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LIDIANE COSTA DE SOUZA

EFEITO DAS PROANTOCIANIDINAS NA LONGEVIDADE DE RESTAURAÇÕES ADESIVAS: ENSAIO CLÍNICO ALEATORIZADO E DUPLO-CEGO

FORTALEZA – CE

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Tese apresentada ao Programa de Pós-Graduação Odontologia da Universidade Federal do Ceará, como requisito parcial para a obtenção do título de Doutor em Odontologia. Área de concentração: Clínica Odontológica.

Orientador: Vicente de Paulo Aragão Saboia

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A Deus.

Aos meus pais, Pedro e Maria José.

Ao meu esposo, Daniel.

À minha família.

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A Deus e a Nossa Senhora, que são minha fortaleza, agradeço por cada dia da minha vida.

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“Não há lugar para a sabedoria onde não há paciência.”

Santo Agostinho

RESUMO

As proantocianidinas (PAs) são agentes naturais capazes de estabelecer ligações cruzadas com o colágeno dentinário, inibir atividades proteolíticas das colagenases e que têm mostrado efeitos positivos na resistência à biodegradação, propriedades mecânicas e estabilidade estrutural da dentina, *in vitro*. Esta tese é constituída por dois capítulos que objetivaram avaliar *in vivo* o efeito das PAs na longevidade de restaurações adesivas de lesões cervicais não cariosas (LCNCs) através de um ensaio clínico aleatorizado e duplo-cego. O capítulo 1 avaliou a PA, na forma de solução aquosa, aplicada na dentina previamente à aplicação do sistema adesivo e após o condicionamento ácido, nas concentrações de 2% (PA2) e 5% (PA5) (em peso). O capítulo 2 avaliou a PA incorporada ao sistema adesivo nas concentrações de 2% (EX2) e 5% (EX5) (em peso). Para ambos, foram selecionados 45 pacientes com 3 lesões cada, dando um total de 135 LCNC por estudo. Um sistema adesivo comercial convencional simplificado, aplicado de acordo com as recomendações do fabricante, foi usado como grupo controle nos estudos. As LCNC de ambos os estudos foram restauradas com resina composta e as restaurações foram avaliadas após o polimento e nos períodos de 6 e 24 meses, utilizando-se os critérios da *United States Public Health Service* (USPHS) modificados e da Federação Internacional de Odontologia (FDI). Para os dois estudos, as diferenças nas avaliações entre os três grupos após 6 e 24 meses foram testadas com a análise de variância de Friedman de medidas repetidas por categoria ($\alpha = 0,05$) e as diferenças nas avaliações de cada grupo no período inicial e após 6 e 24 meses foram avaliadas usando o teste de Wilcoxon ($\alpha = 0,05$). Os resultados do capítulo 1 mostraram que houve uma redução estatisticamente significante na taxa de retenção para o grupo PA5 na avaliação após 6 (17%) e 24 (30%) meses (p=0,03). Os três grupos apresentaram piora significativa na adaptação marginal ao longo do tempo, para o

critério FDI, mas nenhuma restauração foi considerada como tendo uma discrepância clinicamente relevante. Quanto à descoloração marginal, para o critério FDI, observou-se uma diferença significativa entre a avaliação inicial e a avaliação de 24 meses para todos os grupos. Os resultados do capítulo 2 mostraram que o grupo EX5 apresentou uma significativa queda na taxa de retenção (15%) após 6 meses. Após 24 meses, tanto o grupo EX2 (27%) quanto o grupo EX5 (29%) apresentaram taxa de retenção significativamente menor que o grupo controle. Quanto a adaptação marginal, todos os grupos apresentaram discrepância significativa ao longo do tempo, somente para o critério FDI. Todos os grupos apresentaram aumento da pigmentação marginal ao longo do tempo para os dois critérios avaliados, mas somente o grupo EX5 apresentou diferença estatística quando comparado aos demais grupos nos períodos de 6 e 24 meses. Nos dois estudos, nenhuma restauração apresentou sensibilidade pós-operatória ou recorrência de cárie. Desta forma, conclui-se que a PA aplicada previamente ou incorporada ao sistema adesivo não apresentou vantagens clínicas após 24 meses de avaliação.

Palavras-chaves: Dentina. Adesivos dentinários. Ensaio Clínico. Proantocianidinas. Lesões Cervicais não cariosas.

ABSTRACT

Proanthocyanidins (PAs) are natural agents capable of crosslinking dentin collagen, inhibit collagenase proteolytic activities and have shown positive effects on the resistance to biodegradation, mechanical properties and structural stability of dentin *in vitro*. This thesis consists of two chapters that aimed to evaluate *in vivo* the effect of PAs on the longevity of adhesive restorations of non-carious cervical lesions (NCCLs) in a randomized, double-blind clinical trial. Chapter 1 evaluated the PA, as an aqueous solution, applied to the dentin prior to the application of the adhesive system and after the acid etching, at concentrations of 2% (PA2) and 5% (PA5) (by weight). Chapter 2 evaluated the PA incorporated in the adhesive system at concentrations of 2% (EX2) and 5% (EX5) (by weight). For both, 45 patients with 3 lesions each were selected, giving a total of 135 NCCLs per study. A simplified conventional commercial adhesive system, applied according to the manufacturer's recommendations, was used as the control group in the studies. NCCLs from both studies were restored with composite resin and the restorations were evaluated after polishing and in the 6 and 24-month periods using modified United States Public Health Service (USPHS) criteria and the International Federation of Dentistry (FDI). For the two studies, differences in assessments between the three groups after 6 and 24 months were tested with the Friedman variance analysis of repeated measures by category ($\alpha = 0.05$) and differences in the assessments of each group in the initial period and after 6 and 24 months were evaluated using the Wilcoxon test ($\alpha = 0.05$). The results of chapter 1 showed that there was a statistically significant reduction in the retention rate for the PA5 group in the evaluation after 6 (17%) and 24 (30%) months ($p = 0.03$). The three groups showed a significant worsening in marginal adaptation over time for the FDI criterion, but no restoration was considered to have a clinically relevant discrepancy. Regarding the marginal discoloration, for the FDI criterion, a significant difference was observed between the initial evaluation and the 24-month evaluation for all groups. The results of chapter 2 showed that the EX5 group had a significant drop in the retention rate (15%) after 6 months. After 24 months, both the EX2 group (27%) and the EX5 group (29%) had a significantly lower retention rate than the control group. Regarding the marginal adaptation, all groups presented significant discrepancy over time, only for the FDI criterion. All groups presented increased marginal pigmentation over time for the two criteria evaluated, but only the EX5 group presented statistical difference when

compared to the other groups in the periods of 6 and 24 months. In both studies, no restoration showed postoperative sensitivity or recurrence of caries. In this way, it was concluded that PA applied previously or incorporated into the adhesive system did not present clinical advantages after 24 months of evaluation.

Key-words: Dentin. Dentin adhesives. Clinical Trial. Proanthocyanidins. Non-carious cerrvical lesions.

SUMÁRIO

INTRODUÇÃO GERAL

1 INTRODUÇÃO GERAL

A manutenção da estabilidade da união da interface formada entre sistemas adesivos e a dentina é tema de diversos estudos, pois a degradação dessa interface é a principal responsável pelo insucesso clínico de restaurações com resinas compostas (DE MUNCK *et al.*, 2005).

A união de monômeros resinosos ao substrato dentinário é mais crítica quando comparada à união ao esmalte. Isto é uma consequência da complexa composição daquele tecido, constituído, em volume, por 50% de mineral, 30% de componentes orgânicos e 20% de água (MARSHALL, 1993). Dentre os componentes orgânicos, o colágeno tipo I representa 90% da matriz dentinária. As fibras de colágeno apresentam ligações cruzadas exógenas intra e intermoleculares que são responsáveis pela forma e coesão da estrutura dentinária (STENZEL; MIYATA; RUBIN, 1974).

