UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO
MESTRADO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO CLÍNICA ODONTOLÓGICA – MATERIAIS DENTÁRIOS

DENTINA AFETADA POR CÁRIE: AVALIAÇÃO E DESENVOLVIMENTO DE ADESIVO EXPERIMENTAL

PRISCILA RAQUEL SCHIROKY

PORTO ALEGRE, 2016

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Dissertação apresentada como requisito obrigatório para obtenção do título de Mestre em Odontologia na área de concentração Clínica Odontológica.

FABRÍCIO MEZZOMO COLLARES
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“Agir, eis a inteligência verdadeira. Serei o que quiser. Mas tenho que querer o que for. O êxito está em ter êxito, e não em ter condições de êxito. Condições de palácio tem qualquer terra larga, mas onde estará o palácio se o não fizerem ali?”

Fernando Pessoa

RESUMO

A Odontologia minimamente invasiva tornou-se possível em decorrência do atual avanço alcançado na Odontologia adesiva, e a remoção seletiva do tecido cariado passou a ser recomendada para dentes com lesões de cárie profunda. Tal preparo cavitário preserva a dentina afetada por cárie (DAC), camada mais interna da dentina cariada que é remineralizável. Entretanto, esse substrato apresenta propriedades mecânicas inferiores e diversos estudos relatam uma resistência de união reduzida. O objetivo do presente estudo foi revisar sistematicamente a literatura quanto a resistência da união dos sistemas adesivos à DAC quando comparada à dentina hígida (DH), assim como a influência das variáveis metodológicas dos estudos *in vitro*. Além disso, desenvolver e caracterizar uma resina adesiva com a incorporação de um fosfato de cálcio, assim como avaliar o potencial de induzir deposição mineral em dentes com DAC. As buscas foram realizadas nas bases de dados PubMed, Scopus, Web of Science e LILACS. Foram incluídos estudos laboratoriais que investigaram a resistência de união à microtração imediata e longitudinal de sistemas adesivos À DAC natural ou artificial, utilizando DH como controle. Oito artigos preencheram os critérios de inclusão, e uma meta-regressão foi realizada para a associação das variáveis independentes do modelo com o desfecho de resistência de união. O modelo final com 5 variáveis explicou 75,6% da variabilidade entre os grupos. Todas as variáveis independentes apresentaram influência significativa no desfecho, e a DAC resultou em menor resistência de união. Sendo assim, uma resina adesiva com a incorporação de α-fosfato tricálcico (αTCP) foi desenvolvida e caracterizada. Para a formulação das resinas adesivas, 66,66% em peso de BisGMA, 33,33%

em peso de HEMA e um sistema fotoiniciador foram misturados. O αTCP foi adicionado a 2% em peso, originando a resina adesiva teste. Uma resina sem adição de carga foi utilizada como controle. As resinas adesivas foram caracterizadas quanto à taxa de polimerização e grau de conversão, e degradação em solvente. Sessenta dentes permanentes extraídos com lesões de cárie profunda foram submetidos à remoção seletiva do tecido cariado e restaurados utilizando uma das duas resinas adesivas. As amostras foram avaliadas por meio de ensaios imediatos e longitudinais (no decorrer de 6 meses, armazenadas em SBF) de resistência de união à microtração, avaliação da deposição mineral por espectroscopia Raman e dureza Knoop. A dureza Knoop inicial da resina adesiva teste foi maior quando comparada à controle, e não houve diferença entre os grupos em relação ao percentual de variação da dureza Knoop após a imersão em solvente. O grupo teste apresentou maior taxa de polimerização e não houve diferença entres os grupos para o grau de conversão. Não houve diferença entre os grupos para a resistência de união imediata e longitudinal, e ambos os grupos mantiveram estável a resistência de união após 6 meses. A análise da interface em relação à deposição mineral mostrou que houve aumento do conteúdo mineral para os dois grupos. Em relação à dureza Knoop, houve um aumento nos valores após 3 meses, entretanto não houve diferença entre os grupos dentro das diferentes profundidades para cada tempo. Conclui-se que não houve diferença entre os grupos em relação à deposição mineral.

Palavras-chave: adesivos dentinários, cárie dentária, remineralização dentária.

ABSTRACT

The minimally invasive dentistry became possible due to the current advancement in adhesive dentistry, and the selective removal of carious tissue has been recommended for teeth with deep carious lesions. Such cavity preparation preserves the caries-affected dentin (CAD), the inner layer of carious dentin that is remineralizable. However, this substrate presents low mechanical properties and many studies reported reduced bond strength. The objective of this study was to systematically review the literature on the microtensile bond strength (μTBS) of adhesive systems to (CAD) when compared to sound dentin (SD), as well as on the influence of methodological variables of *in vitro* studies. Also, to develop and characterize an adhesive resin with the incorporation of a calcium phosphate, as well as evaluate the potential to induce mineral deposition in teeth presenting CAD. The electronic databases PubMed, Scopus, ISI Web of Science and LILACS were systematically searched. Were included in vitro studies that assessed the immediate and aged microtensile bond strength of adhesive systems to natural or artificially created CAD in comparison to SD. Eight studies met the inclusion criteria and were included in a linear meta-regression of microtensile bond strength. The fully adjusted model with 5 methodological variables explained 75.6% of the variability among groups. All methodological variables were associated with microtensile bond strength, and bond strength to CAD was lower than to SD. Thus, an adhesive resin with the incorporation of α-tricalcium phosphate (αTCP) was developed and characterized. To formulate the experimental adhesive resins, 66.66 wt% BisGMA, 33.33 wt% HEMA and a photo-initiator system were mixed. The αTCP at 2 wt% was added, yielding the test adhesive resin. An adhesive resin without filler was used as control. The adhesive resins were characterized regarding polymerization kinetics and degree of conversion, and softening in ethanol. Sixty extracted human posterior permanent teeth with deep carious lesions were submitted to selective removal of carious tissue and restored using one of the two adhesive resins. Samples were evaluated by immediate and longitudinal (6 months, storage in SBF) following tests: microtensile bond strength, mineral deposition by Raman spectroscopy and Knoop hardness. The test adhesive resin showed higher initial Knoop hardness than the control one, and there was no difference between the groups for the percentage of variation of Knoop hardness after solvent immersion. The test group showed increased polymerization rate, and there was no difference between groups for degree of conversion. There was no difference between the groups for immediate and longitudinal microtensile bond strength, and the microtensile bond strength of both groups remained stable after the 6 months. The interface analysis regarding mineral deposition showed an increasing in mineral content for both groups. For Knoop hardness, there was an increase after 3 months, however the groups where not significantly different considering depth and time. It could be concluded that there was no difference between control and test adhesive resin for mineral deposition.

Key-words: dentin-bonding agents, dental caries, tooth remineralization.

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1. Introdução

A biomineralização que ocorre nas fibras colágenas dos tecidos dentais duros é o processo pelo qual fosfato de cálcio inorgânico impregna a matriz orgânica sob o controle de proteínas não colagenolíticas especializadas presentes nessa matriz (Padovano *et al.*, 2015). Com o intuito de reproduzir esse processo, alguns métodos de remineralização biomimética têm sido investigados com a finalidade de estabilizar a camada híbrida (Liu *et al.*, 2011; Cao *et al.*, 2015). O condicionamento ácido utilizando ácido fosfórico a 37%, primeiro passo do procedimento adesivo, desmineraliza completamente a dentina a uma profundidade de 5-8 µm, e a incompleta infiltração da resina adesiva resultam na permanência de fibras colágenas expostas na base da camada híbrida em regiões ricas em água (Pinna *et al.*, 2015). Sendo assim, a ocorrência de deposição mineral ao longo dessas fibras colágenas desprotegidas evitaria a degradação hidrolítica (Tjaderhane, 2015).

Diversos estudos passaram a testar o uso de análogos biomiméticos das proteínas não colagenolíticas presentes na matriz dentinária, como a DMP1 (*dentin matrix protein 1*), na remineralização biomimética das fibras colágenas (Tay e Pashley, 2008; 2009). Os análogos biomiméticos consistem em compostos que desempenham duas funções: (1) produzem nanoprecursores de fosfato de cálcio amorfo que são pequenos o suficiente e estão estabilizados para que penetrem na matriz colágena desmineralizada antes de serem transformados em nanocristais de apatita, (2) simulam os sítios de ligação dos cristais ao colágeno, guiando a ancoragem dos nanoprecursores à matriz colágena, tanto ao longo das microfibrilas (remineralização intrafibrilar), quanto na superfície das fibras colágenas (remineralização interfibrilar) (Tay e

Pashley, 2008). Com a utilização desses análogos, na presença de um material restaurador que libere íons cálcio e fosfato, é sugerido que uma remineralização intrafibrilar da matriz colágena pode ser alcançada (Abuna *et al.*, 2016). A recuperação do conteúdo mineral desse compartimento da dentina tem sido sugerida como essencial para a recuperação do módulo de elasticidade e da dureza da dentina cariada (Kinney *et al.*, 2003; Balooch *et al.*, 2008). Sendo assim, muitos estudos avaliaram a utilização dos análogos biomiméticos para provar um conceito, utilizando amostras de dentina completamente desmineralizadas ou fibras colágenas reconstituídas (Abuna *et al.*, 2016; Luo *et al.*, 2016), com o intuito de aumentar a longevidade da interface adesiva e melhorar a resistência de união. Entretanto, um estudo recente mostrou que a utilização de um adesivo dentinário que libera íons cálcio e fosfato manteve estável a resistência de união à dentina hígida após 6 meses, independente do uso dos análogos biomiméticos (Abuna *et al.*, 2016).

