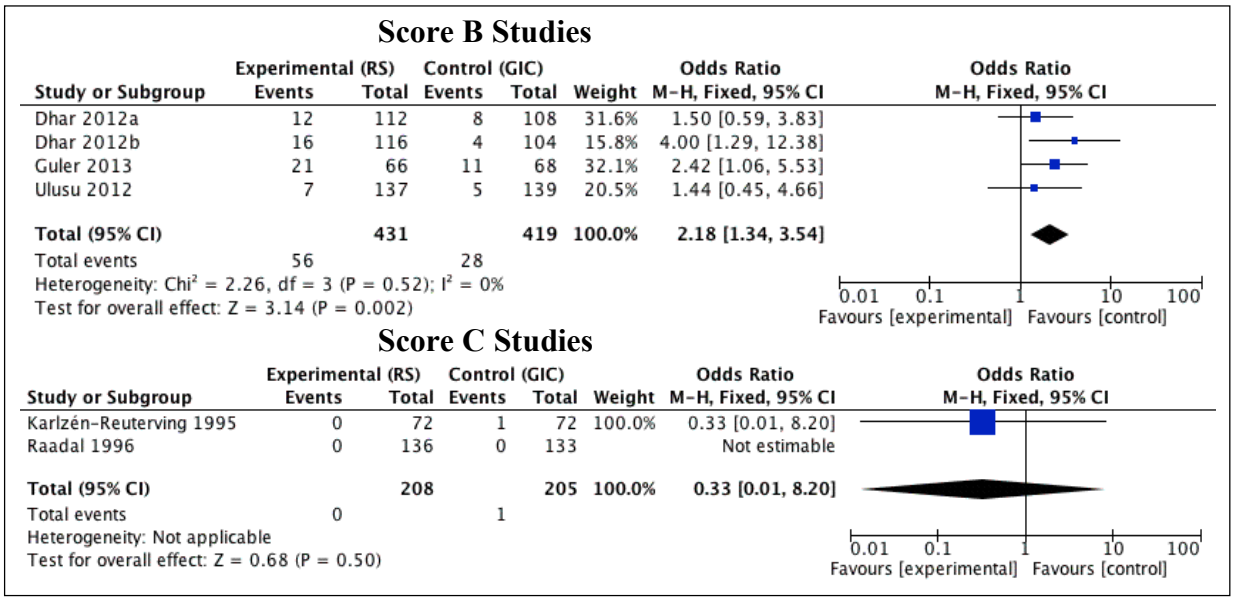

Figure 8. Prevention of dental caries of different sealants after 24 mouths: RS X GIC. GIC- Glass Ionomer Cement. RS- Resin sealant.

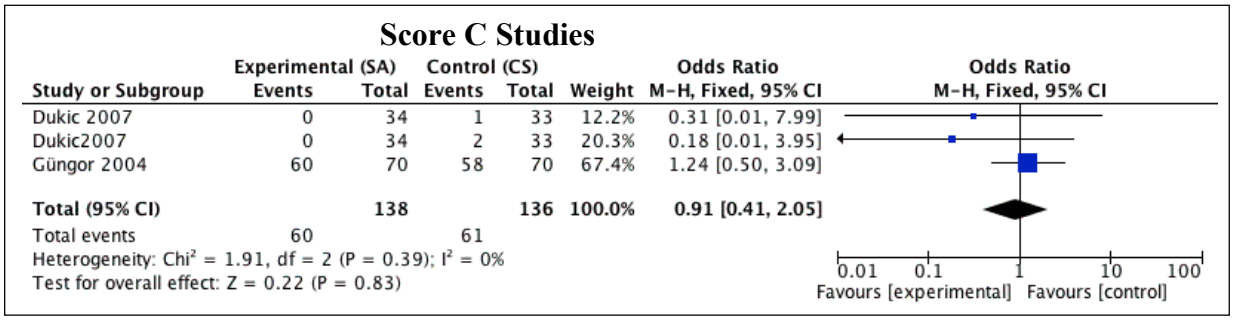

Figure 9. Prevention of dental caries of different techniques after 24 mouths: SA X CS. CS- Conventional Sealing. SA- Sealing with Adhesive.

2.2 ARTIGO: Impact of the Intermediary Layer on Sealant Retention: A Randomized 24-month Clinical Trial*

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*Submetido para o periódico Clinical Oral Investigations (Anexo 6)

Abstract

Objectives: To assess long-term impact of tooth Eruption Stages (ES) on sealant retention on occlusal surfaces previously coated with intermediary bonding layer and to determine caries prevention. Materials and Methods: Sixty-five school children were selected (aged 6-10 years), with four non-carious permanent first molar in different ES (OP- Operculum Present; ME- Marginal Edge; CE- Completely Erupted). Split-mouth and single-blind study design was used. The teeth (260) were randomly according to treatment (sealant/technique): F- Fluroshield; H- Helioseal Clear Chroma; SF- Single Bond + F; EH- Excite + H. Sealant retention, marginal integrity, discoloration and caries prevention were assessed after 6-, 12-, 18-, 24-month by calibrated examiner (Sperman=0.91) using visual inspection. Data were submitted to Cox Proportional Hazard model (survival analysis) and Likelihood ratio $\chi 2$ test (correlation), $p \leq 0.05$. Results: At baseline, ES was 20% in OP, 54% ME and 26% CE. There was no significant difference on sealant retention between the treatments (p=0.2774). However, significant differences were found regarding the ES on sealant retention (p=0.0041). The CE stage showed the highest retention survival rate during 24-month. The overall sealant prevention average was found about 99.3%, showed no difference between the groups. Conclusions: Eruption stages affect sealant retention regardless the intermediate layer and type of sealant. However, there was caries prevention on tooth occlusal surfaces after 24-month, regardless treatment.

Clinical Relevance: Sealing is recommended to prevent occlusal caries of newly-erupted teeth in high-caries-risk patients. However, its application is critical on moisture limited control surfaces independently of sealer material and technique.

Keywords: clinical trial; pit and fissure sealants; tooth eruption; retention; adhesive system; intermediary layer.

Introduction

The pits and fissures of occlusal surfaces are known to be susceptible areas for dental caries development. Their complex morphology is considered a site for harboring bacteria and food remnants, rendering mechanical debridement inaccessible. They also reduce the effectiveness of fluoride's remineralizing activity due to inadequate salivary access to fissures [1,2]. In addition, incomplete maturation of enamel increases caries susceptibility in newly-erupted teeth [3,4].

There is strong evidence that, in both clinical and school settings, sealants can be effective in preventing caries on occlusal surfaces of high-caries-risk children and adolescents [5-7]. However, the effectiveness of resin-based sealants is influenced by tooth eruption stage and the environment surrounding the sealant placement, such as saliva contamination and tissue management, which impedes the retention range of the pit and fissure sealant [5,8,9]. The greatest risk of sealant failure occurs soon after tooth eruption, when contamination with saliva and gingival fluid is almost inevitable [10]. A study showed that even one second of contact between etched enamel and saliva can result in an adherent coating formation that covers the pores created in the enamel, preventing the resin tags formation that is necessary for mechanical adhesion [11].