Na ocorrência de desmineralização, a rede de fibrilas de colágeno e proteínas não-colagenosas é a responsável por preservar a forma e a dimensão da dentina (MACIEL *et al.*, 1996) e é nesse substrato, entre os espaços interfibrilares, que os monômeros resinosos deveriam infiltrar-se para formar uma interface de união compacta e homogênea, chamada camada híbrida (MARSHALL *et al.*, 1997). Entretanto, o que ocorre na realidade é uma incompleta infiltração e encapsulamento das fibrilas de colágeno por esses monômeros, deixando parte delas expostas. Desta forma, as fibrilas ficam mais susceptíveis à degradação (WANG; SPENCER, 2002; WANG; SPENCER, 2003) durante a vida útil da restauração, tanto por processos físicos (mecânicos e térmicos) e químicos (agentes ácidos, saliva) (BRESCHI *et al.*, 2008) quanto biológicos (ação de metaloproteinases da matriz extracelular e catepsinas) (AGEE; ZHANG; PASHLEY, 2000; DE MUNCK *et al.*, 2009). Portanto, a formação de uma rede de colágeno insolúvel, resistente e estável teria um papel importante na longevidade da interface de união dentina/resina frente à degradação inerente às condições do meio bucal (BEDRAN-RUSSO *et al.*, 2009; BEDRAN-RUSSO *et al.*, 2010).

Tem sido demonstrado que a aplicação de agentes exógenos de ligação cruzada a vários tecidos conjuntivos é útil para modificar as estruturas das fibrilas de colágeno, dando-lhes mais estabilidade e melhorando sua resistência à degradação

(CHERUKUPALLI; REDDY, 2016; LIU *et al.,* 2009; MOREIRA *et al.,* 2017; SUNG *et al.,* 2003). Estudos recentes têm utilizado um agente natural capaz de estabelecer ligações cruzadas com o colágeno dentinário: a proantocianidina (PA) (AL-AMMAR; DRUMMOND; BEDRAN-RUSSO, 2009; BEDRAN-RUSSO *et al.,* 2008; BEDRAN-RUSSO *et al.,* 2014; CASTELLAN *et al.,* 2010a; CASTELLAN *et al.,* 2010b; HASS *et al.,* 2016a; MACEDO; YAMAUCHI; BEDRAN-RUSSO, 2009; SCHEFFEL *et al.,* 2014; XIE; BEDRAN-RUSSO; WU, 2008). As proantocianidinas (PAs) são parte de um grupo específico de compostos polifenólicos, formados por subunidades de flavan-3-ol, pertencentes à categoria conhecida como taninos condensados (HAN *et al.,* 2003). São encontradas em uma grande variedade de vegetais, frutas, flores, nozes, sementes e cascas (FERREIRA; SLADE, 2002). As PAs apresentam atividades antibacteriana, anti-inflamatória e antialérgica, bem como ações vasodilatadoras (AFANAS'EV *et al.,* 1989; BUENING *et al.* 1981; KOLODZIEJ *et al.* 1995), o que tem levado ao aumento do interesse pelo estudo deste composto em áreas da saúde. Além disso, as PAs são capazes de inibir significantemente atividades proteolíticas das enzimas como as colagenases e elastases (MAFFEI e*t al.,* 1994) e a progressão de cáries artificiais em dentina radicular (WALTER *et al.* 2008, XIE *et al.,* 2008). As PAs são agentes antioxidantes naturais e também podem aumentar a síntese de colágeno, pois, embora tenham uma atividade inibitória para a maioria das enzimas, são capazes de facilitar a hidroxilação da prolina através da ativação da enzima hidroxilase (MAFFEI e*t al.,* 1994).

Outras vantagens inerentes a PA são sua baixa citotoxicidade, seu baixo custo e sua fácil obtenção (AL-AMMAR; DRUMMOND; BEDRAN-RUSSO, 2007; BEDRAN-RUSSO *et al.,* 2009), uma vez que são encontradas abundantemente na natureza, como em sementes de uva e de cacau, açaí, canela e oxicoco (COS *et al.,* 2004). Por todas essas características, vários estudos *in vitro* têm investigado o efeito de extratos ricos em PAs sobre a resistência à biodegradação, propriedades mecânicas e estabilidade estrutural de dentina coronal (BEDRAN-RUSSO *et al.,* 2011; CASTELLAN *et al.,* 2010b)

A PA interage com colágeno de quatro diferentes maneiras: pela ligação covalente com as proteínas (PIERPOINT, 1969), por ligações iônicas (LOOMIS, 1974), pela formação de ponte de hidrogênio (HAGERMAN; BUTLER, 1981) ou interações hidrofóbicas (HAN *et al.,* 2003). Todas estas diferentes interações mantêm

o colágeno intacto, mesmo depois de ter sido clivado por uma enzima (WEADOCK; OLSON; SILVER, 1983).

Em restaurações adesivas, a PA pode ser utilizada de algumas maneiras, como: um *primer* adicional, aplicado após o condicionamento ácido e antes da aplicação do adesivo (AL-AMMAR *et al.*, 2009; CASTELLAN *et al.*, 2013; FANG *et al*., 2012), adicionada ao sistema adesivo (EPASINGHE *et al*., 2012; EPASINGHE *et al*., 2015; GREEN *et al*., 2010; LIU; WANG, 2013; VENIGALA *et al.*, 2016) ou até mesmo adicionada ao agente de condicionamento ácido (HASS *et al*., 2016b). HECHLER *et al.* (2012) avaliaram o desempenho a longo prazo da PA aplicada como *primer* ou incorporada ao sistema adesivo utilizando o Single Bond (3M ESPE, St. Paul, MN) e verificaram que, após 52 semanas de exposição à digestão por colagenase, a resistência à microtração da interface resina/dentina foi significativamente maior em relação ao controle quando a PA foi utilizada como um *prime*r, ao passo que a PA incorporada ao sistema adesivo não mostrou diferença significativa em relação ao controle no tempo avaliado.

Embora as metodologias de testes *in vitro* com protocolos de envelhecimento possam prever o desempenho de um material (AMARAL *et al.,* 2007; HEINTZE; ROUSSON, 2011; VAN MEERBEEK *et al.,* 2010), ensaios *in vivo* continuam sendo imprescindíveis para avaliar a melhor eficácia clínica de adesivos e/ou técnicas.

Em virtude da demanda restauradora e da facilidade de acesso e visualização para posterior avaliação, as lesões cervicais não cariosas (LCNCs) têm sido largamente usadas em estudos clínicos para materiais adesivos. As LCNCs são comuns na cavidade oral e têm sido encontradas em mais de 85% dos pacientes que procuram tratamento odontológico (LEVITCH *et al.,* 1994). Sabe-se que a dentina esclerótica das LCNCs pode ser mais desafiadora para a união do que a dentina coronal, por isso tal substrato proporciona uma boa superfície para se testar as qualidades de um adesivo (TAY *et al.*, 2000*)* ou de um protocolo de adesão.

Um estudo que avaliasse em longo prazo o efeito da PA na estabilização das interfaces resina/dentina, através da verificação do comportamento clínico de restaurações de resina composta, seria de fundamental importância para a comprovação da eficácia deste composto na técnica adesiva.

PROPOSIÇÃO

2 PROPOSIÇÃO

2.1 Objetivo Geral

- Avaliar clinicamente o efeito na longevidade de restaurações de lesões cervicais não cariosas (LCNCs) de uma solução aquosa contendo proantocianidinas a 2% ou a 5%, aplicada como pré-tratamento da dentina, ou da inclusão de proantocianidina nessas mesmas concentrações (em peso) em um sistema adesivo convencional simplificado.