Com o atual panorama da Odontologia minimamente invasiva, a remoção seletiva do tecido cariado tem sido recomendada para dentes com lesões de cárie profunda, e suas bases foram recentemente estabelecidas (Schwendicke *et al.*, 2016). A remoção seletiva do tecido cariado tem como objetivo remover a dentina infectada por cárie, deixando o máximo de dentina afetada por cárie para a remineralização terapêutica, permitindo uma maior conservação dos tecidos dentários e diminuindo o risco de exposição pulpar (Schwendicke *et al.*, 2016). Com essa abordagem, há uma redução de 77% da incidência de exposição pulpar quando comparado à remoção não seletiva do tecido cariado, em dentes decíduos e permanentes, e não há diferença entre as duas abordagens quanto a presença de sinais e sintomas pós-operatórios indicativos de comprometimento pulpar (Ricketts *et al.*, 2013). O subsequente selamento por meio da restauração das cavidades, com margens em tecido sadio para um melhor desempenho do sistema adesivo e manutenção do selamento (De Almeida Neves *et al.*, 2011) resulta em uma redução gradual da atividade bacteriana e consequentemente da progressão da lesão de cárie (Paddick *et al.*, 2005; Ricketts *et al.*, 2013). Isso ocorre especialmente devido à redução do número de bactérias viáveis (Maltz *et al.*, 2002; Lula *et al.*, 2009; Maltz *et al.*, 2012) e à sobrevivência de uma microbiota bacteriana menos complexa e menos cariogênica, pois as mesmas ficam isoladas da fonte de nutrição essencial para sua manutenção e proliferação (Paddick *et al.*, 2005). Além do enfoque microbiológico, estudos longitudinais já mostraram clínica e radiograficamente que o adequado selamento controla as lesões de cárie em dentes permanentes (Maltz *et al.*, 2007; Orhan *et al.*, 2010).

A dentina afetada por cárie apresenta-se apenas parcialmente desmineralizada, com fibras colágenas que mantiveram sua estrutura natural, sendo passível de ser remineralizada (Fusayama, 1979; Pugach *et al.*, 2009). Sendo assim, surgiu a necessidade de promover também a remineralização desse substrato (Toledano *et al.*, 2015). Os cristais minerais intrafibrilares remanescentes na dentina parcialmente desmineralizada podem atuar como sítios para a nucleação e o crescimento dos cristais de apatita, promovendo a remineralização e, consequentemente, o restabelecimento das propriedades mecânicas e da resistência de união (Bertassoni *et al.*, 2011). Considerando isso, a utilização de um sistema adesivo que contenha um composto fonte de íons cálcio e fosfato, à exemplo dos utilizados na remineralização da camada híbrida, poderia desempenhar igual papel na remineralização da dentina afetada por cárie, melhorando a capacidade de recuperação desse substrato, e sem a necessidade da utilização dos análogos biomiméticos devido a presença de cristais de hidroxiapatita remanescentes.

2. Objetivo

O objetivo desse estudo foi realizar uma revisão sistemática da literatura para avaliar a resistência de união de sistemas adesivos à dentina afetada por cárie quando comparada à dentina hígida, e desenvolver e caracterizar uma resina adesiva com a incorporação de α-fosfato tricálcico, bem como avaliar o seu potencial de estimular deposição mineral em dentes com dentina afetada por cárie.

3. Artigo I

Bond strength of adhesive systems to caries-affected dentin and the influence of methodological variables: a meta-regression analysis

A meta-regression on bonding to caries-affected dentin

Priscila Raquel Schiroky[a]
Vicente Castelo Branco Leitune[a]
Roger Keller Celeste[b]
Susana Maria Werner Samuel[a]
Fabrício Mezzomo Collares[a]

[1] Conservative Dentistry Department, Dental Materials Laboratory, School of Dentistry, Federal University of Rio Grande do Sul, R. Ramiro Barcelos, 2492, Porto Alegre, Brazil
[2] Preventive and Social Dentistry Department, School of Dentistry, Federal University of Rio Grande do Sul, R. Ramiro Barcelos, 2492, Porto Alegre, Brazil

Corresponding author:

Fabrício Mezzomo Collares

Dental Materials Laboratory, School of Dentistry

Universidade Federal do Rio Grande do Sul

Rua Ramiro Barcelos, 2492 - Rio Branco

90035-003 - Porto Alegre, RS, Brazil

Phone: + 55 51 3308 5198

E-mail: fabricio.collares@ufrgs.br

Key words: Adhesive systems. Caries-affected dentin. Management of dental caries. Meta-regression. Microtensile bond strength. Minimally invasive dentistry.

Abstract

Objectives: To systematically review the literature on the microtensile bond strength (μTBS) of adhesive systems to caries-affected dentin (CAD) when compared to sound dentin (SD), as well as on the influence of methodological variables of *in vitro* studies.

Data: The following search strategy was used: ("dental caries" OR "caries affected dentin" OR "carious affected dentin") AND "bond strength". Were included *in vitro* studies that assessed the immediate and aged μTBS of adhesive systems to natural or artificially created caries-affected dentin, compared to sound dentin.

Sources: PubMed, Scopus, ISI Web of Science and LILACS databases were systematically searched.

Study selection: Eight studies met the inclusion criteria and were included in a linear meta-regression of μTBS. Extraction data resulted in the establishment of five methodological variables: substrate condition (SD, natural CAD or artificial CAD), tooth type (permanent or deciduous), aging method, type of adhesive and time of acid conditioning (time of acid etching/acid primer application), adhesive area (mm^2) and test machine crosshead speed (mm/min). The fully adjusted model explained 75.6% of the variability among groups. The bond strength to CAD is lower when compared to SD. Artificial CAD produces lower bond strength than natural CAD. All methodological variables were associated with microtensile bond strength.

Conclusions: CAD promotes lower bond strength than SD. The use of artificial lesions can underestimate results. All the evaluated variables play an important role in microtensile bond strength.

Clinical significance: Research efforts should endeavor to develop reliable materials and strategies to remineralize the residual carious lesions.

1. Introduction

The best current scientific evidence on managing deep carious lesions recommends a selective removal of carious tissue in order to preserve healthy and remineralizable tissues, and maintain pulp vitality [1,2]. In performing carious tissue removal, large areas of the cavity substrate will consist of caries-affected dentin (CAD), making it a clinically relevant substrate for adhesive procedures [3]. Bonding to CAD represents a challenge and is compromised due to chemical and morphological changes of this substrate as a consequence of caries progression [3,4]. This dentin exhibits reduced mineral content and increased permeability, changes in the collagen structure and increased wetness [4,5]. As consequences, the CAD presents lower mechanical properties and leads to a formation of a hybrid layer much thicker and incompletely resin infiltrated [6]. Bond strength to caries-affected dentin has been found to be lower when compared to sound dentin (SD) [7,8]. However, there is conflicting results for some types of adhesive systems [9].

The *in vitro* studies accessing the bond strength to CAD are generally performed using artificial carious lesions [10-13]. Recently, a systematic review on the bonding of resin adhesives to CAD included only studies that used teeth with natural dental caries [14]. Therefore, a relevant number of *in vitro* studies using teeth with carious lesions artificially created, in order to overcome possible difficulties of using teeth with natural caries, have not been assessed and their inclusion needs a meta-analytical approach that involves all data available in current literature regarding the CAD. Additionally, methodological variations between different studies could affect the outcome of bond-strength tests [15], and make the comparison of reports difficult. The research question that led to this systematic review was: “Do the adhesive systems produce lower bond strength to caries-affected dentin when compared to sound dentin?” Thus, this systematic review of literature aimed to evaluate the bond strength to natural and artificial caries-affected dentin when compared to sound dentin, as well as the association methodological variables.

2. Materials and methods

This study was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [16].

2.1 Search strategy

A systematic literature search was carried out in PubMed, Scopus, ISI Web of Science and LILACS databases, using the search strategies shown in Table 1, to identify the relevant studies included. The search was limited by language (English, Portuguese, and Spanish). The last search was performed in May 2016.

2.2 Eligibility criteria

Laboratory studies assessing the immediate and aged microtensile bond strength of adhesive systems to natural or artificially created caries-affected dentin were included. Two reviewers, PRS and VCBL, independently screened the titles and abstracts of all the identified articles. Articles considered eligible were obtained for a full-text assessment. Studies that did not include a control group with bonding to sound dentin substrate were excluded.