Studies have reported that the use of an intermediate bonding layer between humidity contaminated enamel and sealant improves bond strength, reduces microleakage, and enhances resin flow into fissures [3,12,13]. Other clinical studies have confirmed the benefit of using bonding agents beneath sealants on contaminated or non-contaminated enamel to increase sealant bond success range in application conditions that are less than ideal [9,14-17]. On the other hand, even if contamination of etched surfaces is not apparent, sealant application under high relative humidity exhibits a significant reduction in its bond strength [12]. Conversely, another study has shown that sealant success, without bonding agent, is dependent on optimal placement conditions since no effect on sealant success by a bonding agent was evident under such conditions [18]. However, long-term clinical trials data are limited related to sealant success, with or without an intermediate bonding layer, under less than ideal placement conditions.

Therefore, the aims of this study were: (a) to assess the impact of tooth eruption stages on long-term sealant retention on occlusal surfaces previously coated with an intermediate bonding layer and sealant material, and (b) to evaluate

caries prevention evolved. The first hypothesis tested was that there are significant differences in long-term sealant retention outcome for different sealing techniques (with or without the intermediate bonding system) and tooth eruption stages. The second hypothesis was that the sealing has effect in long-term caries prevention, regardless tooth eruption stages.

Materials and Methods

This clinical trial follows the CONSORT guidelines and was conducted after approval from the Ethics Committee of Piracicaba Dental School, State University of Campinas (protocol number 143/2003).

Two hundred children (age 6-10 years) were examined in an attending public school (E. M. José Pousa de Toledo), located in the low socio-economic level zone of Piracicaba, São Paulo, Brazil. The children did not receive any preventive health care prior to the study. The fluoride content in drinking water was 0.7 ppm [19]. Every student in the school received oral hygiene instructions, but study participants only received a dental kit (toothbrush, toothpaste and dental floss) and additional dental treatment (e.g. restorative treatment or pulp therapy) if necessary. In these cases, the children were referred to the Pediatric Dental Clinic of Piracicaba Dental School, University of Campinas. The study was conducted in a dental office inside the school. The purpose and clinical procedures of the study were explained by one of the investigators and informed written consent was obtained from the parents and/or guardians of all children enrolled. The population studied was at high-caries-risk patients, showing initial caries lesions on smooth surfaces and/or cavities in primary teeth, and absence of enamel condition-fluorosis. Children were selected based on the following criteria: four caries-free erupted first molar permanent teeth; no systemic diseases; and, no dental material allergies.

The sample size of the study was calculated based on a difference of 15% between treatments ($\alpha=0.05$; $\beta=0.1$), with 90% power. Of two hundred children who were initially examined for the study, 67 were excluded due to inclusion criteria, 37 because of moved to another city and 31 who refused to continue the study. Sixty-five children (a total of 260 teeth) were randomly allocated for the study at baseline. The mean age was 8 years (ranging from 6 to 10 years), 53% male. The clinical trial was a single-blind and randomized study (lottery method) that involved a 24-month

follow-up of a split-mouth design. Two variables were under evaluation: (1) sealant technique and (2) tooth eruption stage.

The teeth were randomly categorized into four groups (n=65) according to the sealant materials involved (with and without fluoride), i.e. sealant and/or adhesive system: F- Fluroshield; H- Helioseal Clear Chroma; SF- Single Bond + Fluroshield; EH- Excite + Helioseal Clear Chroma. The fissure sealing was performed in accordance with the manufacturer's instructions. The stage of tooth eruption was scored following Dennison et al. criteria [8] at baseline (Table 1).

At examination, a visual inspection on dry tooth surfaces was conducted under dental operating light. For the treatments, buccal/lingual and occlusal surfaces were first cleaned by pumice/water slurry to remove the dental biofilm and stains. Moisture control was maintained by cotton-roll-isolation aided by a chair-side assistant. All sealant materials were applied from the central fissure for the cusps in order to prevent bubbles. Both surfaces were sealed at the same visit if the buccal/lingual fissures required sealant treatment.

For F group, the enamel surface was etched using 37% phosphoric acid (H_3PO_4) gel for 30 s, rinsed for 10 s, and air-dried. The material was applied, using a sharp explorer in order to avoid excessive material spreading, and light cured for 40 s. For H group, the enamel surface was etched using 37% phosphoric acid (H_3PO_4) gel for 30 s, rinsed for 10 s, and air-dried. The material was applied with plastic tip incorporated into a sealant syringe and light cured for 20 s. For SF and EH groups, an adhesive system layer was applied to the surface with a microbrush (3M/ESPE, Sumaré, São Paulo, Brazil) and air-thinned prior to sealant application. Sealant and adhesive were light cured together (SF for 40 s and EH for 20 s). Brand names, type, composition, manufacturers, and batch numbers of sealant materials and adhesive systems are shown in Table 2. Light curing was conducted using the Elipar Tri-light unit (ESPE-America, Seefeld, Bavaria, Germany) with an 800 mW/cm^2 light intensity. After sealing application, the occlusions were checked with carbon markers. Any premature contacts were removed to ensure that the sealants had not produced occlusal interference.

Recall examinations were conducted after 6-, 12-, 18-, and 24-month. All procedures at each appointment, including calibration, were performed by the same calibrate investigator (KRK). A preliminary pilot study was performed for intra-examiner reproducibility of the teeth selection, sealing procedure, and criteria for

evaluation. Thirteen children were examined twice, a week apart, to measure the intra-examiner coincidence level. The value of the Spearman's correlation test was 91%.

During the follow-up examinations, the groups were assessed for sealant retention (anatomical form), marginal integrity and discoloration and caries prevention using the adapted criteria proposed by Feigal et al. [14] (Table 3). Before the visual and tactile inspection, a prophylaxis using pumice and water slurry was performed. All evaluations were conducted under normal clinical conditions with a dental operating light, a mouth mirror and a dental explorer with a tip of blind probe. Sealants with a failure score were repaired using the same materials as in the original sealant application. Teeth were excluded from the statistical analysis when the first sealant failure (partial or total loss) was observed.

The data analysis was performed using non-parametric Wilcoxon k-sample test based on weighted comparisons of the estimated hazard rate of the individual population, adjusted through the Tukey-Kramer methodology by multiple comparisons to evaluate the differences in retention rates and the stage of tooth eruption between the sealant techniques involved. Survival analysis was performed by the Cox Proportional Hazard model. Failure time data were adjusted by using an appropriate censoring method. Contingency tables and corresponding chi-square tests were used to test the association between sealant materials and the survival time. Likelihood ratio $\chi 2$ test was performed to correlate integrity marginal, marginal discoloration and caries prevention with the treatment. All statistical analyses were carried out using SAS System, release 9.3 (SAS Institute Inc., Cary, NC, USA, 2010) and in all tests the 5% significance level was adapted.