2.2 Objetivos Específicos

- Avaliar clinicamente, através dos critérios da *United States Public Health Service* (USPHS) modificados e da Federação Internacional de Odontologia (FDI), o efeito da aplicação de solução aquosa de proantocianidinas a 2 % ou a 5% antes da aplicação de um sistema adesivo convencional simplificado, ou da incorporação de proantocianidinas 2% ou 5% (em peso) no sistema adesivo, após a confecção das restaurações e nos períodos de 6 e 24 meses.
- Comparar a taxa de retenção, pigmentação marginal, adaptação marginal, sensibilidade pós-operatória e recorrência de cárie em restaurações de LCNCs quando uma solução aquosa de proantocianidinas a 2% ou a 5% é aplicada antes da aplicação de um sistema adesivo convencional simplificado, nos períodos após a confecção das restaurações e após 6 e 24 meses.
- Comparar a taxa de retenção, pigmentação marginal, adaptação marginal, sensibilidade pós-operatória e recorrência de cárie em restaurações de LCNCs quando proantocianidinas a 2% ou a 5% são incorporadas ao sistema adesivo convencional simplificado, nos períodos após a confecção das restaurações e após 6 e 24 meses.

CAPÍTULOS

3 CAPÍTULOS

Esta tese está baseada no artigo 46 do Regimento Interno do Programa de Pós-graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratarem de pesquisas envolvendo seres humanos, ou partes deles, o projeto de pesquisa deste trabalho foi submetido à apreciação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, através da submissão no site da plataforma Brasil, tendo sido aprovado (Anexos A) e foi também inscrito no site do Registro Brasileiro de Ensaios Clínicos (Anexo B). Assim sendo, esta tese é composta por 2 capítulos citados abaixo:

- Capítulo 1

  Título: Two-year clinical evaluation of proanthocyanidin-primer performance in non-carious cervical lesions: a double-blind randomized clinical trial

  Autores: SOUZA LC, RODRIGUES NS, CUNHA DA, FEITOSA VP, SANTIAGO SL, REIS A, LOGUERCIO AD, SABOIA VPA.

  Periódico: Clinical Oral Investigations*

- Capítulo 2

  Título: Two-year clinical evaluation of proanthocyanidin incorporation in two-step etch-and-rinse adhesive system

  Autores: SOUZA LC, RODRIGUES NS, CUNHA DA, FEITOSA VP, SANTIAGO SL, REIS A, LOGUERCIO AD, SABOIA VPA.

  Periódico: Journal of Dentistry**

Normas das revistas

* http://www.springer.com/medicine/dentistry/journal/784?detailsPage=pltci_1060698

** https://www.elsevier.com/journals/journal-of-dentistry/0300-5712/guide-for-authors

CAPÍTULO 1

Two-year clinical evaluation of proanthocyanidin-primer performance in non-carious cervical lesions: a double-blind randomized clinical trial

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Running Title: Proanthocyanidin pre-treatment on resin/dentin adhesion

Keywords: Dentin-bonding agents; Non-carious cervical lesions; Proanthocyanidin; Longevity; Clinical trial.

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Two-year clinical evaluation of proanthocyanidin-primer performance in non-carious cervical lesions: a double-blind randomized clinical trial

ABSTRACT

Objective: This double-blind randomized clinical trial evaluated the influence of pre-treatment with proanthocyanidin (PA) from grape seed extract on clinical behavior of simplified etch-and-rinse adhesive placed in non-carious cervical lesions (NCCL) over 6- and 24-month, using two evaluation criteria: FDI and USPHS.

***Methods*:** A total of 135 restorations were randomly placed in 45 patients. The NCCLs were phosphoric acid etched for 15 s and distributed into 3 groups: Control (PA0) - adhesive ExciTE F (Ivoclar Vivadent) applied following the manufacturer's recommendations; PA2 and PA5 groups – 2wt% and 5wt% PA solution, respectively, were applied for 60 s and washed for 30 s prior to application of the adhesive ExciTE F. The resin composite was placed incrementally and light-cured. The restorations were evaluated at baseline, 6 and 24 months. Statistical analyses were performed using appropriate tests (α=0.05).

Results: The retention rates were 98% (PA0), 98% (PA2) and 83% (PA5) after 6-month and 93% (PA0), 89% (PA2) and 70% (PA5) after 24-month. Only PA5 showed significant difference when comparing with baseline findings for 6 and 24 months (p=0.03). All groups presented significant worsening for marginal adaptation over time only for FDI criteria, but none of them was considered clinically unacceptable. Concerning the marginal discoloration, a significant difference between baseline vs. 24-month recall was observed for all groups using FDI criteria.

Conclusion: The application of proanthocyanidin as primer did not present clinical advantages after 24 months of clinical evaluation, regardless of the concentration used.

Clinical Relevance: Scientific literature shows that the collagen crosslinking agent, proanthocyanidin, can stabilize and reinforce the collagen fibrils of the dentin matrix in vitro, increasing the durability of the dentin-resin interface. However, no improvements were found clinically after 24 months herein.

Keywords: Dentin-bonding agents; Non-carious cervical lesions; Proanthocyanidin; Longevity; Clinical trial.

1. Introduction

The degradation of resin–dentin bond interface created with hydrophilic bonding agents occurs by hydrolytic, enzymatic and fatigue degradation processes [1, 2]. Significant number of resin-sparse collagen fibrils can be found at the hybrid layer [3-5] and this matrix is one of the main degradation patterns found in unsuccessful adhesive restorations [2, 6-9].

In recent years, several alternatives have been proposed to preserve the durability of the resin-dentin interface *in vitro* and *in vivo*. Among them, the application of collagen cross-linkers is an emerging and interesting option to increase the longevity of resin/dentin interfaces, as they can increase the resistance of collagen fibrils from dentin matrix alone with inhibitting inactivate host-derived metalloproteases (MMPs) [10-12].

Of those investigated so far, proanthocyanidins (PAs), from grape-seed extract, is by far the most extensively tested in dentistry [10, 13], mainly due to it is a

natural polyphenolic compound known as a potent antioxidant and great scavenger of proteins, with low toxicity [14, 15].

Grape seed extract is one of the most used sources of proanthocyanidin. [14, 16, 17]. The use of grape seed extract improved the ultimate tensile strength, stiffness [18, 19], and long-term stability [20, 21] of dentin collagen. In addition to its cross-linking effect, proanthocyanidin has also been shown to inhibit the synthesis of several MMPs from macrophages and inhibit the catalytic activity of MMP-1 and MMP-9 [14, 22].

However, the application of PA-based agents, often made as an extra bonding step with 10 min, 1 h or longer durations [18, 20, 23-25], which turns makes clinical application unfeasible. Recently, PA preconditioners were used in shorter treatment duration (60 s or 120 s), a clinically applicable time [26], showing increase of the cross-linking degree and ultimate tensile strength of demineralized dentin [27]. Unfortunately, to extent of our knowledge, no clinical trials were conducted to predict the effect of PA applied as pre-treatment on clinical performance of adhesive restorations in non-carious cervical lesions (NCCLs).

Therefore, the objective of this double-blind randomized clinical trial was to evaluate the influence of pre-treatment with PA on the clinical behavior of etch-and-rinse adhesive system placed in non-carious cervical lesions (NCCL) over the course of 6- and 24-month, using two evaluation criteria: World Dental Federation (FDI) and United States Public Health Service (USPHS) criteria. The null hypothesis tested was that bonding to NCCLs with or without PA before simplified etch-and-rinse adhesive application yields similar retention rates over 6- and 24-month of clinical service.