2.3 Data extraction

The data were extracted independently by the two aforementioned reviewers. Data on sample size, substrate condition (sound dentin, natural caries-affected dentin or artificial-caries affected dentin), tooth type (permanent or deciduous), aging method, type of adhesive, time of acid conditioning (time of acid etching/acid primer application), adhesive area (mm^2), test machine crosshead speed (mm/min), bond strength (MPa) and standard deviation were obtained. Discrepancies in the data extraction were solved by consensus. Corresponding authors were requested up to three times for additional data when necessary. If no answer was received, the study was excluded from analysis.

2.4 Risk of bias assessment

To assess the individual risk of bias of each study, the reporting of 8 methodological items in the studies were analyzed: (1) teeth storage medium after extraction, (2) teeth randomization, (3) caries-affected dentin removal criteria, (4) using materials according to manufacturer’s instructions, (5) storage medium after bonding, (6) time of tooth storage before debonding, (7) crosshead speed of the testing machine, and (8) failure mode determination. Item 3 was only considered for studies that employed teeth with natural carious lesion, because the artificial carious lesions do not undergo selective removal of carious tissue. The studies were classified as high risk of bias, when failed to report 6 items or more, moderate risk of bias, when failed to report 3 to 5 items, or low risk of bias, when failed to report 2 items or less. Studies classified as high risk of bias would be excluded from the analysis.

2.5 Summary measure and Statistical analysis

The summary measure was the mean microtensile bond strength (μTBS) in MPa. Descriptive analyses are presented in Table 2. To avoid collinearity of different characteristics, variables were combined in order to create unique mutually exclusive categories (time of acidic conditioning/type of adhesive, adhesive area/crosshead speed).

Random linear meta-analysis using DerSimonian-Laird random-effects was used as a preliminary step to assess heterogeneity. Then, data were analyzed in linear meta-regression with restricted maximum likelihood estimator (REML). To test if group-level variables were significantly related to mean-group μTBS, a backward stepwise multiple meta-regression was run, removing variables with $p>0.25$. Due to small number of included studies, the p-values were estimated from Monte-Carlo non-parametric method (with 1000 permutations) adjusted for multiple testing [17]. Diagnostic procedures were performed to check for outliers, leverage and normality of residuals. To estimate explained variance (R^2), it was calculated the heterogeneity parameter (tau2) using empirical Bayes estimator that denotes the standard deviation of study-level variance. All analyses were performed in Stata 13.1 (Stata; College Station, TX, USA).

3. Results

The search strategy identified 616 potentially relevant articles. After screening the titles and abstracts, 559 articles were excluded and 57 full text articles were evaluated for eligibility criteria. The language limitation excluded 4 articles. A total number of 10 articles met the eligibility criteria. Of them, 2 were excluded because the corresponding author did not reply the 3 contacts for additional relevant data. The entire progress of studies selection and the reasons for exclusions are presented in Figure 1. Eight articles were included, and the descriptive characteristics are shown in Table 2. One study performed distilled water aging using teeth halves instead of sticks/beams [13], therefore the longitudinal groups were excluded from the meta-regression analysis. The risk of bias analysis is presented in Table 3, and all studies were classified as low or moderate risk of bias.

The lowest mean for the bond strength in a group was 5.83 MPa, and the highest value was 44.2 MPa. Based on the pooled data and due to a high heterogeneity among studies (I^2 = 97.6%), a meta-regression analysis was conducted. The results from included studies were based on the means and standard errors of 74 groups from 586 specimens, and are presented in Table 4. Five methodological variables were defined after data extraction: substrate condition, tooth type, aging method, type of adhesive/time of acid conditioning, and adhesive area/teste machine crosshead speed.

All methodological variables of the studies were associated with the outcome. The results of the meta-regression analysis showed that the bond strength to caries-affected dentin is lower when compared to sound dentin ($p < 0.01$). Moreover, the artificial caries-affected dentin produces lower bond strength than natural caries-affected dentin ($p < 0.01$). The use of deciduous teeth represents a bond strength 10.69 MPa lower than permanent teeth ($p = 0.02$). The aging method used, as well as the time of conditioning and type of adhesive, significantly influences the bond strength ($p < 0.01$). Aging by thermocycling combined to load cycling resulted in the lowest bond strength. The two-step etch-and-rinse adhesive with an etching time of 7 seconds presented the highest bond strength, which is 17.06 MPa higher than that of the three-step etch-and-rinse adhesive with an etching time of 15 seconds ($p < 0.01$). An adhesive area 0f 0.8 mm^2 and a test machine crosshead speed of 1 mm/min presented a bon strength 11.12 MPa higher than the parameters of 1 mm^2 and 0.5 mm/min ($p = 0.01$).

The fully adjusted model explained 75.6% of the variability among groups. Analysis of standardized residuals showed that they were normally distributed (Shapiro-Wilk test, $p = 0.06$) with zero mean and no leverage, but with one group as outlier [13].

4. Discussion

Bonding to caries-affected dentin has been the focus of researchers since complete caries removal (nonselective removal to hard dentine) is no longer recommended for deep carious lesion [2]. Conservative management of carious lesions aims to preserve the tooth structure, maximizing the restoration longevity [2]. Considering it, many *in vitro* studies were conducted to evaluate the microtensile bond strength of adhesive system to caries-affected dentin (CAD) when compared to sound dentin (SD). From the eight included studies, seven presented low risk of bias, and only one study presented moderate risk of bias. Thus, no study was excluded after the risk of bias assessment. Based on the present systematic review, caries-affected dentin substrate produced lower bond strength than sound dentin substrate. All the five variables (substrate condition, tooth type, aging method, type of adhesive/time of acid conditioning, and adhesive area/test machine crosshead speed (mm/min)) presented association, significantly influencing the bond strength.

Studies that employed in vitro models to induce caries-like lesions in human teeth were included. Two methods of caries induction were employed by the included studies: pH-cycling model and microbiological model. The findings showed that the use of artificial caries-affected dentin reduced the microtensile bond strength when compared to natural caries-affected dentin. When compared to natural carious dentin, the caries induced by pH-cycling model present similar Knoop hardness until depth of 40 µm, while that for lesions created by microbiological model the Knoop hardness could not be measured until 50 µm duo to the excessive demineralization, and the values kept lower than the natural carious dentin from 100 to 500 µm [18]. In all included studies the natural lesions where submitted to selective removal of carious tissue before the bonding procedure, while the artificial ones were not. Therefore, as substrates have difference in hardness, this can explain differences in bonding strenght.

A modified dentin is created when affected by carious lesions, reflecting alterations in its fundamental components and arrangement of this tissue [19]. Considering that primary and permanent sound dentin exhibits variations regarding morphological structure and chemical composition [20], they will behave differently when undergoing carious process. Primary sound dentin exhibits higher dentin tubule density than permanent dentin from superficial to deeper dentin [20]. The greater tubule density represents a reduced area of intertubular dentin available to constitute the hybrid layer [19]. Additionally, the tubule diameter increases as the dentin depth increases, for both primary and permanent teeth, also reducing the superficial area for bonding [19,20]. It has been shown that thicker hybrid layers are formed in primary dentin when compared with permanent teeth [21]. Primary teeth exhibit a reduced buffering capacity as a consequence of its lower mineral content, which probably increase its susceptibility to acidic conditioners [22]. All these aspects may contribute to the lower bond strength of primary caries-affected dentin when compared to permanent caries-affected dentin.

Whereas the immediate adhesion effectiveness of most current adhesive systems is quite satisfactory, their long-term bond durability and resistance to degradation can be limited [23]. Various *in vitro* aging methods have been proposed to predict long-term interface degradation [24,25]. Therefore, the present systematic review only included studies that used *in vitro* aging models, in addition to assessment of immediate bond strength. Long-term water storage is the most common method used [25,26]. All the included studies that conducted distilled water storage employed a storage time of 6 or 12 months. After 6 and 12 months of water storage, the bond strength reduced when compare to 24 h of water storage. This longitudinal degradation is in accordance with the findings in current available literature [15,25,27]. Thermocycling is another aging method widely used [15,25]. Included studies used regimens of 3,000 and 5,000 cycles in water between 5 and 55°C, the last being associated with mechanical loading. Both regimens reduced, but not statistically significantly, the bond strength when compared to 24 h water storage. This result is in agreement with a previous systematic review that evaluated the parameters involved in dentin bonding, and could be explained by the low number of cycles employed by the studies [15]. Considering that 10,000 cycles might represent 12 months of intraoral functioning [28], 3,000 cycles seems to be not sufficient to produce an aging effect reliable to predict the long-term degradation. Additionally, in the study that used 3,000 cycles, flat dentinal surfaces were restored instead of cavities, leading to a lower C-factor (about 1/6). Since the thermal contraction/expansion of composite resins is high as compared to that of tooth tissue, repetitive stresses are induced at the composite-dentin interface [28]. Combined with chemical degradation, these are the two proposed paths in what thermocycling aging works [25]. Thus, the conformation of the restoration may lead to similar results before and after thermocycling [29]. The decreasing in bond strength was greater when thermocycling was associated to cyclic loading, due to the increased effect of combined treatments. Regarding the cyclic loading method, different protocols (number of cycles, load and frequency) have been applied, and the number of cycles is the most variable parameter [24]. It has been shown that just when thermocycling and mechanical loading are performed concomitantly a significant decrease in microtensile bond strength is achieved [30]. As well as thermocycling, the cyclic loading regimens used by the included studies were not sufficient to simulate the long-term challenging of bond longevity. The pH cycling method showed a similar degradation, when compared to immersion in distilled water or 24 h, that immersion in distilled water during 6 months. Therefore, in predicting long-term bonding durability, the synergistic interaction of thermal and mechanical cycling, as well as the water aging for 12 months better challenged the bond integrity and may disclose more valuable clinical information because of the complexity of intraoral conditions.