Results

The number of children who returned for the 6-, 12-, 18-, and 24-month evaluations was, respectively: 53 (82%), 47 (72%), 43 (66%), 37 (57%). The dropouts were due children's moving out. CONSORT flow diagram, including treatment regimen of subjects randomized teeth and follow-up phases of the study is shown in Figure 1. Study results indicated no significant interaction between tooth eruption stages and sealant techniques on sealant retention ($p=0.2249$). In addition, there were no significant differences on sealer material retention between the sealant techniques ($p=0.2774$) (Table 4). Regarding the baseline, the distribution of the

cohort's tooth eruption stages was: OP= 20%; ME= 54%; CE= 26%. There was significant effect of ES on sealant retention (p=0.0041). The CE Stage (Completely Erupted - 22.1±0.6 months) showed the highest retention survival rate at 24-month recall period, followed by Stage OP (Operculum Present – 20.1±1.0 months) and then Stage ME (Marginal Edge- 19.2±0.7 months) (p=0.0035). Therefore, it can be observed that, the longer the period of time is, higher is the failure found, considering the ES (Figure 2).

During this study, the sealant marginal integrity failure and discoloration were present in 2% and 4% of the teeth at 24-month follow-up, respectively, but it was not related with caries presence. The overall sealant prevention average was found about 99.3%. There was no significant difference between groups (p>0.05) (Table 5).

Discussion

Dental sealants are attested to be effective in preventing dental caries due to their ability to adhere to enamel surfaces [7]. They have proven to be beneficial for high caries risk children. A critical application issue is the moisture tolerance of conventional resin-based sealants, since in most of the cases, the relative isolate is required when sealing of permanent molars in the newly stage of eruption [20], which are more susceptible to caries formation due to the difficulty of cleaning this area [21]. However, the use of an intermediary layer on sealant technique has been controversial [13,18]. The first hypothesis was partially rejected, since there was no interaction between studied factors (sealant technique, with or without an intermediary layer, and tooth eruption stages). There are few relevant published papers showing relationship between the use of intermediary layer and different tooth eruption stages. Concerning sealant technique used in this study, there was no significant difference between them. This is probably due to the similar materials composition. This result is consistent with earlier reports in dental literature that studied, in a clinical trial, the application of an adhesive system beneath the sealant [18,22,23].

In addition, other investigators have conducted clinical studies on the retention rate of sealants that were filled and unfilled using bonding agents [24-27]. After two years, they concluded that bonding agents did not increase the retention of either types of sealant [24,26]. Similar to a 12-month study that compared the

retention and caries prevention rates of a moisture-tolerant resin-based sealant (Embrance) with a conventional resin-sealant, with or without a bonding agent, or glass ionomer cement. Results concluded that the use of a bonding agent as an intermediary layer between enamel and sealant was not significantly effective for the retention of fissure sealants or for caries prevention even with the use of a moisture tolerant sealant [27].

Data from this present study indicated no significant difference with regard to sealant retention between the filled (Fluroshield) and unfilled (Helioseal Clear Chroma) sealants used. This similarity may be explained by the fact that operatory procedures, such as enamel etching with phosphoric acid, created a similar substrate pattern for bonding. Similar findings have been reported by other groups, which assessed clinical performance of filled and unfilled sealer materials [28-32].

In the present study, all materials used were composed by methacriylates with similar properties, providing low viscosity. Despite of higher amount of diluents on Adper Single Bond 2 composition, it is important to consider that it cannot influence on the stability of sealant retention. In addition, the Adper Single Bond 2 content components maybe are related to deep penetration into pit and fissures and the sealant retention are related with enamel resintags formation provided by enamel etching acid [11].

On the other hand, long-term clinical trials have been demonstrating that an intermediate layer of primer and adhesive can increase the retention of sealants under contaminated conditions [3,10,14,33,34]. The benefit of primer and adhesive layer beneath the sealant seems to be based on a combination of moisture-chasing effects of the hydrophilic primer, increased flow dispersion imparted by the less viscous primer and adhesive and increased flexibility of the combined and polymerized primer/adhesive/resin complex once reaction is complete [35]. Although these studies indicate the usefulness of this intervention, the clinician should consider the increase in cost and time required. Thus, this combined sealant/adhesive system application technique has to be wisely recommended for the clinical practice and depending on the tooth eruption stage.

Considering Eruption Stages, the obtained results indicated that the lowest retention success rate was related to partially erupted teeth, most common in the Stage OP (Operculum Present) and then Stage ME (Marginal Edge). Highest retention rates were achieved on fully erupted teeth regardless of the sealant

technique performed. The different retention results concerning eruption stages may be due to inadequate moisture control on partially erupted teeth (OP and ME). In addition, it is known that resin-based sealants require a completely dry field to achieve adequate adhesion, as Bis-GMA-based materials are primarily hydrophobic in nature [36,37]. Similar to the present study, Dennison et al. [8] conducted a 36-month study using a self-curing pit and fissure sealant on occlusal surfaces of newly erupting permanent molars. The research reported that molars partially erupted had twice the probability for retreatment than teeth not treated until the entire marginal ridge was exposed. In addition, a 24-month study that compared resin and glass-ionomer sealant in partially erupted permanent molars observed similar retention between the materials and suggested that partially erupted teeth might be affected by etching efficacy of the acid in resin-based sealants [38].

Presently, this study showed that the failure rate of sealant technique at 24-month evaluation it is also related to elapsed time (Figure 2). So, it was observed that for all tooth eruption stages there was a decrease survival rate and the highest survival rate found was for CE (22 months). Similarly, Jodkowska [1] reported that the retention rate of fissure sealant on the occlusal surface depends, among others factors, on the duration of observation.

However, in this randomized clinical trial, we found sealant retention failure (partial and total loss) approximately 1-5% in the first year of application and 15-34% in second year follow-up, with the greatest loss at 18-month, and high caries prevention was observed for all studied groups during the 24-month follow-up. The application of resin sealants can increase the occlusal caries prevention with or without fluoride, and therefore may be used to treat populations similar to that of this study. These current results corroborate those that showed no additional benefits of the fluoride inclusion in resin sealants in order to prevent caries, due to the low fluoride release [39].

It is important, therefore, that sealant adhesion in pits and fissures be improved particularly in newly-erupted teeth with incomplete maturation of enamel, which can increase caries susceptibility and biofilm stagnation [3,4,21], so that sealants' beneficial effect on caries prevention can be observed [1,40]. So, further clinical studies longer than 24-month observation periods and practice-based research may contribute to evaluate the effective sealant retention performance, since the longer the follow-up, the higher difference between similar materials can be

observed.