2. Materials and methods

The description of the experimental design followed the Consolidated Standards of Reporting Trials (CONSORT) statement [28].

2.1 Ethics approval

The local Ethics Committee on Investigations Involving Human Subjects reviewed and approved the protocol and issued a consent form for this study (protocol #640.695). Written informed consent was obtained from all patients prior to starting the treatment.

2.2 Protocol registration

This clinical trial was registered in http://www.ensaiosclinicos.gov.br/ clinical registry under protocol #RBR-366MBJ. All participants were informed about the nature and objectives of the study.

2.3 Trial design, settings and location of data collection

This was a double-blind, equal allocation rate, split-mouth randomized clinical trial. The study was carried out in the clinics of the School of Dentistry at the local University from November 2014 to January 2017.

Recruitment

Patients were recruited as they seek for treatment in the clinics of Dentistry of the local university. No advertisement was made for participant recruitment. Patients were recruited in the order in which they reported for the screening session, thus forming a sample of convenience.

Eligibility criteria

A total of 62 participants were examined by two calibrated postgraduated dental students to check if they met the inclusion and exclusion criteria (Figure 1).

The evaluations were performed using a mouth mirror, an explorer and a periodontal probe. Participants had to be in good general health, older than 18 years old, have an acceptable oral hygiene level and present at least 20 teeth under occlusion.

Participants were required to have at least three NCCLs to be restored in three different teeth. These lesions had to be non-retentive, deeper than 1 mm, and involving both the enamel and dentin of vital teeth without mobility. The cavosurface margin could not involve more that 50% of enamel [29]. Patients with extremely poor oral hygiene, severe or chronic periodontitis, or heavy bruxism habits were excluded from the study as they would receive other treatments before restorative intervention.

2.4 Sample size calculation

The adhesive two-step etch-and-rinse ExcitTE F (Ivoclar Vivadent AG, Schaan, Liechtenstein) was used in the present study. The reported percentage of retention of the Excite adhesive system is 73% after 5 years of clinical evaluation [30]. Considering 5% alpha, an 80% power and a monocaudal two-sided test, the minimum sample size was 43 restorations per group to find a 22% difference between the tested groups.

2.5 Random sequence generation and allocation concealment

The randomization was done on an intra-individual basis so that each subject ended up with three restorations. These randomization schemes were performed using software available at http://www. sealedenvelope.com.

A staff member not involved in the research protocol performed the randomization process with computer-generated tables. Details of the allocated groups were recorded on cards contained in sequentially numbered, opaque, sealed envelopes. Opening the envelope only on the day of the restorative procedure

guaranteed the concealment of the random sequence. In all cases, the tooth with the highest tooth number received the treatment described first, while the tooth with the next number in sequence received the treatment mentioned second and the next tooth received the treatment mentioned third.

2.6 Interventions: restorative procedure

Forty-five patients were selected for this study and all received dental prophylaxis with a suspension of pumice and water in a rubber cup and signed an informed consent before the restorative procedures.

The degree of sclerotic dentin from the NCCLs was measured according to the criteria described by Swift and others [31] (Table 1). The cavity dimensions in millimeters (height, width, and depth), the geometry of the cavity (evaluated by profile photograph and labeled at $<45^{o}$, 45^{o}-90^{o}, $90^{o}<135^{o}$, and $>135^{o}$)[32], the presence of an antagonist, and the presence of attrition facets were observed and recorded. Pre-operative sensitivity was also evaluated by applying an air-blast for 10 s from a dental syringe placed 2 cm from the tooth surface and with an explorer. These features were recorded to allow comparison of the baseline features of the dentin cavities among experimental groups.

To calibrate the restorative procedure, the study director placed one restoration of each group to identify all steps involved in the application technique. Then, one operator, who has more than five years of clinical experience in operative dentistry, placed three restorations, one of each group, under the supervision of the study director in a clinical setting. The restoration failures were shown to the operator prior to starting the study. At this point, the operator was considered calibrated to perform the restorative procedures.

One operator restored all teeth. All participants received three restorations, one of each experimental group in different lesions previously selected according to the inclusion criteria.

Before restorative procedures, the operator cleaned all lesions with pumice and water in a rubber cup, followed by rinsing and drying. Then, shade selection was made using a shade guide (Ivoclar Vivadent AG, shade guide, Schaan, Liechtenstein, German). The tooth to be restored was isolated with cotton rolls and a light cured gingival barrier (Top Dam, FGM, Joinville, Santa Catarina, Brazil). The operator did not prepare any additional retention or bevel in the class V cavity. The teeth were distributed in 3 groups and the adhesive ExciTE F (Ivoclar Vivadent, Schaan, Liechtenstein) was applied as described below. The materials, compositions and application modes are described in Table 2.

- *PA0 (Control)* – The 37% phosphoric acid (Condac acid, FGM, Brazil) was applied for 15 s. Then, cavities were rinsed thoroughly for 15 s, keeping dentin visible moist slightly with absorbent paper. One coat of adhesive was gently scrubbed on the entire enamel and dentin surface for approximately 10 s each, according to the manufacturer's recommendations (Table 2). Then, the solvent was evaporated by gentle air stream for 5 s and light cured for 10 s at 1250 mW/cm^2 (Emitter A Schuster, Santa Maria, RS, Brazil).

- *PA2* – After the phosphoric acid procedure, 2% proanthocyanidin (V. vinifera, Meganatural Gold, Madera, CA, USA) solution was applied for 1 minute in the dentin using a disposable applicator, washed for 30 s, removing excess moisture with absorbent paper. Then, the adhesive system ExciTE F was applied according to the control group.

- *PA5* - After the phosphoric acid, 5% proanthocyanidin (V. vinifera, Meganatural Gold, Madera, CA, USA) solution was applied for 1 minute in the dentin using a disposable applicator, washed for 30 s, removing excess moisture with absorbent paper. Then, ExciTE F was applied according to the control group.

The resin composite Empress Direct (Ivoclar Vivadent, Schaan, Liechtenstein) was used in up to three increments, each one being lightly cured for 20 s at 1250 mW/cm^2. The restorations were finished immediately with fine and extra-fine #3195 diamond burs (KG Sorensen, Barueri, SP, Brazil) under constant water-cooling. After one-week, each one was finished and polished with slow-speed polishing points (Jiffy Polishers, Ultradent, South Jordan, UT, USA).

2.7 Calibration procedures for clinical evaluation

For training purposes, two experienced and calibrated examiners observed 10 photographs that were representative of each score for each criterion. They evaluated 10 patients each on two consecutive days. These subjects had cervical restorations but were not part of this project. An intra-examiner and inter-examiner agreement of at least 85% was necessary before beginning the evaluation [33]. In case of disagreement between the examiners, consensus was obtained.

2.7.1 Blinding

The examiners, who were not involved with the restoration procedures and therefore blinded to the group assignment, performed the clinical evaluation. Patients were also blinded to group assignment in a double-blind randomized clinical trial design.

2.7.2 Clinical evaluation

An individual standardized paper case report form was used for each evaluator at each recall time so that evaluators were kept blinded to earlier evaluations during the follow-up recalls. Intraoral color photographs were collected at baseline and at the recall appointments to aid in the evaluation, if necessary. Clinical photographs consisted of digital images obtained using a Nikon D90X camera with a 105-mm Medical Nikon lens (Nikon Inc., Melville, NY, USA).

The restorations were evaluated by World Federation criteria (FDI) [34] and the classical United States Public Health Service (USPHS) criteria (adapted by Bittencourt and others [35] and Perdigão and others [36]) at baseline and after 6 and 24 months of clinical service. Only the clinically relevant measures for evaluation of adhesive performance were used and scored (Tables 3 and 4). The primary clinical endpoint was restoration retention/fracture, but the following secondary endpoints were also evaluated: marginal staining, marginal adaptation, postoperative sensitivity, and recurrence of caries.