In order to overcome the incomplete penetration of adhesive resin in the thicker hybrid layer formed at this more reactive substrate, some studies have proposed a different protocol for conditioning primary dentin [11,21]. One study evaluated the bond strength after a short etching time [31], and the two categories that presented the higher bond strengths (2-step etch-and-rinse 7 seconds and 2-step self-etching 10 s) are only composed of groups from this study. The 2-step self-etching adhesive with 10 seconds of primer application showed the second higher bond strength value. This category of type of adhesive is composed by a single mild self-etch adhesive (Clearfil SE Bond, Kuraray). The 2-step self-etching adhesives have shown clinical effectiveness, evaluated by the retention rate of non-carious cervical restorations, similar to the “gold-standard” 3-step etch-and-rinse adhesives [23]. Also, a previous systematic review reported that the microtensile bond strength of self-etch and etch-and-rinse after 12 months of water storage had no statistically significant difference [32].

5. Conclusions

It could be concluded that the caries-affected dentin promotes lower bond strength than sound dentin. Artificial CAD results in even lower values when compared to natural CAD. Hence, the use of artificial lesions can underestimate results. All the evaluated variables (substrate condition, tooth type, aging method, type of adhesive/time of acid conditioning, and adhesive area/test machine crosshead speed) play an important role in bond strength outcome. Considering that the main goal of the conservative management of carious lesions is to enhance teeth lifetime, creating conditions for long-lasting success restorations [2,33], the compromised bond strength to CAD could represent a challenge for longevity. Research efforts should endeavor to develop reliable materials and strategies to remineralize the residual carious lesions, thus it could be possible to guarantee durability.

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Tables

Table 1 Systematic review search strategy for electronic databases

PubMed, Scopus, ISI Web of Science and LILACS	
#1	“dental caries”
#2	“caries-affected dentin”
#3	“carious affected dentin”
#4	“bond strength”
#5	(#1) OR (#2) OR (#3)
#6	(#5) AND (#4)

Table 2 Descriptive data of each study included in meta-regression analysis

Article	n	Substrate condition*	Tooth type	Aging method**	Type of adhesive***	Time of acid etching/acid primer application (seconds)	Adhesive area (mm^2) and test machine crosshead speed (mm/min)
Lenzi (2014) [31]	6	SD and ACAD	Deciduous	DW 24 h / DW 12 m	2ER/2SE	15 or 7/20 or 10	0.8 and 1
Aggarwal (2011) [7]	10	SD and NCAD	Permanent	SS <24 h / Termocycling (5,000 cycles, 5 ± 2°C to 55 ± 2°C, 30 s) and cyclic loading (150,000 cycles, 60 N, 5 to 7 Hz)	2ER/1SE	15/15	1 and 0.5
Marquezan (2010) [12]	5	SD and ACAD	Deciduous	DW 24h / Cyclic loading (50,000 cycles, 90 N, 3 Hz) / pH-cycling	2ER	15	1 and 0.5
Erhardt (2008) [8]	12	SD and NCAD	Permanent	DW 24 h / DW 6 m	2ER/2SE/2SE	15/20/30	1 and 0.5
Omar (2007) [34]	10	SD and NCAD	Permanent	DW 24 h / Termocycling (3,000 cycles, 5°C to 55°C, 30 s)	3ER/2SE/1SE	15/20/20	1 and 0.5
Lenzi (2015) [10]	6	SD and ACAD	Deciduous	DW 24 h / DW 12 m	2ER/2SE	15/20	0.8 and 1
Lenzi (2014) [11]	5	SD and ACAD	Deciduous	DW 24 h / DW 6 m	2ER	15	0.8 and 1

Tedesco (2014) [13]	6	SD and ACAD	Deciduous	DW 24 h / DW 24 m	2ER/2SE	15/20	0.8 and 1

*Substrate condition: SD, sound dentin; ACAD, artificial caries-affected dentin; NCAD, natural caries-affected dentin.

**Aging method: DW, distilled water; SS, saline solution.

***Type of adhesive: 3ER, 3-step etch-and-rinse; 2ER, 2-step etch-and-rinse; 2SE, 2-step self-etching; 1SE, 1-step self-etching.

Table 3 Risk of bias assessment

Article	Teeth storage medium after extraction	Teeth randomization	Caries-affected dentin removal criteria*	Using materials according to manufacturer's instructions	Storage medium after bonding	Time of tooth storage before debonding	Crosshead speed of the testing machine	Failure mode determination	Classification
Lenzi (2014) [31]	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	LOW
Aggarwal (2010) [7]	Yes	No	Yes	Yes	No**	No**	Yes	No	MODERATE
Marquezan (2010) [12]	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	LOW
Erhardt (2008) [8]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Omar (2007) [34]	Yes	Yes	Yes	Yes	Yes	Yes	No**	No	LOW
Lenzi (2015) [10]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Lenzi (2014) [11]	Yes	Yes	-	No	Yes	Yes	Yes	Yes	LOW
Tedesco (2014) [13]	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	LOW

***** This item was only considered for studies that employed teeth with natural carious lesion, because the artificial carious lesions do not undergo selective removal of carious tissue.

****** For meta-regression analysis, data were obtained by contacting the authors.

Table 4 Estimates of mean microtensile bond strength (µTBS) for six variables from multiple linear meta-regression among 74 groups of 8 studies

Variable	Variable categories	Regression coefficient	Confidence interval 95%		p-value
Substrate condition	Sound dentin	Referent			<0.01
	Natural caries-affected dentin	-5.88	-9.51	-2.26	
	Artificial caries-affected dentin	-16.64	-19.94	-13.34	
Tooth type	Permanent	Referent			0.02
	Deciduous	-10.69	-20.30	-1.08	
Aging method	Saline solution storage < 24 h	Referent			<0.01
	Water storage 24 h	8.70	1.23	16.16	
	Water storage 6 months	5.29	-2.33	12.90	
	Water storage 12 months	-0.20	-8.49	8.09	
	Thermocycling (3,000 cycles, 5-55°C, 30 s)	1.86	-6.87	10.60	
	Cyclic loading (150,000 cycles, 60 N, 5-7 Hz)	9.21	-4.30	22.71	
	Thermocycling (5,000 cycles, 5-55°C, 30 s) and cyclic loading (50,000 cycles, 90 N, 3 Hz)	-6.50	-13.48	0.49	
	pH cycling	5.20	-8.15	18.55	
Type of adhesive and time of acid conditioning	3-step etch-and-rinse 15 s	Referent			<0.01
	2-step etch-and-rinse 15 s	11.82	4.96	18.68	
	2-step etch-and-rinse 7 s	17.06	8.61	25.51	
	2-step self-etching 30 s	4.21	-3.78	12.20	
	2-step self-etching 20 s	10.34	4.02	16.66	
	2-step self-etching 10 s	16.72	8.23	25.21	
	1-step self-etching 20 s	-0.60	-7.91	6.72	
	1-step self-etching 15 s	8.10	-1.69	17.89	
Crosshead speed and adhesive area	0.5 mm/min and 1 mm^2	Referent			0.01
	1 mm/min and 0.8 mm^2	11.12	2.47	19.78	