Conclusions

Based on this study, partially erupted (OP and ME) teeth showed lower retention rate than those completely erupted (CE), regardless of the intermediate layer and sealant material involved. There was caries prevention on tooth occlusal surfaces after 24-month, regardless treatment. Thus, the occlusal eruption stage affects the sealant retention.

Compliance with Ethical Standards

Conflict of Interest: Author Kelly Maria Silva Moreira declares that she has no conflict of interest. Author Kamila Rosamilia Kantovitz declares that she has no conflict of interest. Author Juliana Pedrini Dias Aguiar declares that she has no conflict of interest. Author Ana Flávia Sanches Borges declares that she has no conflict of interest. Author Fernanda Miori Pascon declares that she has no conflict of interest. Author Regina Maria Puppin-Rontani declares that she has no conflict of interest.

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Ethical approval: This article does contain any studies with human participants performed by any of the authors. This clinical trial study was conducted after approval from the Ethics Committee of Piracicaba Dental School, State University of Campinas (protocol number 143/2003).

Informed consent: For this type of study, formal consent is required. Thus, informed consent was obtained from all individual participants included in the study.

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Table 1. Criteria for tooth eruption stage evaluation.

Rating	
OP	Operculum Present. The entire tooth occlusal surface in the gum completely immersed, but the operculum tissue extends in the distal margin of tooth groove.
ME	Marginal Edge. The entire tooth occlusal surface is immediately adjacent to the marginal edge.
CE	Completely Erupted. Occlusal sulcus completely supragingival.

Adapted from: Dennison JB, Straffon LH and More FG. Evaluating tooth eruption on sealant efficacy. JADA (1990); 121:11.

Table 2. Brand, composition, batch number of the materials used in present study.

Materials	Types	Composition	Manufacturers and Batch #
FluroShield	Filled resin sealant with fluoride	Urethane modified Bis-GMA dimetacrylate; Barium aluminoborosilicate glass (30%), Polymerizable dimetacrylate resin, Bis-GMA, Sodium fluoride, Dipentaerythritol pentaacrylate phosphate, Titanium dioxide, Silica amourphous.	Dentsply, Milford, DE, USA # 317131
Helioseal Clear Chroma	Unfilled resin sealant with no fluoride	Bis-GMA, Triethylene glycol dimethacrylate (>99wt.%). Additional contents are stabilizers, catalyts and pigments (<1wt.%)	Ivoclar/Vivadent Schaan Liechtenstein # F54463
Adper Single Bond 2	Adhesive system	BisGMA, HEMA, Dimethacrylates, Ethanol, Water, Photoinitiator system, Methacrylate functional copolymer of polyacrilic and polyitaconic acids.	3M/ESPE St. Paul, MN USA # 4BM
Excite	Adhesive system	Bis-GMA, HEMA, methacrylates, silicon dioxide, ethanol, catalysts and stabilizers	Ivoclar/Vivadent Schaan Liechtenstein # G03541

(FluroShield-Dentisply, Milford, DE, USA or Helioseal Clear Chroma-Ivoclar/Vivadent Schaan Liechtenstein) associated or not with an intermediary layer (Single Bond-3M/ESPE, St. Paul, MN, USA or Excite-Ivoclar/Vivadent, Schaan, Liechtenstein).

Table 3. Criteria for sealants evaluation.

	Evaluation of *Marginal Integrity*
0	Sealant material adjacent to the tooth and not detectable with an explorer
1	Margin detectable with the explorer
2	Crevice along the margin of visible width and depth
3	Crevice formation with exposure of central fissure
	Evaluation *of Marginal Discoloration*
0	No color change at the tooth-sealant interface
1	Discoloration noted along the margin in one area
2	Discoloration noted along the margin in multiple areas
3	Severe discoloration with evidence of penetration and leakage
	Evaluation of *Anatomical Form (Sealant Retention)*
0	Harmonious and continuous with occlusal form and structure
1	Change in anatomical form but all pits and fissures covered
2a	Loss of sealant from one or two pits or accessory grooves (partial loss), but no need to repair or replace sealant)
2b	Loss of sealant from pits or accessory grooves (partial loss), with a need for replacement or repair of the sealant
3	Loss of sealant from all pits (total loss)
	Evaluation of *Caries Prevention*
0	Caries absence at the sealed surfaces (White spots and cavited lesions) on marginal or sealant lost area
1	Caries presence at the sealed surfaces (White spots or/and cavited lesions) on marginal or sealant lost area

Adapted from: Feigal RJ, Musherure P, Gillespie B, Levy-Polack M, Quelhas I, Hebling J. Improved sealant retention with bonding agents: a clinical study of two-bottle and single-bottle systems. J Dent Res. (2000) 79(11):1850-6.