These variables were ranked according to FDI criteria into clinically very good, clinically good, clinically sufficient/ satisfactory, clinically unsatisfactory but repairable, and clinically poor (replacement required) [34] and in the USPHS criteria into Alfa, Bravo and Charlie. [35]. Both examiners evaluated all the restorations once and independently. When disagreements occurred during the evaluations, they had to reach a consensus before the participant was dismissed. The restoration retention rates were calculated according to the ADA guidelines [37]. Cumulative failure percentage = [(PF + NF) / (PF + RR)] X 100%, where PF is the number of previous failures before the current recall, NF is the number of new failures during the current recall, and RR is the number of currently recalled restorations.

2.8 Statistical Analysis

The statistical analyses followed the intention-to-treat protocol according to CONSORT (Consolidated Standards of Reporting Trials) suggestion [28]. Descriptive statistics were used to describe the distributions of the evaluated criteria. Statistical analysis for each individual item was performed for each evaluation criteria (FDI and USPHS criteria).

The differences in the ratings of the three groups after 6 and 24 months were tested with Friedman repeated-measures analysis of variance by rank (α=0.05), and differences in the ratings of each group at baseline and after 6 and 24 months were evaluated using the Wilcoxon test (α=0.05). Cohen's kappa statistics was used to test inter-examiner agreement. In all statistical tests, we pre-set the level of significance to 5%.

3. Results

The restorative procedures were implemented exactly as planned and no modification was performed. Seventeen out of 62 patients were not enrolled in the study because they did not fulfill the inclusion criteria (Figure 1). Thus, 45 subjects were selected. All baseline details relative to the research subjects and characteristics of the restored lesions are displayed in Table 5. All research subjects were evaluated at the baseline and at 6 months and two patients with three restorations each did not attend the 24-month recall rate (Figure 1), because one moved to another city and other could not return due to health problems.

3.1 Retention

Ten restorations were lost at 6 months. According to FDI and USPHS criteria, the 6-month retention rates (95% confidence interval) were 98% (88 – 99%) for PA0; 98% (88 – 99%) for PA2; and 83% (69 – 91%) for PA5. Twenty-one restorations

were lost at 24 months. According to FDI and USPHS criteria, the 24-month retention rates (95% confidence interval) were 93% (82 – 98%) for PA0; 89% (76 – 95%) for PA2; and 70% (55 – 88%) for PA5.

When the data from 6-month results from each group were compared with their baseline findings, a significant difference was found only for PA5 ($p = 0.03$; Tables 6 and 7). Also, when the retention rate of the PA5 was compared with PA0 and PA2, significant differences in the retention rates were detected after 6 months ($p = 0.001$; Tables 6 and 7). When the data from 24-month results from each group were compared with their baseline findings, a significant difference was found only for PA5 ($p = 0.03$; Tables 6 and 7). Also, when the retention rate of the PA5 was compared with PA0, significant differences in the retention rates were detected after 24 months ($p = 0.001$; Tables 6 and 7).

3.2 Post-operative sensitivity

No restorations showed post-operative sensitivity immediately after restorative procedures according to the FDI and USPHS criteria. After 6- and 24-month, no restoration showed post-operative sensitivity using both the FDI and USPHS criteria (Tables 6 and 7).

3.3 Marginal adaptation

According to the FDI criteria, 94 restorations at the 6-month recall were considered to have some discrepancies in marginal adaptation. After 24-month recall, 103 restorations were considered to have some discrepancies in marginal adaptation. No significant difference was detected between any pair of groups at the 6- and 24-month recall for both criteria ($p > 0.05$; Tables 6 and 7).

However, significant worsening for all three groups of marginal adaptation was observed within all groups over time (baseline vs. 6-month and baseline vs. 24-month) ($p < 0.05$; Tables 6 and 7). Despite the high number of the restorations with lack of marginal adaptation in the FDI criteria, none of them was considered to have clinically relevant discrepancies in the marginal adaptation even after 6- and 24-month of clinical evaluation (Table 6).

When the USPHS criteria were used, only 8 restorations were scored as *Bravo* for marginal adaptation ($p > 0.05$) at the 6-month recall. After 24-month recall, 8 restorations were scored as *Bravo* for marginal adaptation ($p > 0.05$). No significant difference was detected between any pair of groups at the 6- and 24-month recalls and between recall times within group ($p > 0.05$).

3.4 Marginal discoloration

For the FDI criteria, 12 restorations at the 6-month recall were considered to have minor discrepancies. After 24-month recall, 42 restorations were considered to have minor discrepancies (clinically good and satisfactory).

A significant difference between baseline vs. 6-month recall was observed for the group PA2 using FDI criteria ($p < 0.05$; Tables 6). However, a significant difference between baseline vs. 24-month recall was observed for all groups using FDI criteria ($p < 0.05$; Tables 6). It worth to mention that, after 24-month recall, no significant differences were observed between groups ($p>0.05$; Tables 6 and 7).

When the USPHS criteria were used, only 7 restorations at 6-month recall were scored as *Bravo* for marginal staining ($p > 0.05$). After 24-month recall, 21 restorations were scored as *Bravo* for marginal staining. A significant difference between baseline vs. 24-month recall was observed for PA0 and PA2 groups using

USPHS criteria ($p < 0.05$; Tables 6). After 24-month recall, only PA2 showed a significant higher marginal staining when compared with PA5 ($p = 0.001$; Tables 6 and 7).

3.5 Recurrence of caries

No restoration showed recurrence of caries at the 6- and 24-month clinical recall using the FDI and the USPHS criteria.

3.6 General Overview

When the FDI criteria for 'acceptable' *vs*. 'not acceptable' restorations were applied, only 21 restorations were ranked as 'not acceptable' due to loss of the restorations, the majority for the PA5 group (Table 8).

4. Discussion

Among the clinical parameters for the evaluation of an adhesion protocol or the performance of any restorative material in the NCCL, the retention rate is the most important, since the restoration loss do not allow an evaluation of other parameters [38]. Regarding this parameter, PA5 group showed significant reduction in the retention rate (17%) when compared with its baseline and when compared with PA0 (2%) and PA2 (2%) groups, after 6-month. After 24-month, this reduction was even greater for PA5 group (30%) and also increased for PA2 group (11%), although it was not statistically significant, which leads to rejection of the null hypothesis.

In this study, PA was obtained from grape-seeds extract (GSE), since it has a higher PA concentration (at least 95%) and is soluble in water [20], facilitating the formulation of the solutions as primer. The literature reports the use PA in 0.5 to 15wt% [19-21, 39, 40]. A recent study used PA (1% or 5%) and other cross-linking agents for 5 min and demonstrated that the long-term effect is both crosslinker and

dose dependent [41]. In this study, concentrations of 2% (PA2) and 5% (PA5) were used and a greater drop in retention rate was observed when the highest concentration of PA was used. A justification for this can be that PA has a free radical scavenging effect, which can disturb the free radical polymerization of the resin, inhibiting the ideal resin polymerization [42], especially within collagen mesh.

The use of non-carious cervical lesions is particularly valuable for clinical studies because it is difficult to study this type of lesion *in vitro*. These lesions have quite variable etiology and their prevalence is increasing as the adult population continues to age [43, 44]. Besides, the sclerotic dentin on the surface of the NCCLs is more challenging than coronal dentin to adhesion, so such a substrate provides a good surface to test the qualities of an adhesive system [45].