Figures

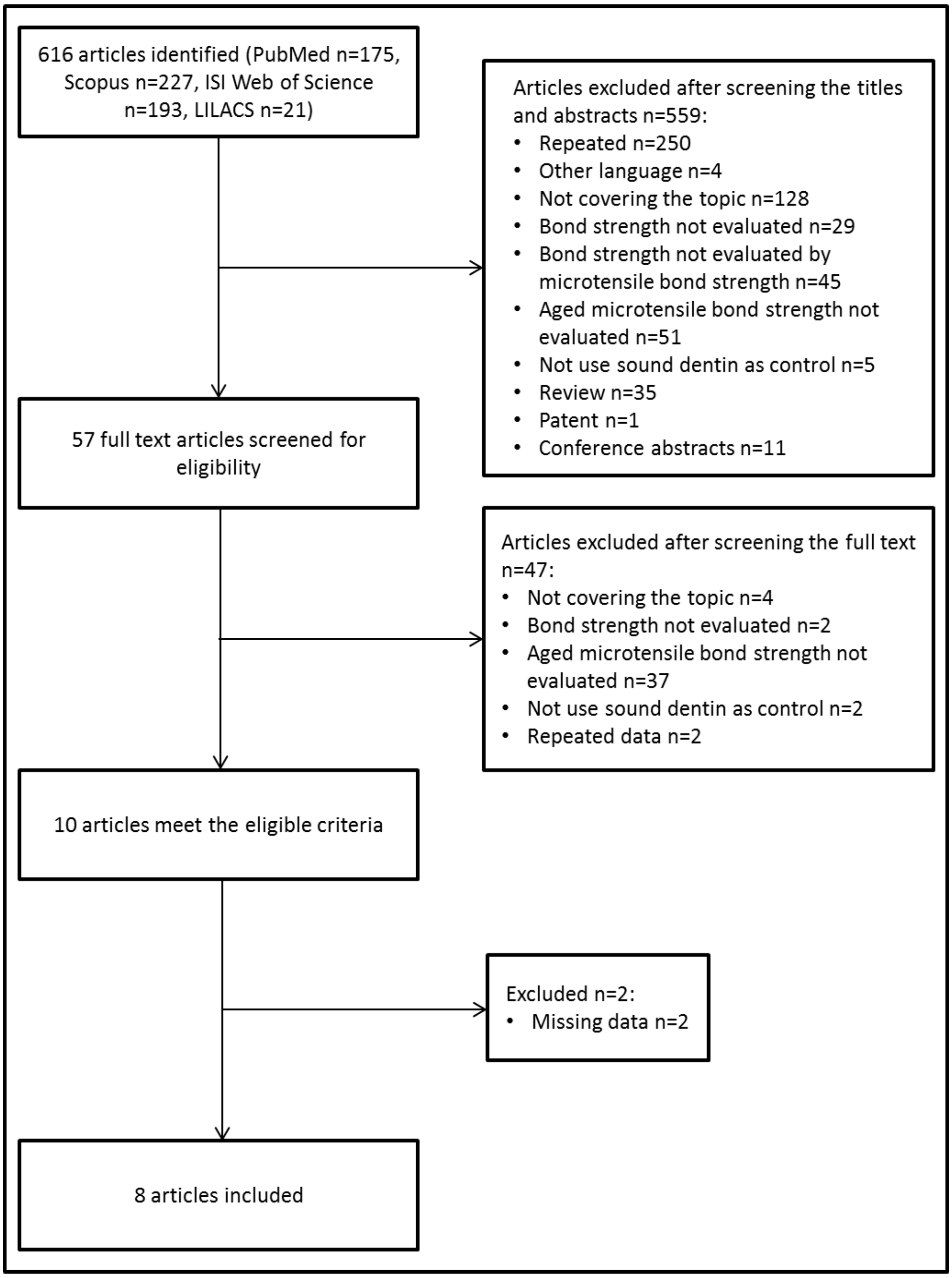

Fig. 1 Flow chart of articles selection.

4. Artigo II

Influence of an adhesive resin with α-tricalcium phosphate on the remineralization of caries-affected dentin

Priscila Raquel Schiroky [1]

Vicente Castelo Branco Leitune [1]

Susana Maria Werner Samuel [1]

Fabrício Mezzomo Collares [1]

[1] Conservative Dentistry Department, Dental Materials Laboratory, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Corresponding author:

Fabrício Mezzomo Collares

Dental Materials Laboratory, School of Dentistry

Universidade Federal do Rio Grande do Sul

Rua Ramiro Barcelos, 2492 - Rio Branco

90035-003 - Porto Alegre, RS, Brazil

Phone: + 55 51 3308 5198

E-mail: fabricio.collares@ufrgs.br

Abstract

Objectives Develop and characterize an adhesive resin with the incorporation of α-tricalcium phosphate (αTCP), as well as evaluate the potential to induce mineral deposition in caries-affected dentin (CAD).

Materials and methods To formulate the experimental adhesive resins, 66.66 wt% BisGMA, 33.33 wt% HEMA and a photo-initiator system were mixed. The αTCP was added at 2 wt%, yielding the test adhesive resin. Adhesive resins were characterized by softening in ethanol, polymerization kinetics and degree of conversion. Sixty extracted human posterior permanent teeth with deep carious lesions were submitted to selective removal of carious tissue and restored using one of the two adhesive resins. Samples were evaluated by immediate and longitudinal (6 months, storage in SBF) following tests: microtensile bond strength, Knoop hardness, and mineral deposition by Raman spectroscopy.

Results The test adhesive resin showed higher initial Knoop hardness than the control, and there was no difference between the groups for the percentage of variation of Knoop hardness after solvent immersion, as well as for degree of conversion. Polymerization rate of test group was higher than control. There was no difference between the groups for immediate and longitudinal microtensile bond strength, and it remained stable after the 6 months. The analysis for mineral deposition showed an increasing in mineral content in both groups. For Knoop hardness, the groups where not significantly different considering depth and time.

Conclusions There was no difference between control and test adhesive resin for mineral deposition.

Clinical relevance Different methods to remineralize the CAD, improving the bond strength and further restoring the mechanical properties, should be investigated.

Key words: Calcium phosphates. Caries-affected dentin. Dentin remineralization. Mineral deposition.

Introduction

The actual consensus on management of permanent teeth with deep carious lesions and pulp vitality recommends a selective removal of carious tissue [1]. Adopting this strategy, the bonding substrate will consist mostly of caries-affected dentin (CAD) [2]. When compared to sound dentin (SD), CAD has a reduced mineral content, greater porosity and greater water content [3], which lead to reduced mechanical properties and the formation of a hybrid layer less stable, more prone to degradation [2], and presenting lower bond strength [4]. The dentin matrix of the CAD presents residual apatite crystallites along the collagen fibrils, since it is a partially demineralized dentin [5]. The presence of these remnant apatite seed crystallites can act as centers for homogeneous epitaxial nucleation, making it a tissue that can be remineralized [6]. The use of an adhesive resin that can release calcium and phosphate ions could induce intrafibrillar collagen remineralization on CAD [7]. By means of a remineralization of partially mineral-depleted dentin collagen within the CAD substrate, the bonding longevity of the resin-dentin interface could be enhanced and the mechanical properties restored [7, 8]. Thus, the objectives of this *in vitro* study were to develop and characterize an adhesive resin with the incorporation of α-tricalcium phosphate (αTCP), and evaluate the bonding performance and potential to induce mineral deposition in teeth submitted to selective removal of carious tissue, which in turn presents caries-affected dentin as bonding substrate.

Materials and methods

Experimental adhesive resins

The reagents bisphenol A glycol dimethacrylate (BisGMA), 2-hydroxyethyl methacrylate (HEMA), camphorquinone (CQ), ethyl 4-dimethylaminobenzoate (EDAB) and hydroxytoluene butylated (BHT) were used (Sigma-Aldrich, St. Louis, MO, USA). The experimental adhesive resins were formulated by mixing 66.66 wt% BisGMA and 33.33 wt% HEMA. CQ and EDAB were added, as the photo-initiator system, at 1 mol% according to the moles of monomer used; and BHT at 0.01 wt% as polymerization inhibitor. The test ion-releasing adhesive resin was obtained with the incorporation of the inorganic filler αTCP at 2 wt%, synthesized according to a previous study [9] and presenting a mean particle size of 6,03 µm [10]. An experimental adhesive resin without inorganic filler was used as control. All formulations were weighed with an analytical balance (AUW220D; Shimadzu, Kyoto, Japan), mixed and ultrasonicated (L100; Schuster, Santa Maria, RS, Brazil) to obtain homogenous solutions. To perform photoactivation, light source device used was Radii cal (1200 mW/cm^2, SDI, Bayswater, Victoria, Australia).

Softening in ethanol

To determine the resistance to degradation in solvent, 5 specimens per group (n=5) with 4 mm of diameter and 1 mm thick were used. The specimens were obtained with a silicon matrix that was covered with a polyester strip and photoactivated for 20 seconds in both sides. Specimens were than embedded in an acrylic resin and polished in a polisher (Model 3v, Arotec, Cotia, SP, Brazil) with silicon carbide sandpapers (1000 and 1200 granulation) and a felt disc saturated with alumina suspension (Alumina, 0.5 µm, Arotec, Cotia, SP, Brazil). After, the specimens were dried at 37 °C for 24 h. Five indentations, 100 µm apart from each other, were assessed (at 10 g for 5 s) in each specimen with a microhardness tester (HMV 2; Shimadzu, Tokyo, Japan) in order to obtain the initial Knoop hardness number (KHN1). The specimens were immersed in a solution of 50% ethanol (Labsynth, Diadema, SP, Brazil) and 50% distilled water for 1 h and washed with distilled water. Another five indentations were assessed to determine the final Knoop hardness number (KHN2). The percentage difference of KHN1 and KHN2 was also calculated (ΔKHN%).