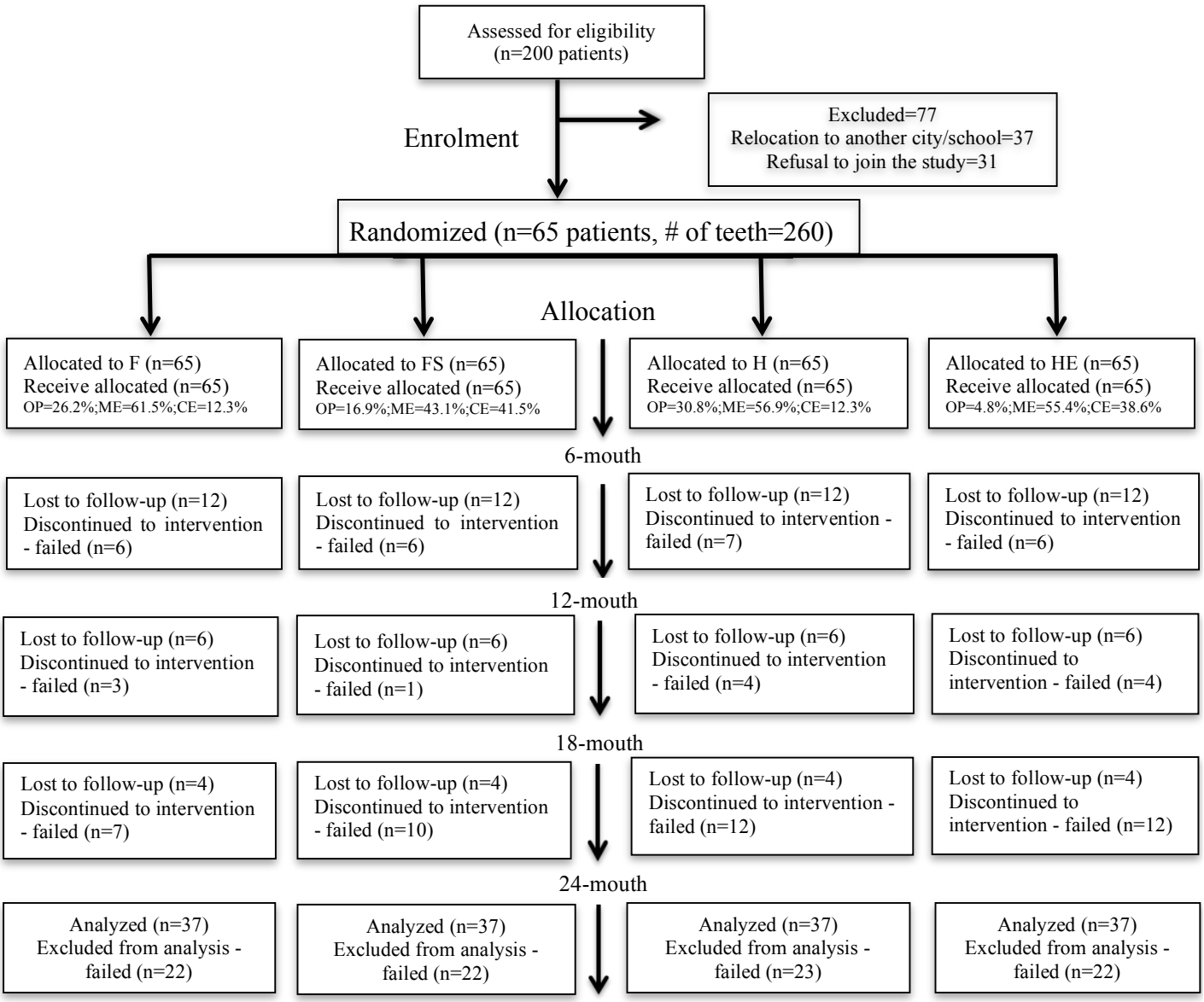
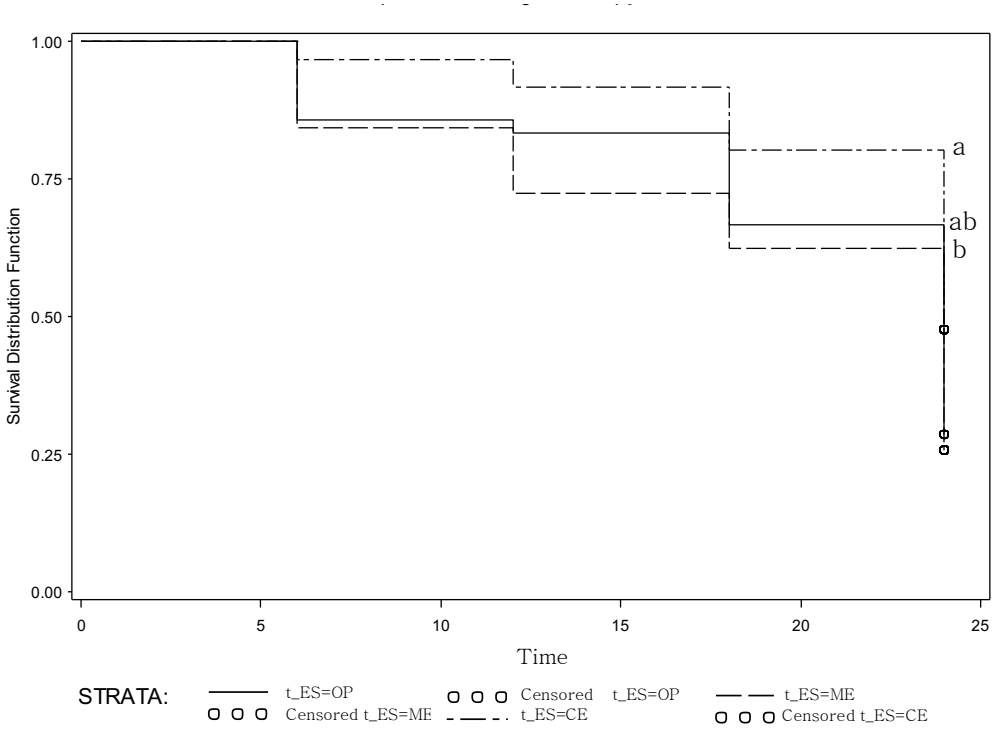


Figure 1 - Flow diagram of follow-up phases of the study.

Table 4 - Retention rate of the sealant materials/techniques during follow-up.

Materials/ Techniques	Follow-up Retention Rate				
	baseline	6-month	12-month	18-month	24-month
	n=65 % (# teeth failed/total teeth)	n=53 % (# teeth failed/total teeth)	n=47 % (# teeth failed/total teeth)	n=43 % (# teeth failed/total teeth)	n=37 % (# teeth failed/total teeth)
Fluroshield	100% (0/65)	92.5% (4/53)	97.9% (1/47)	93.0% (3/43)	43.2% (21/37)
Fluroshield + Single Bond	100% (0/65)	100% (0/53)	97.9% (1/47)	93.0% (3/43)	70.3% (11/37)
Helioseal Clear Chroma	100% (0/65)	94.3% (3/53)	100% (0/47)	90.7% (4/43)	29.7% (26/37)
Helioseal Clear Chroma + Excite	100% (0/65)	94.3% (3/53)	97.9% (1/47)	93.0% (3/43)	51.4% (18/37)

n – total of person treated in this study according to the follow-up period;
% - percentage of teeth evaluated at each follow-up period.
There was no significant difference between groups and follow-up.



*ES=Eruption Stage; OP=Operculum Present; ME=Marginal Edge; CE=Completely Eruption

Figure 2 - Sealant retention over time in the different stages of eruption.
Different letters represent statistically significant difference by the Cox Proportional Hazard model ($p<0.05$).

Table 5 - Caries prevention for the treatments during follow-up.

(Materials/ Techniques)	Follow-up Caries Prevention					
	baseline	6-month	12-month	18-month	24-month	Mean for treatment
	n=65 % (# total failed/total teeth)	n=53 % (# total failed/total teeth)	n=47 % (# total failed/total teeth)	n=43 % (# total failed/total teeth)	n=37 % (# total failed/total teeth)	
Fluroshield	100% (0/65)	100% (0/53)	100% (0/47)	98% (1/43)	100% (0/37)	$\bar{x}$=99.5%
Fluroshield + Single Bond	100% (0/65)	100% (0/53)	100% (0/47)	98% (1/43)	100% (0/37)	$\bar{x}$=99.5%
Helioseal Clear Chroma	100% (0/65)	98.1% (1/53)	100% (0/47)	98% (1/43)	100% (0/37)	$\bar{x}$=99%
Helioseal Clear Chroma + Excite	100% (0/65)	100% (0/53)	100% (0/47)	98% (1/43)	100% (0/37)	$\bar{x}$=99.5%
Mean of the treatments	$\bar{x}$=100%	$\bar{x}$=99.5%	$\bar{x}$=100%	$\bar{x}$=98%	$\bar{x}$=100%	$\bar{x}$=99.4%

n - total of person treated in this study according to the follow-up period;
% - percentage of teeth evaluated at each follow-up period.
There was no significant difference between groups and follow-up.