FDI criteria, as well the USPHS criteria, are parameters for evaluating dental restorations. FDI criteria were published in 2007 by FDI [34, 46] and since then, some studies [38, 47, 48] have used them. It has been concluded that the FDI criteria is more sensitive than the USPHS criteria modified for identifying small variations in the clinical outcomes when evaluating restorations of NCCLs [38, 47, 48]. This finding was corroborated in the present study, as the marginal adaptation was only statistically significant for all three groups over time (baseline vs. 6-month) when FDI criteria were used.

One hundred and three restorations exhibited some marginal adaptive discrepancies in the 24-month recall. All groups showed a significant worsening in this criteria over time (baseline *vs* 6-month *vs* 24-month), however, none restauration was considered to have a clinically unsatisfactory marginal adaptation. Some clinical trials [38, 47-50] showed that marginal discrepancies of a composite restoration usually develop rather rapidly. However, the most the marginal defects were small

and clinically acceptable [51] and the simple procedure of restoration re-polishing can amend these discrepancies without causing any damage to the integrity of the restoration [52].

When using FDI criteria, PA2 group showed more marginal discoloration after 6 months compared to baseline and this difference was not seen for the other groups. After 24 months no differences were observed among the groups but all of them showed increase in marginal discoloration compared to baseline. When USPHS was used, the groups PA0 and PA2 presented a significant difference compared their baseline with 24-month recall and PA2 showed a significant higher marginal discoloration than PA5, in this period. The PA5 group had great loss of restorations, which may have underestimated the statistical results for this assessment, since these criteria were not evaluated in the lost restorations. Moreira et al. (2017) [53] showed that the PA-treated dentin samples were brownish in color.

PA grape-seed solutions have a darker color and it can be attributed to their oxidative properties and the presence of high different molecular weight polymer polyphenols, which may justify the color change [10, 39, 54]. Studies have attempted to purify PA by extracting oligomeric or dimeric substances that would be more related to the benefits of PA in dental procedures and [55,56] might cause less undesirable changes.

In order to follow-up the clinical performance of PA-primer application in NCCL, additional recall evaluations are planned for this study. Over 24-month, the use of PA as primer before adhesive application did not prove advantageous for adhesion in the NCCL especially for PA5 group. Although, it is early to conclude that PA pre-treatment should not have been take in account to preserve adhesion, once the

possible benefits from pre-treatment with 2% PA, as observed in some *in vitro* studies, only will be noted after more follow-up time.

5. Conclusion

The application of proanthocyanidin as primer did not present clinical advantages after 24 months of clinical evaluation, regardless of the concentration used.

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6. References

[1] Reis A, Carrilho M, Breschi L, Loguercio AD (2013) Overview of clinical alternatives to minimize the degradation of the resin-dentin bonds. Oper Dent 38: E1–E25. doi:10.2341/12-258-LIT.

[2] Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E (2008) Dental adhesion review: Aging and stability of the bonded interface. Dent Mater 24: 90–101. doi:10.1016/j.dental.2007.02.009.

[3] Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H (2000) In vivo Degradation of Resin-Dentin Bonds in Humans Over 1 to 3 Years. J Dent Res 79: 1385–1391. doi:10.1177/00220345000790060601.

[4] Hashimoto M, Ohno H, Sano H, Tay FR, Kaga M, Kudou Y, Oguchi H, Araki Y, Kubota M (2002) Micromorphological changes in resin-dentin bonds after 1 year of water storage. J Biomed Mater Res 63: 306–311. doi:10.1002/jbm.10208.

[5] Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H (2003) In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission

electron microscopy. Biomaterials 24: 3795–3803. doi:10.1016/S0142-9612(03)00262-X.

[6] Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjäderhane L, Toledano M, Pashley EL, Tay FR (2006) Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. Biomaterials 27: 4470–4476. doi:10.1016/j.biomaterials.2006.01.040.

[7] Mazzoni A, Carrilho M, Papa V, Tjäderhane L, Gobbi P, Nucci C, Di Lenarda R, Mazzotti G, Tay FR, Pashley DH, Breschi L (2011) MMP-2 assay within the hybrid layer created by a two-step etch-and-rinse adhesive: Biochemical and immunohistochemical analysis. J Dent 39: 470–477. doi:10.1016/j.jdent.2011.04.004.

[8] Kostoryz EL, Dharmala K, Ye Q, Wang Y, Huber J, Park JG, Snider G, Katz JL, Spencer P (2009) Enzymatic biodegradation of HEMA/BisGMA adhesives formulated with different water content. J Biomed Mater Res - Part B Appl Biomater 88: 394–401. doi:10.1002/jbm.b.31095.

[9] Tay FR, Pashley DH (2003) Have dentin adhesives become too hydrophilic? J Can Dent Assoc 69: 726–731. doi:10.1016/S0109-5641(03)00110-6.

[10] Bedran-Russo AK, Pauli GF, Chen SN, McAlpine J, Castellan CS, Phansalkar RS, Aguiar TR, Vidal CMP, Napotilano JG, Nam JW, Leme AA (2014) Dentin biomodification: Strategies, renewable resources and clinical applications. Dent Mater 30: 62–76. doi:10.1016/j.dental.2013.10.012.

[11] Liu Y, Liu W, Sun G, Wei X, Yi D (2009) Calcification resistance of procyanidin-treated decellularized porcine aortic valves in vivo. Heart Surg Forum 12: 24-29. doi:10.1532/HSF98.20081103.

[12] Sung HW, Chang WH, Ma CY, Lee MH (2003) Crosslinking of biological tissues using genipin and/or carbodiimide. J Biomed Mater Res A 64: 427–438. doi:10.1002/jbm.a.10346.

[13] Perdigão J, Reis A, Loguercio AD (2013) Dentin adhesion and MMPs: A comprehensive review. J Esthet Restor Dent 25: 219–241. doi:10.1111/jerd.12016.

[14] Han B, Jaurequi J, Tang BW, Nimni ME (2003) Proanthocyanidin: a natural crosslinking reagent for stabilizing collagen matrices. J Biomed Mater Res A 65: 118–124. doi:10.1002/jbm.a.10460.

[15] Ye X, Krohn RL, Liu W, Joshi SS, Kuszynski CA, McGinn TR, Bagchi M, Preuss HG, Stohs SJ, Bagchi D (1999) The cytotoxic effects of a novel IH636 grape seed proanthocyanidin extract on cultured human cancer cells. Mol Cell Biochem 196: 99–108. http://www.ncbi.nlm.nih.gov/pubmed/10448908.

[16] Liu Y, Chen M, Yao X, Xu C, Zhang Y, Wang Y (2013) Enhancement in dentin collagen's biological stability after proanthocyanidins treatment in clinically relevant time periods. Dent Mater 29: 485–492. doi:10.1016/j.dental.2013.01.013.

[17] Castellan CS, Bedran-Russo AK, Antunes A, Pereira PNR (2013) Effect of dentin biomodification using naturally derived collagen cross-linkers: One-year bond strength study. Int J Dent. doi:10.1155/2013/918010.

[18] Bedran-Russo AKB, Pashley DH, Agee K, Drummond JL, Miescke KJ (2008) Changes in stiffness of demineralized dentin following application of collagen crosslinkers. J Biomed Mater Res - Part B Appl Biomater 86: 330–334. doi:10.1002/jbm.b.31022.

[19] Bedran-Russo AKB, Pereira PNR, Duarte WR, Drummond JL, Yamauchi M (2007) Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Biomed Mater Res B Appl Biomater 80: 268–272. doi:10.1002/jbm.b.30593.

[20] Castellan CS, Pereira PN, Grande RHM, Bedran-Russo AK (2010) Mechanical characterization of proanthocyanidin-dentin matrix interaction. Dent Mater 26: 968–973. doi:10.1016/j.dental.2010.06.001.