Polymerization kinetics and degree of conversion

The polymerization kinetics and degree of conversion for each experimental adhesive resin were evaluated via Fourier-transform infrared spectroscopy with a spectrometer (Vertex 70, Bruker Optics, Ettlingen, Germany) coupled to a horizontal attenuated total reflectance (ATR) device consisting of a diamond crystal (Platinum ATR-QL, Bruker Optics, Ettlingen, Germany). A support device was used to affix the light-curing unit at a distance of 1 mm from the top of the sample. The samples (n = 3) were directly dispensed onto the ATR crystal and photoactivated for 30 s by a light-emitting diode Radii Cal (1200 mW/cm², SDI, Bayswater, Australia). One hundred scans were made at 10 kHz velocity and 4 cm^{-1} resolution, with an instrument aperture of 6 mm. Absorbance spectra were obtained using Opus software (Opus 6.5, Bruker Optics, Ettlingen, Germany), with Blackman-Harris 3-Term apodization over the range of 4000 to 400 cm^{-1}. The degree of conversion was calculated considering the intensity of carbon-carbon double bond stretching vibration (correspondent peak at 1640 cm^{-1}) and using the aromatic carbon-carbon double bond stretching vibration at 1610 cm^{-1} from the polymerized and unpolymerized samples as an internal standard. Data were plotted and curve fitting was applied. In addition, the polymerization rate (Rp (s^{-1})) was calculated as the degree of conversion at time *t* subtracted from the degree of conversion at time t-1.

Tooth selection and preparation

Sixty extracted human posterior permanent teeth with deep carious lesions, radiographically involving the inner pulpal third or quarter of dentine, were selected. X-ray images were obtained using a digital system with phosphorous plates (VistaScan, Dürr Dental GmbH & Co. KG, Bietigheim-Bissingen, Germany) at 70 kV and 8 mA, with an exposure time of 0.8 s. The teeth were obtained under a protocol approved by the Research Ethics Committee of Federal University of Rio Grande do Sul, Brazil. They were stored in distilled water at 4°C until use. The teeth were submitted to selective removal to soft dentin by one experienced operator. The necrotic tissue at the pulpal wall was removed with dentin excavators, and the cavity margins were prepared to hard dentin with low-speed round burs and dentin excavators. When necessary, the cavities were also assessed and prepared using high-speed round diamond burs under water cooling.

Bonding and restoring procedures

Specimens were randomly allocated, using a software (Research Randomizer 4.0 Urbaniak, G. C., and Plous, S., 2013), into two groups (n=30) according to the adhesive resin used: (1) control, (2) test. Cavities were conditioned with 37% phosphoric acid during 30 s for enamel and 15 s for dentin, a commercial primer (Primer Scotch Bond Multi Purpose, 3 M ESPE, St.

Paul, MN, USA) was actively applied to dentin for 20 s and air-dried until solvent was visually volatilized. Adhesives resins were applied and light-cured for 20 s using a LED unit Radii Cal (1200 mW/cm2, SDI, Australia), followed by placement of two 2 mm-thick increments of a resin composite (Filtek Z350XT, 3M-ESPE, St. Paul, USA), light-cured for 40 s each. After resin composite restoration, the teeth were stored in distilled water ate 37 °C for 24 h.

Microtensile bond strength and failure pattern analysis

The teeth were longitudinally sectioned in slices using a diamond saw (Isomet, Buehler, Lake Bluff, USA). One slice per teeth from the middle of the cavity was kept and the others were further sectioned in sticks (0.7 mm x 0.7 mm). The sticks from each group were then randomly reassigned, using a software (Research Randomizer 4.0 Urbaniak, G. C., and Plous, S., 2013), into two subgroups (n=15) according to the storage time before testing: (1) no storage, (2) 6 months in simulated body fluid (SBF). The SBF was renewed at each 15 days. The sticks were fixed in a metallic device by using cyanoacrylate adhesive, and submitted to the microtensile bond strength at a universal testing machine (EZ-SX Series; Shimadzu) with a crosshead speed of 1 mm/min. The fracture pattern was evaluated with a stereomicroscope (HMV2; Shimadzu) and classified as adhesive, mixed, cohesive in dentin or cohesive in composite.

Interface analysis for mineral deposition

Micro-Raman spectroscopy was performed, using a SENTERRA Raman Microscope (Bruker Optics, Ettlingen, Germany), to assess mineral deposition of one stick per teeth. The same side of the stick was analyzed at baseline, and after 1 and 2 months of storage in SBF. The following micro-Raman parameters were used: a 100 mW diode laser with 785 nm wavelength and spectral resolution of ~3.5 cm^{-1}, with 5 coadditions and an accumulation time of 3 s. A two-dimensional mapping was performed over a square of 500 µ x 500 µ, at intervals of 20 µm using a computerized XYZ stage. It covered the composite resin, adhesive layer, hybrid layer and caries-affected dentin. Spectra were acquired between 110 and 1540 cm^{1}, and with the software Opus7.2 (Buker Optics, Ettlingen, Germany) the correspondent peak of PO_4 (960 cm^{-1}) were used for integration.

Knoop hardness

One slice per teeth was kept and stored in SBF during 6 months. Initially, the slices were hand-polished using 600#, 1200#, 1500# and 2000#-grits SiC abrasive papers and felt disc saturated with an alumina suspension (Alumina, 1 µm, Arotec, Cotia, SP, Brazil), under water irrigation (60 s each step). The specimens were subjected to a microhardness test in which five lines of indentations (10 g/5 s) starting 10 µm from the cavity floor toward the pulp chamber wall and 100 µm apart of each other were evaluated. A 100-µm distance was left between each point in lines, resulting in 4 measurement points at 10, 100, 200 and 300 µm from the cavity floor. Microhardness was assessed using a digital microhardness tester (HMV 2, Shimadzu, Tokyo, Japan). This analysis was done at baseline, after 3 months and after 6 months of SBF storage. The slices were maintained vertically suspended in SBF to avoid that the precipitated hydroxyapatite to be deposited on the specimens' surface.

Statistical analyses

Data normality was evaluated using the Kolmogorov-Smirnov test. Statistical analysis was performed using t test and paired t test for softening in ethanol, t test for degree of conversion, two-way ANOVA followed by Tukey's post hoc test for microtensile bond strength, and two-way repeated measures ANOVA followed by Tukey's post hoc test for Knoop hardness. The level of significance was 0.05 for all tests.

Results

The test adhesive resin showed an initial Knoop hardness (KHN1) of 22.82 MPa, that was higher than the KHN1 of 20.02 MPa of control group. After the immersion in solvent, all groups showed decreased values of microhardness (KHN2). There was no difference between the groups for percentage of reduction (ΔKHN%). Degree of conversion of control (62.69%) and test (62.87%) adhesive resins were also not significantly different. All the Knoop microhardness and degree of conversion values are shown in Table 1. Polymerization kinetics of experimental adhesive resins are shown in Figure 1.

Microtensile bond strength values are presented in Table 2. For sticks that presented premature failure, the lowest value found in each group was assigned. There was no statistical difference between the groups for immediate an after aging bond strengths. The microtensile bond strength of both groups did not reduce after aging. The adhesive fracture pattern was predominant in the control group immediately (57.45%) and after 6 months (56.86%). For test group, both adhesive and mixed patterns (44.90%) were most predominant immediately, while after 6 months the mixed pattern (54.90%) was predominant (Figure 2).

For each group, one representative image of interface analysis for mineral deposition by Raman spectroscopy, using the the correspondent peak of PO4 (960 cm-1) for integration, is shown in Figure 3. According the scale, the color change from blue to red represent an increase in mineral content.

Considering the two groups, there was no difference of Knoop hardness between them for the same depth and time of evaluation. From 100 µm depth to 200 µm depth it could be observed an increasing in Knoop hardness for both groups when comparing 24 h and 3 m values. Also, the values for 6 m remained stable when comparing 3 m to 6 m. All the values are shown in Table 3.

Discussion

Selective removal of carious tissue has been shown to be advantageous when managing deep carious lesions, reducing the risk of pulpal exposure or symptoms [1, 11]. As a consequence, it preserves the potential for remineralization of carious tissue [1]. Bonding to CAD results in reduced bond strength when compared to sound dentin [4] as a consequence of its lower mineral content and changes in collagen structure [12]. Additionally, the partially demineralized dentin left under the restoration could result in an increased cuspal deflection of restored teeth [13]. By means of a remineralization of partially demineralized dentin collagen within the CAD substrate, the bonding longevity of the resin-dentin interface could be enhanced and the mechanical properties restored [7, 8]. The present study developed an adhesive resin with the incorporation of αTCP, a compound that can release ionized calcium and phosphate, and evaluated the capability to improve the potential for remineralization of CAD.