3 DISCUSSÃO

Apesar dos vários meios utilizados para a prevenção da cárie dentária, esta ainda se constitui em um problema de saúde pública. E, diante da era preventiva, várias são as intervenções realizadas em prol da erradicação desta doença. As superfícies oclusais são locais altamente susceptíveis, sobretudo no período de irrupção dentária, quando o acúmulo de biofilme é favorecido e o acesso à adequada higienização é limitado (Carvalho, 2014). Assim, métodos preventivos auxiliares são requeridos para estas regiões (Jodkowska, 2008), em pacientes com risco à cárie dentária (Ahovuo-Saloranta et al., 2013).

O uso de selantes de fóssulas e fissuras tem sido considerado um método promissor, viável e eficaz para a prevenção da cárie oclusal (Wendt et al., 2001; Tagliaferro et al., 2011). Nesta dissertação, por meio da Revisão Sistemática e Metanálise, artigo 1, foi confirmada a evidência clínica da eficácia deste método preventivo, em pacientes com risco à cárie dentária, quando comparado a não realização de tratamento. Além disso, no Estudo Clínico Randomizado, artigo 2 desta dissertação, a aplicação de selantes também se mostrou eficaz, uma vez que a prevenção de cárie nas superfícies oclusais foi de ≅ 99% para uma população de risco à cárie (Apêndices 1-2).

No entanto, é importante ressaltar que a eficácia do selamento oclusal pode estar intimamente ligada ao material e técnica utilizada. Esta por sua vez, tem sido apontada como um dos principais fatores de fracasso do selamento (Sundfeld et al., 2007, 2010). Em relação aos materiais utilizados, os de uso corriqueiro são os selantes resinosos e os cimentos de ionômero de vidro. Ao avaliar a retenção destes materiais seladores e técnicas de selamento na Revisão Sistemática e Meta-análise, os selantes resinosos apresentaram maior taxa de retenção aos 24 meses do que os ionômericos, corroborando com resultados prévios da literatura (Dhar et al., 2012; Ulusu et al., 2012). E, quando a técnica de selamento incluiu a utilização de uma camada intermediária adesiva previamente ao selante resinoso, em estudos de baixa evidência, a retenção do selante foi favorecida, após 24 meses de análise como descrito por demais autores (Feigal et al., 2000; Lygidakis et al., 2009). Entretanto no Estudo Clínico Randomizado (Apêndice 3), não foi encontrada diferença significativa nas técnicas de aplicação, sem e com camada intermediária, como Mascarenhas et al. (2008) e Nazar et al., (2013) também relataram. Neste estudo clínico o que afetou a retenção ao longo do tempo foi o estágio de irrupção dentária, sendo que o estágio mais avançado de irrupção apresenta maior percentual de retenção dos selantes, como já havia sido reportada por outros autores esta correlação direta entre a retenção dos selantes e o estágio de irrupção dentária (Feigal et al.,

1993; Ahovuo-Saloranta et al., 2008). Este fato pode explicar a controvérsia dos resultados encontrados pelas pesquisas desta dissertação referente à técnica usando camada intermediária, dado que os estudos analisados na Revisão Sistemática e Meta-análise incluíram somente dentes completamente irrompidos, nos quais o controle da humidade é mais propício de ser alcançado devido à menor interferência de fluídos provenientes da gengiva adjacente. Esta questão, portanto, ainda é muito discutida na literatura.

No que concerne à prevenção da cárie dentária, os resultados da Revisão Sistemática e Meta-análise demonstraram que não houve diferença entre os selantes resinosos e os ionoméricos, após 12 meses. Já aos 24 meses, os cimentos ionoméricos convencionais favoreceram a prevenção da cárie dentária, estando de acordo com Ulusu et al. (2012), Antonson et al. (2012) e Guler e Yilmaz (2013), enquanto Messer et al. (1997), Francis et al. (2008) e Jodkwska (2008) reportaram melhor efeito preventivo dos selantes resinosos. Além disso, há também achados de que os selantes resinosos parecem não diferir dos selantes ionoméricos na proteção contra à cárie (Pavinato e Imparato, 2013). Para a técnica utilizada, a inclusão da camada intermediária adesiva não interferiu na capacidade preventiva dos selantes resinosos após 24 meses de acompanhamento, assim como no Estudo Clínico que houve prevenção da cárie independente da técnica utilizada. Na literatura, não há estudos focados na influência da aplicação prévia da camada intermediária adesiva na prevenção da cárie oclusal, somente associando a mesma à retenção dos materiais seladores. Assim, o Estudo Clínico Randomizado realizado mostrou ser uma importante referência para outros estudos sobre a técnica de selamento, uma vez que nele foi avaliado tanto a retenção das diferentes técnicas quanto à prevenção à cárie dentária.

Já para a comparação entre os selantes com composições básicas semelhantes, não houve estudos compatíveis para a avaliação na Revisão Sistemática e Meta-análise. No Estudo Clínico Randomizado, ao comparar dois selantes resinosos com e sem carga, não foi observada diferença em relação à retenção desses materiais na superfície oclusal e à prevenção da cárie dentária. Esta semelhança pode ser explicada pelo fato de que os procedimentos operatórios, tais como o condicionamento do esmalte com ácido fosfórico, criam um padrão de união semelhante para o substrato dentário. Achados semelhantes foram relatados por outros estudos, que avaliaram o desempenho clínico de selantes resinosos (Koch et al., 1997; Reddy et al., 2015), em contrapartida, outros autores relataram imperfeições na adaptação marginal do selante com carga, mostrando que a composição do material selador pode interfirir no módulo de elasticidade, na viscosidade e molhabilidade do material e,

consequentemente, contribuir para a presença de "gaps" e insucesso do selamento oclusal (Irinoda et al., 2000; Sahafi et al., 2001; Kantovitz et al., 2008).

Perante os resultados encontrados nesta dissertação, não é exclusivamente a retenção do selante na superfície oclusal que indica a capacidade de prevenção à cárie dentária. No entanto, mais estudos clínicos bem delineados abrangendo diferentes estágios de irrupção dentária precisam ser realizados para que o material e técnica de selamento de fóssulas e fissuras, requisitos críticos para a longevidade deste procedimento clínico, sejam distintamente utilizados de forma criteriosa e concernentes aos respectivos princípios científicos e a evidência clínica.