[21] Castellan CS, Bedran-Russo AK, Karol S, Pereira PNR (2011) Long-term stability of dentin matrix following treatment with various natural collagen cross-linkers. J Mech Behav Biomed Mater 4: 1343–1350. doi:10.1016/j.jmbbm.2011.05.003.

[22] Song SE, Choi BK, Kim SN, Yoo YJ, Kim MM, Park SK, Roh SS, Kim CK (2003) Inhibitory effect of procyanidin oligomer from elm cortex on the matrix metalloproteinases and proteases of periodontopathogens. J Periodontal Res 38: 282–289. http://www.ncbi.nlm.nih.gov/pubmed/12753366.

[23] Karol S, Bedran-Russo AK (2010) Nanomechanical properties of demineralized dentin treated with collagen cross linkers. Dent Mater. doi:10.1016/j.dental.2009.11.083.

[24] Bedran-Russo AKB, Castellan CS, Shinohara MS, Hassan L, Antunes A (2011) Characterization of biomodified dentin matrices for potential preventive and reparative therapies. Acta Biomater 7: 1735–1741. doi:10.1016/j.actbio.2010.12.013.

[25] Aguiar TR, Vidal CMP, Phansalkar RS, Todorova I, Napolitano JG, McAlpine JB, Chen SN, Pauli GF, Bedran-Russo AK (2014) Dentin Biomodification Potential Depends on Polyphenol Source. J Dent Res 93: 417–422. doi:10.1177/0022034514523783.

[26] Fang M, Liu R, Xiao Y, Li F, Wang D, Hou R, Chen J (2012) Biomodification to dentin by a natural crosslinker improved the resin-dentin bonds. J Dent 40: 458–466. doi:10.1016/j.jdent.2012.02.008.

[27] Liu R, Fang M, Xiao Y, Li F, Yu L, Zhao S, Shen L, Chen J (2011) The effect of transient proanthocyanidins preconditioning on the cross-linking and mechanical properties of demineralized dentin. J Mater Sci Mater Med 22: 2403–2411. doi:10.1007/s10856-011-4430-4.

[28] Schulz KF, Altman DG, Moher D, Group C (2010) CONSORT 2010 statement : Updated guidelines for reporting parallel group randomised trials. doi:10.4103/0976-

500X.72352.

[29] Loguercio AD, Reis A, Barbosa AN, Roulet JF (2003) Five-year double-blind randomized clinical evaluation of a resin-modified glass ionomer and a polyacid-modified resin in noncarious cervical lesions. J Adhes Dent 5: 323–332. http://www.ncbi.nlm.nih.gov/pubmed/15008339.

[30] Franco EB, Benetti AR, Ishikiriama SK, Santiago SL, Lauris JRP, Jorge MFF, Navarro MFL (2006) 5-year Clinical Performance of Resin Composite Versus Resin Modified Glass Ionomer Restorative System in Non-carious Cervical Lesions. Oper Dent 31: 403–408. doi:10.2341/05-87.

[31] Swift EJ, Perdigão J, Heymann HO, Wilder AD, Bayne SC, May KN, Sturdevant JR, Roberson TM (2001) Eighteen-month clinical evaluation of a filled and unfilled dentin adhesive. J Dent 29: 1–6. http://www.ncbi.nlm.nih.gov/pubmed/11137632.

[32] Da Costa TRF, Loguercio AD, Reis A (2013) Effect of enamel bevel on the clinical performance of resin composite restorations placed in non-carious cervical lesions. J Esthet Restor Dent 25: 346–356. doi:10.1111/jerd.12042.

[33] Schmalz G, Ryge G (2005) Reprint of Criteria for the clinical evaluation of dental restorative materials. Clin Oral Investig 9: 215–232. doi:10.1007/s00784-005-0018-z.

[34] Hickel R, Peschke A, Tyas M, Mjör I, Bayne S, Peters M, Hiller KA, Randall R, Vanherle G, Heintze SD (2010) FDI World Dental Federation - clinical criteria for the evaluation of direct and indirect restorations. Update and clinical examples. J Adhes Dent 12: 259–272. doi:10.3290/j.jad.a19262.

[35] Dalton Bittencourt D, Ezecelevski IG, Reis A, Van Dijken JW, Loguercio AD (2005) An 18-months' evaluation of self-etch and etch & rinse adhesive in non-carious cervical lesions. Acta Odontol Scand 63: 173–178. doi:10.1080/00016350510019874.

[36] Perdigão J, Sezinando A, Monteiro PC (2012) Laboratory bonding ability of a multi-purpose dentin adhesive. Am J Dent 25: 153–158.

http://www.ncbi.nlm.nih.gov/pubmed/22988685.

[37] ADA Council on Scientific Affairs (2001) Revised American Dental Association acceptance program guidelines: dentin and enamel adhesives. American Dental Association, Chicago, pp 1–9.

[38] Perdigão J, Kose C, Mena-Serrano A, De Paula E, Tay L, Reis A, Loguercio A (2014) A New Universal Simplified Adhesive: 18-Month Clinical Evaluation. Oper Dent 39: 113–127. doi:10.2341/13-045-C.

[39] Scheffel D, Hebling J, Scheffel R, Agee K, Turco G, de Souza Costa C, Pashley D (2014) Inactivation of Matrix-bound Matrix Metalloproteinases by Cross-linking Agents in Acid-etched Dentin. Oper Dent 39: 152–158. doi:10.2341/12-425-L.

[40] Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, Tay FR (2011) Limitations in Bonding to Dentin and Experimental Strategies to Prevent Bond Degradation. J Dent Res 90: 953–968. doi:10.1177/0022034510391799.

[41] Seseogullari-Dirihan R, Mutluay MM, Pashley DH, Tezvergil-Mutluay A (2017) Is the inactivation of dentin proteases by crosslinkers reversible? Dent Mater 33: 62–68. doi:10.1016/j.dental.2016.09.036.

[42] Kalachandra S, Turner DT (1989) Water sorption of plasticized denture acrylic lining materials. Dent Mater 5: 161–164. http://www.ncbi.nlm.nih.gov/pubmed/2637211.

[43] Bartlett DW, Shah P (2006) A Critical Review of Non-carious Cervical (Wear) Lesions and the Role of Abfraction, Erosion and Abrasion. J Dent Res 85: 306–312. doi:10.1177/154405910608500405.

[44] Wood I, Jawad Z, Paisley C, Brunton P (2008) Non-carious cervical tooth surface loss: A literature review. J Dent 36: 759–766. doi:10.1016/j.jdent.2008.06.004.

[45] Tay FR, Kwong SM, Itthagarun A, King NM, Yip HK, Moulding KM, Pashley DH (2000) Bonding of a self-etching primer to non-carious cervical sclerotic dentin:

interfacial ultrastructure and microtensile bond strength evaluation. J Adhes Dent 2: 9–28. http://www.ncbi.nlm.nih.gov/pubmed/11317411.

[46] Hickel R, Roulet JF, Bayne S, Heintze SD, Mjör IA, Peters M, Rousson V, Randall R, Schmalz G, Tyas M, Vanherle G (2007) Recommendations for conducting controlled clinical studies of dental restorative materials. Science Committee Project 2/98--FDI World Dental Federation study design (Part I) and criteria for evaluation (Part II) of direct and indirect restorations includi. J Adhes Dent 9: 121–147. http://www.ncbi.nlm.nih.gov/pubmed/18341239.

[47] Mena-Serrano A, Kose C, De Paula EA, Tay LY, Reis A, Loguercio AD, Perdigão J (2013) A new universal simplified adhesive: 6-month clinical evaluation. J Esthet Restor Dent 25: 55–69. doi:10.1111/jerd.12005.