The experimental adhesive resins were submitted to softening in ethanol test. The initial value of Knoop hardness was higher for test group when compared to control group. Actually, the filler addition represents a way to improve the material's mechanical properties, since they act like reinforce particles [14]. However, the percentage of variation in Knoop hardness after immersion in ethanol was not significantly different when comparing the groups. Softening in ethanol represents a method to measure the polymer crosslink density [15, 16]. Thus, the adhesive resins are supposed to present similar crosslink density. Considering the higher initial Knoop hardness for test group, the similar percentage of variation in Knoop hardness for both adhesive resins could be explained by the αTCP characteristics, as it is a calcium phosphate that presents high solubility, an important aspect considering it a biomaterial [17].

The incorporation of fillers may also affect the polymerization behavior, by acting like rigid centers for chain initiation [14]. In this study, the incorporation of αTCP did not influence the degree of conversion of adhesive resin, possibly because it was added at a concentration of 2 wt%. Regarding the polymerization kinetics, the polymerization rate of test group was higher than control group. The filler addition increases the viscosity of the mixture, and the limited mobility results in an autoacceleration at a higher rate and more rapid [18, 19]. Also, the αTCP may have increased the polymerization reaction due to diffraction and scattering effects, which influence the availability of light energy within the polymer [19].

Especially after conservative caries treatment, the mineral-depleted collagenous matrix is not entirely infiltrated by adhesive resin, considering that thicker hybrid layers are formed at CAD [3, 12]. In such a situation, the unprotected collagen fibrils at the bottom of the hybrid layer lack the protection of polymerized resin and are susceptible to time-dependent hydrolytic degradation [20, 21]. The collagen degradation of collagen fibrils will occur over time, with studies showing degraded hybrid layers and reduced bond strengths after a period of 3 months [20, 22]. Considering that even the microtensile bond strength of the control group remained stable after SBF storage for 6 months, it could suggest that the period of storage may have been short to show a difference between control and test adhesive resins. As the mineral distribution of CAD is highly variable and the lesion depth can extend hundreds of microns below the cavity surface [23], a high standard deviation in microtensile bond strength was expected. Due to the increased porosity in the intertubular dentin, CAD presents lower mechanical properties and cohesive strength [2, 12]. Specimens of resin-bonded caries-affected dentin failed predominantly in adhesive interface, but the percentage of mixed fracture pattern was also relevant. Thus, the weaker portions of the specimens are presumably the CAD itself and the bonding interface.

The interface evaluation for mineral deposition showed similar results for control and test groups. All specimens presented an increasing in mineral content over time at the CAD. The remineralization depends on the mineral content of the lesion surface layer at the baseline, which results in different magnitudes of mineral content for different specimens [24]. In Raman spectroscopy interface images, it is possible to observe that the hybrid layer at the interface dentin/adhesive resin remains not remineralized. This part of collagen matrix is devoid of seed crystallites [25]. For partially demineralized dentin, remineralization is achieved by a combination of epitaxial growth over remnant seed crystallites at the basal part of the collagen matrix [25, 26]. Epitaxial growth may be regarded as a top–down approach according to the classical crystallization theory, via ion-by-ion addition to pre-existing seed crystallites [26]. Conversely, the bottom–up mineralization approach is based on the non-classical theory of crystallization, which involves the use of biomimetic analogs for generating metastable amorphous mineral precursors and mesocrystals [27]. The association of a biomimetic remineralization mechanism, as the use of biomimetic analogs, could have resulted in biomimetic remineralization at the hybrid layer [26]. Thus, the CAD could be completely remineralized, and the microtensile bond strength improved.

The mineral deposition showed by both groups was also confirmed by an increasing in Knoop hardness especially in the first 3 months of SBF storage. The started restoration of mechanical properties, represented by the Knoop hardness, is directly related to the precipitation of minerals [28], and it occurred regardless the adhesive resin used.

Control and test adhesive resins showed similar patterns in CAD remineralization. It could be concluded that the addition of a compound source of calcium and phosphate ions to an adhesive resin did showed better results in the improvement of remineralization capability of CAD in a period of 6 months, when compared to a control adhesive resin. In longer evaluation periods, the use of a bioactive adhesive resin could show difference and better results than the conventional.

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Tables

Table 1. Microhardness values before (KHN1) and after the immersion in solvent (KHN2), the percentage variation of microhardness values (ΔKHN%), and degree of conversion (DC%) for experimental adhesive resins.

	KHN1	**KHN2**	**ΔKHN%**	**DC%**
C	20.02 (± 0.88) Ba	12.48 (± 0.68) b	37.69 (± 1.74) A	62.69 (± 0.93) A
T	22.82 (± 0.33) Aa	13.618 (± 0.78) b	40.30 (± 4.22) A	62.87 (± 0.66) A

C: control group, T: test group.

Different capital letter indicates statistical difference in same column ($p<0.05$).

Different small letter indicates statistical difference in same row ($p<0.05$).

Table 2. Microtensile bond strength (MPa) immediate and after 6 months of SBF storage.

	24 h	**6 m**
C	23.92 (±9.42) Aa	20.68 (± 11.23) Aa
T	25.30 (± 8.96) Aa	20.62 (± 7.63) Aa

C: control group, T: test group.

Different capital letter indicates statistical difference in same column ($p<0.05$).

Different small letter indicates statistical difference in same row ($p<0.05$).

Table 3. Knoop hardness values according depth and time.

	Depth (μm)	24 h	3 m	6 m
C	10	5.62 (± 7.80) Aa	7.65 (± 10.35) Aa	8.77 (± 5.88) Aa
T	10	6.63 (± 7.06) Aa	7.96 (± 5.37) Aa	8.22 (± 5.05) Aa
C	100	10.48 (± 10.42) Ab	14.08 (± 13.01) Aa	12.805 (± 7.57) Aab
T	100	10.72 (± 9.84) Ab	14.75 (± 9.96) Aa	13.04 (± 8.49) Aab
C	200	14.70 (± 11.46) Ab	17.51 (± 12.80) Aa	15.34 (± 7.70) Aab
T	200	13.30 (± 9.66) Ab	17.76 (± 10.37) Aa	14.94 (± 8.53) Aab
C	300	19.50 (± 12.94) Aab	22.43 (± 13.91) Aa	16.73 (± 7.93) Ab
T	300	18.50 (± 10.85) Aab	21.16 (± 11.53) Aa	16.58 (± 9.20) Ab

C: control group, T: test group.

Different capital letter indicates statistical difference in same column for the same depth ($p<0.05$). Different small letter indicates statistical difference in same row ($p<0.05$).

Figures

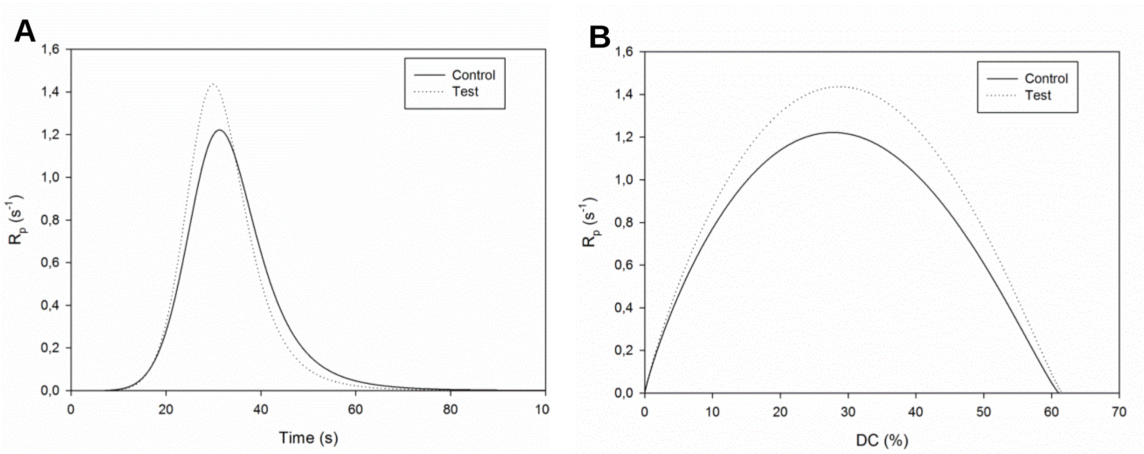

Figure 1. Polymerization rate as a function of time (A) and degree of conversion (B) of experimental adhesive resins.