4 CONCLUSÃO

Pode-se concluir que:

- Selantes são eficazes em prevenir lesão de cárie em pacientes alto de risco.
- Selantes resinosos apresentam maior taxa de retenção.
- Selantes ionoméricos convencionais mostram maior prevenção da cárie dentária.
- A retenção do material na superfície não é indicativo de prevenção à cárie.
- Independente da técnica de selamento, dentes completamente irrompidos apresentaram o maior percentual de retenção dos selantes.
- Na Meta-análise, estudos de baixa evidência mostraram que a camada intermediária favoreceu a retenção do material selador na superfície oclusal, enquanto no Estudo Clínico Randomizado a mesma não favoreceu a retenção, independente do estágio de irrupção oclusal. Mais estudos clínicos randomizados devem ser realizados para se constatar o verdadeiro papel da retenção do selante na prevenção da cárie oclusal.
- Houve prevenção da cárie dentária nas superfícies oclusais após 24 meses de acompanhamento.

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APÊNDICE 1- Ficha clínica do Estudo Clínico Randomizado.
Nome do paciente:____Série:______
Nome do responsável:____
Endereço:____Telefone:_________________
Data de nascimento:_____________Idade:______N° paciente___________________________

	1° consulta	6 meses	12 meses	18 meses	24 meses
n° dente					
Estágio irrupção					
Integridade Marginal					
Estabilidade cor					
Cárie secundária					
Forma					
n° dente					
Estágio irrupção					
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Forma					
n° dente					
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Estabilidade cor					
Cárie secundária					
Forma					

APÊNDICE 2 - Fotografias ilustrativas da sequência da aplicação e acompanhamento do selante fluorshield, do artigo 2.

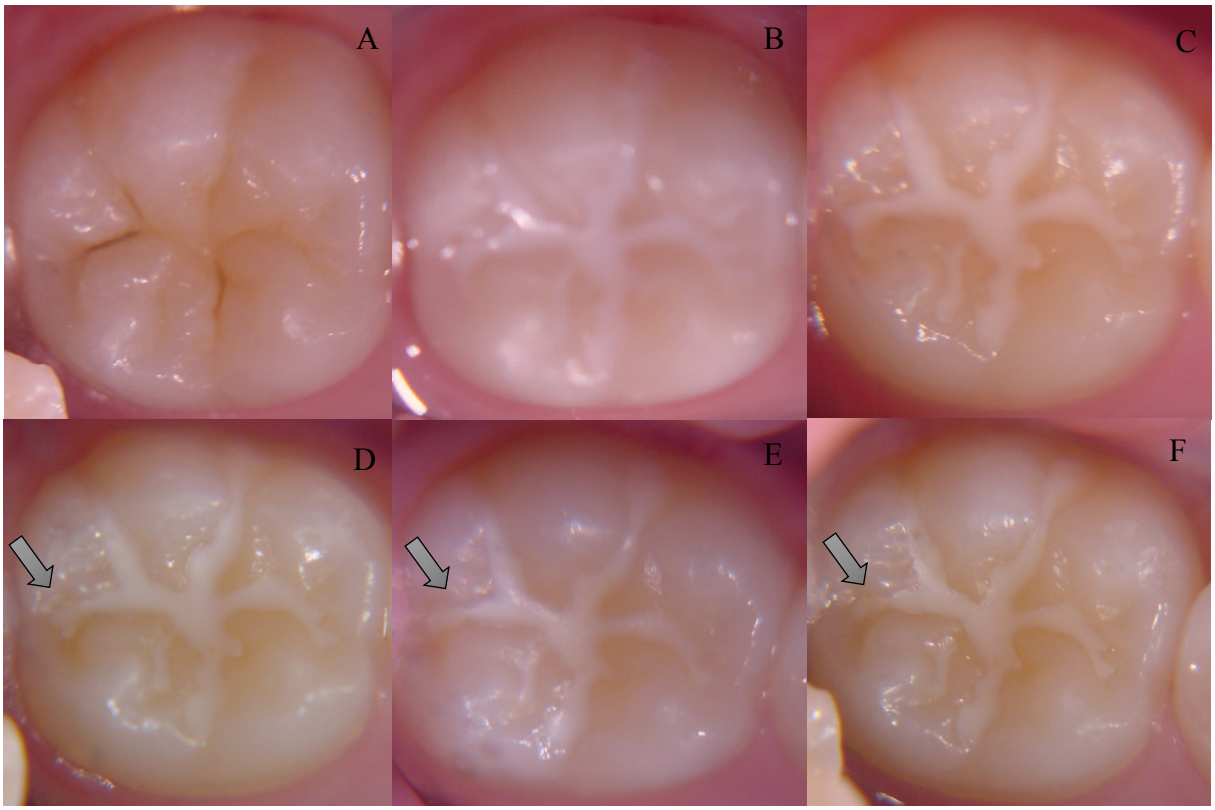
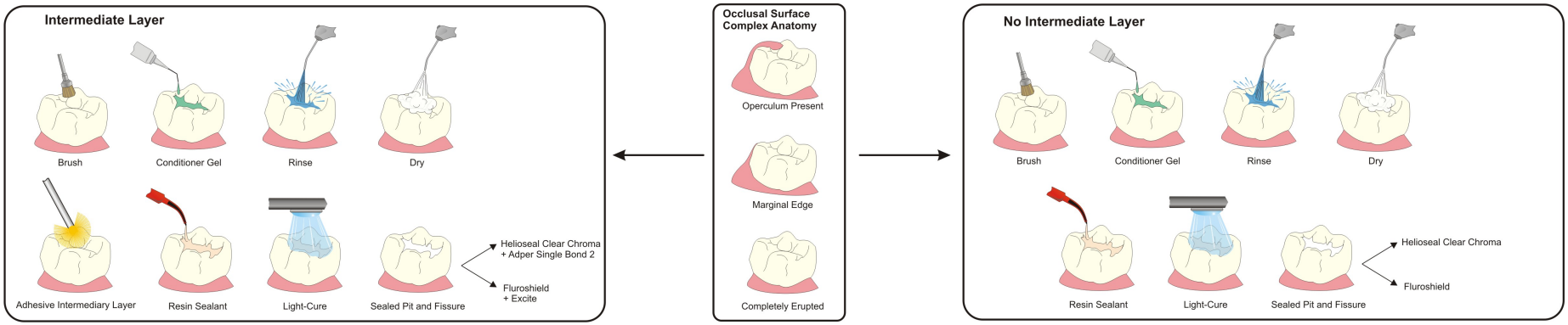


Figura 1. Selamento de fóssulas e fissuras oclusais com selante fluroshield opaco. A) Dente 36 antes da realização do selamento. B) Dente 36 imediatamente após a realização do selamento. C) Dente 36 após 6 meses da realização apresentando integridade do selamento. D) Dente 36 após 12 meses da realização do selamento oclusal, com perda mínima do selante na fóssula principal próximo à crista marginal distal, sem evidência de cárie. E) Dente 36 após 18 meses da realização do selamento, com pequena perda do selante na fóssula principal próximo à crista marginal distal, sem evidência de cárie. F) Dente 36 após 24 meses da realização do selamento com semelhança ao relatado após 18 meses, sem evidência de cárie.

APÊNDICE 3 – Graphical abstract referente ao artigo 2.



ANEXO 1 - Certificado e Emenda 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 clínico de selantes de fóssulas e fissuras"**, protocolo nº 143/2003, dos pesquisadores Regina Maria Puppin Rontani, Kamila Rosamilia Kantovitz e Kelly Maria Silva Moreira, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 09/06/2004, com alterações em 03/10/2014.

The Ethics Committee in Research of the Piracicaba Dental School - University of Campinas, certify that the project **"Clinical evaluation of pit and fissure sealants"**, register number 143/2003, of Regina Maria Puppin Rontani, Kamila Rosamilia Kantovitz and Kelly Maria Silva Moreira, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee on Jun 09, 2004; with alterations on Oct 03, 2014.

Prof. Dr. Felippe Bevilacqua Prado
Secretário
CEP/FOP/UNICAMP

Profa. Dra. Lívia Maria Andaló Tenuta
Coordenadora
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 2 - Declaração da transferência do direito autoral.

Declaração

As cópias dos documentos de minha autoria ou de minha coautoria, já publicados ou submetidos para publicação em revistas cientificas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertacão de Mestrado, intiulada "SELANTES DE FÓSSULAS E FISSURAS: EFEITO DE MATERIAIS/TÉCNICAS E ESTÁGIO DE IRRUPÇÃO DENTÁRIA - METANÁLISE E ESTUDO CLÍNICO RANDOMIZADO", não infringem os dispositivos da Lei nº 9.610/98, nem o direito autoral de qualquer editora.

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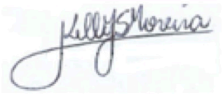

Autora: Kelly Maria Silva Moreira
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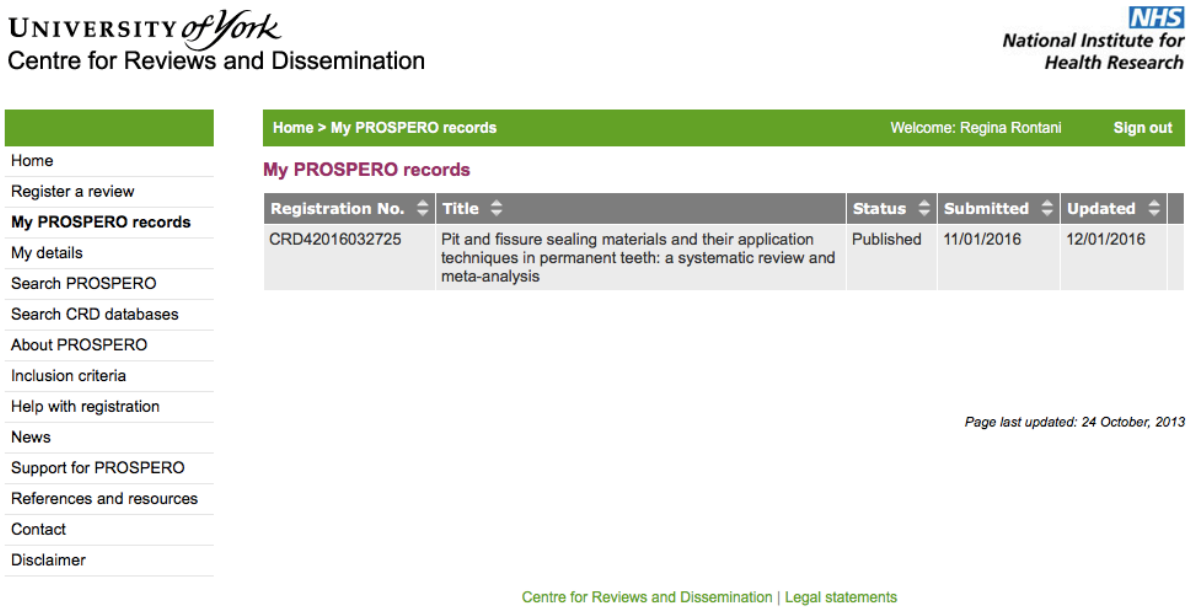
Orientadora: Regina Maria Puppin Rontani
RG 10723931

ANEXO 3 – Registro da Revisão Sistemática e Metanálise, artigo 1.



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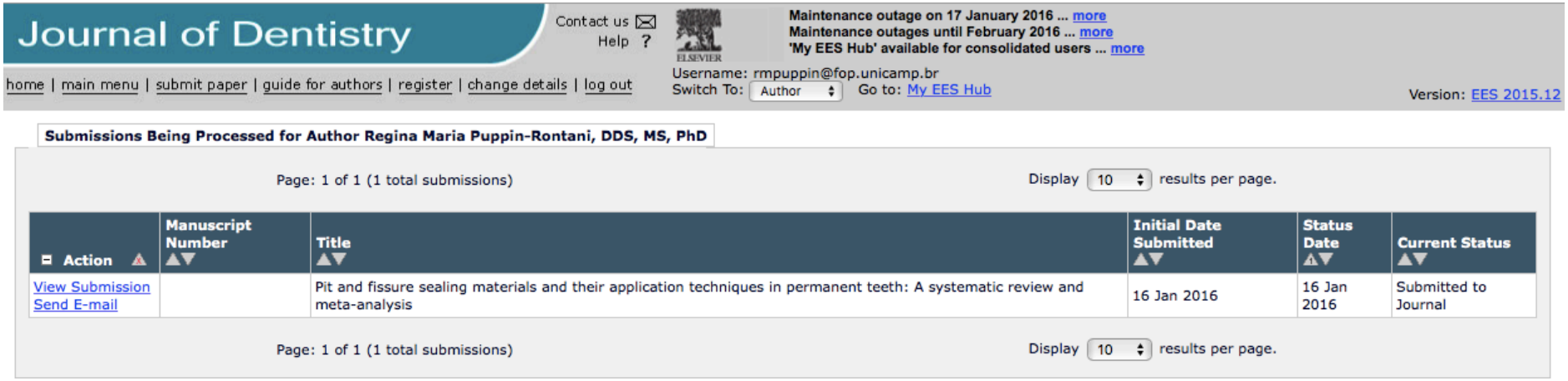
ANEXO 4 – Dados da submissão do primeiro artigo apresentado nesta dissertação para o periódico Journal of Dentistry.



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Page: 1 of 1 (1 total submissions) Display 10 results per page.

ANEXO 5 – Registro do Estudo Clínico Radomizado, artigo 2.

ANEXO 6 – Dados da submissão do segundo artigo apresentado nesta dissertação para o periódico Clinical Oral Investigations.

Clinical Oral Investigations

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