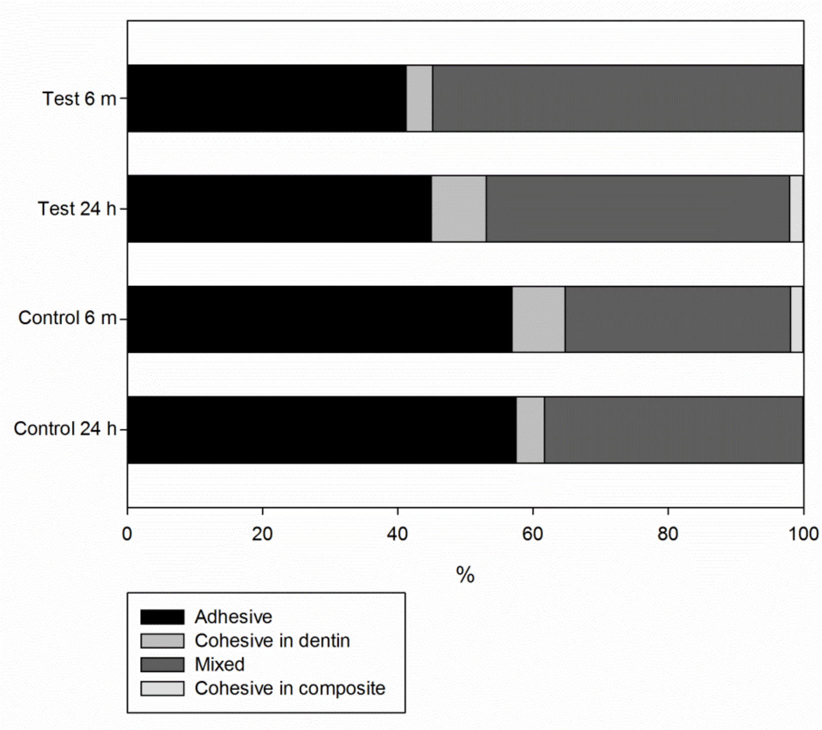

Figure 2. Fracture pattern after microtensile bond strength test.

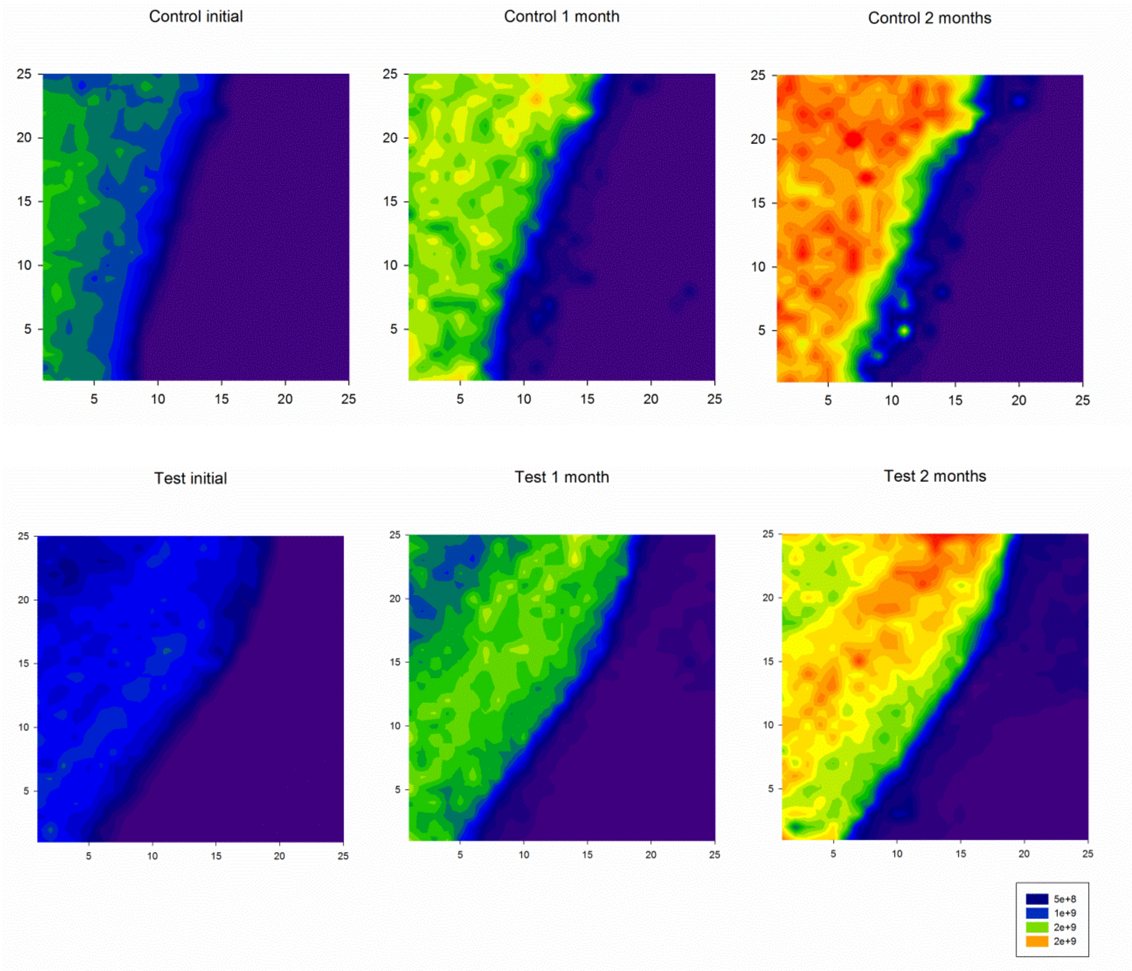

Figure 3. Representative images of mineral deposition at dentin/adhesive resin interfaces of control and experimental evaluated by Raman spectroscopy using the correspondent peak of PO_4 (960 cm^{-1}) for integration. The images covered, from left to right, caries-affected dentin, hybrid layer and adhesive layer/composite resin.

5. Considerações finais

Com o estabelecimento da Odontologia minimamente invasiva e a forte recomendação para a realização de tratamento conservadores, os dentes com lesões de cárie profunda estão sendo submetidos à remoção seletiva do tecido cariado. O principal objetivo deste tratamento consiste em conservar a dentina afetada por cárie, substrato passível de ser remineralizado, diminuindo o risco de exposição pulpar (Schwendicke *et al.*, 2016). A dentina afetada por cárie passa a ser, então, o principal substrato para o procedimento de adesão. Considerando suas propriedades mecânicas reduzidas (Nakajima *et al.*, 2011), a resistência de união a esse substrato é menor do que a resistência de união à dentina hígida, e isso foi mostrado pela revisão sistemática da literatura seguida de uma meta-regressão. Sendo assim, existe a necessidade de desenvolver estudos que investiguem técnicas e materiais que possam melhorar essas características. Uma maneira de melhorar as condições desse substrato seria através da utilização de um sistema adesivo que aumentasse o seu potencial de ganho mineral, caracterizando assim um método de reminralização.

Os resultados do segundo artigo, entretanto, mostraram que a utilização de uma resina adesiva com a incorporação de um fosfato de cálcio, o α-fosfato tricálcico, não resultou em maior deposição mineral quando comparada à resina adesiva controle. Se o período de envelhecimento das amostras fosse superior a 6 meses, talvez poderiam ser encontradas diferenças na utilização do material experimental.

6. Perspectivas futuras

A utilização de uma técnica de remineralização biomimética com o uso de análogos biomiméticos poderia ter resultado na ocorrência de deposição mineral na camada híbrida, zona totalmente desmineralizada onde não existem remanescentes de cristais de hidroxiapatita, melhorando a resistência de união à dentina afetada por cárie ao longo do tempo (Liu *et al.*, 2011).

Os análogos biomiméticos consistem em análogos das proteínas não colagenolíticas presentes na matriz dentinária. Na biomineralização da matriz colágena, a mesma funciona como um modelo para a deposição mineral na presença das proteínas não-colagenolíticas. A remineralização biomimética ocorre independentemente da existência de remanescentes de cristais de hidroxiapatita. Na presença dos análogos, os nanoprecursores de fosfato de cálcio amorfo formados conseguem penetrar na matriz colágena. Esses nanoprecursores apresentam potencial de se transformarem em nanocristais de hidroxiapatita. Enquanto isso, outros análogos apresentam a função de ligarem-se ao fosfato de cálcio e ao colágeno, permitindo que os nanocristais penetrem nos espaços intrafibrilares da matriz colágena. Através de um mecanismo de cristalização não-clássico mediado por partículas, os nanocristais se transformam em cristais de hidroxiapatita maiores dentro e ao longo das fibras colágenas. Inicialmente os ácidos poliacrílico e polivinilfosfônico foram empregados como análogos biomiméticos. O ácido poliacrílico estabiliza o fosfato de cálcio amorfo na forma de nanopartículas que podem infiltrar nos espaços intrafibrilares. O ácido polivinilfosfônico apresenta sítios de ligação às fibrilas colágenas, atraindo guiando a deposição dos nanoprecursores (Tay e Pashley, 2008). Diversos estudos mostraram o conceito de remineralização biomimética em dentes submetidos ao condicionamento ácido (Tay e Pashley, 2009; Mai *et al.*, 2010). Recentemente, o desenvolvimento de primers de sistemas adesivos contendo os análogos biomiméticos passou a ser investigada, aproximando a técnica ao uso clínico (Abuna *et al.*, 2016).

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