[48] Piva F, Coelho-de-Sousa FH, Ribeiro CS (2009) A deciduous teeth composite restoration clinical trial using two methods. J Dent Res.

[49] Loguercio AD, Andrade E, Paula D, Hass V, Luque-martinez I, Reis A, Perdigão J (2015) A new universal simpli fi ed adhesive : 36-Month randomized double-blind clinical trial. J Dent 43: 1083–1092. doi:10.1016/j.jdent.2015.07.005.

[50] Lawson NC, Robles A, Fu C, Paul C, Sawlani K, Burgess JO (2015) Two-year clinical trial of a universal adhesive in total-etch and self-etch mode in non-carious cervical lesions. J Dent 43: 1229–1234. doi:10.1016/j.jdent.2015.07.009.

[51] Peumans M, De Munck J, Mine A, Van Meerbeek B (2014) Clinical effectiveness of contemporary adhesives for the restoration of non-carious cervical lesions. Dent Mater 30: 1089–1103. doi:10.1016/j.dental.2014.07.007.

[52] Hickel R, Brüshaver K, Ilie N (2012) Repair of restorations – Criteria for decision making and clinical recommendations. Dent Mater 29: 28–50. doi:10.1016/j.dental.2012.07.006.

[53] Moreira MA, Souza NO, Sousa RS, Freitas DQ, Lemos MV, De Paula DM, Maia FJN,

Lomonaco D, Mazzetto SE, Feitosa VP (2017) Efficacy of new natural biomodification agents from Anacardiaceae extracts on dentin collagen cross-linking. Dent Mater 33: 1103–1109. doi:10.1016/j.dental.2017.07.003.

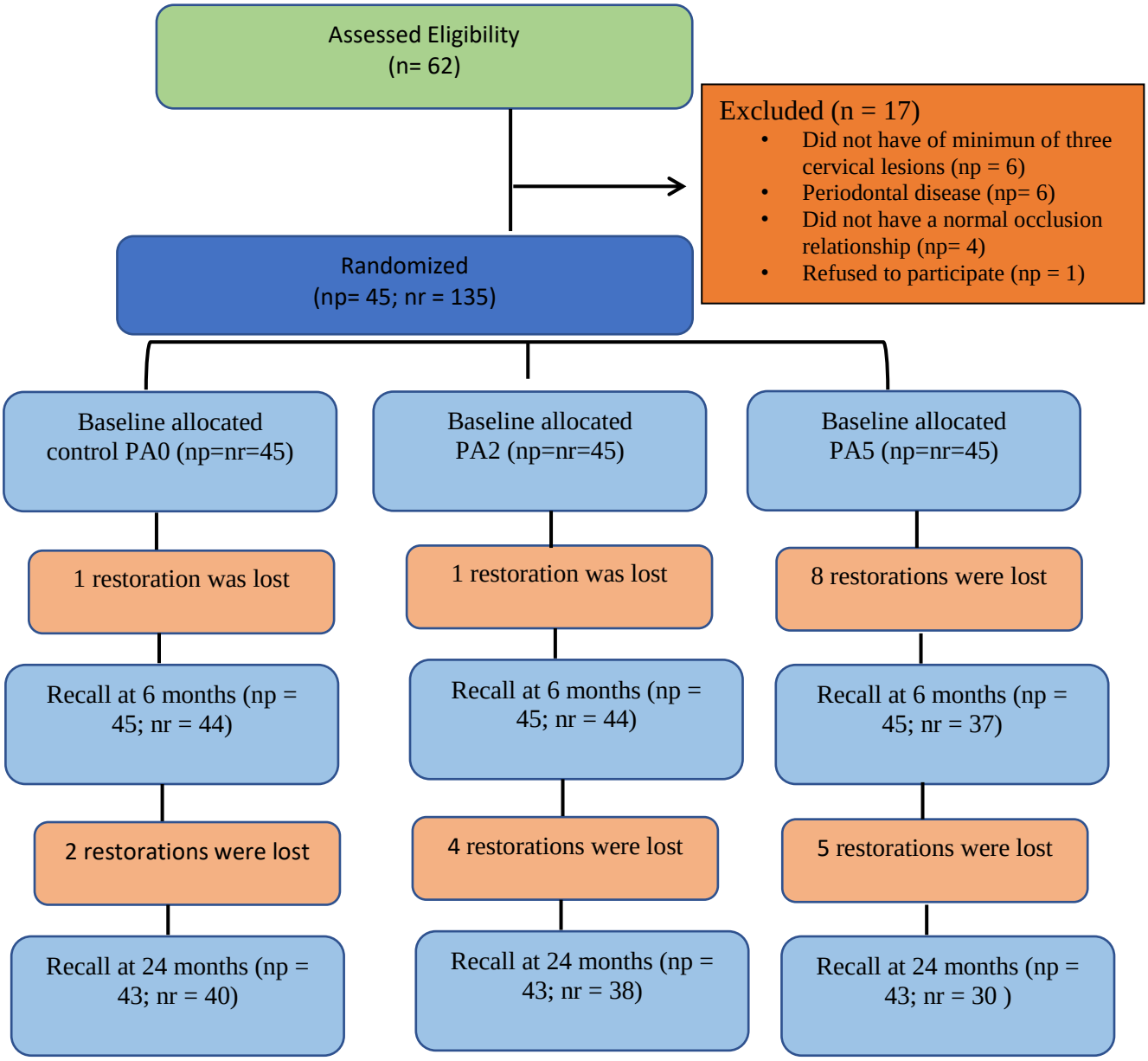
[54] Machado AC, Dezan Junior E, Gomes-Filho JE, Cintra LTA, Ruviére DB, Zoccal R, Damante CA, Jardim Junior EG (2012) Evaluation of tissue reaction to Aroeira (Myracrodruon urundeuva) extracts: a histologic and edemogenic study, J. Appl. Oral Sci. 20 (2012) 414–418. doi:10.1590/S1678-77572012000400005.

[55] Phansalkar RS, Nam JW, Chen SN, McAlpine JB, Napolitano JG, Leme A, Vidal CM, Aguiar T, Bedran-Russo AK, Pauli GF (2015) c. Fitot. 101:169-78. doi: 10.1016/j.fitote.2014.12.006.

[56] Nam JW, Phansalkar RS, Lankin DC, McAlpine JB, Leme-Kraus AA, Vidal CM, Gan LS, Bedran-Russo A, Chen SN, Pauli GF (2017) J Org Chem. 82(3):1316-1329. doi: 10.1021/acs.joc.6b02161.

Legends of figure:

Figure 1 – Flow diagram. Np: number of patients, Nr: number of restorations. PA2 = 2% proanthocyanidin solution; PA5: 5% proanthocyanidin solution.



Legends of Tables:

Table 1 - Dentin sclerosis scale.

Table 2 – Materials, composition and application mode.

Table 3 - World Dental Federation (FDI) criteria used for clinical evaluation [34].

Table 4 - Modified United States Public Health Service (USPHS) criteria according to Bittencourt and others [35] and Perdigão and others [36].

Table 5 - Distribution of noncarious cervical lesions according to research subject (gender and age) and characteristics of Class V lesions (shape, cervicoincisal size of the lesion, degree of sclerotic dentin, presence of antagonistic, presence of attrition facets, presence of preoperative sensitivity, and tooth and arch distribution).

Table 6 - Number of evaluated restorations for each experimental group (PA0 [no pretreatment with PA], PA2 [2% proanthocyanidin applicated before the adhesive system] and PA5 [5% proanthocyanidin applicated before the adhesive system] classified according to the World Dental Federation (FDI) criteria[34].

Table 7 - Number of evaluated restorations for each experimental group (PA0 [no pretreatment with PA], PA2 [2% proanthocyanidin applicated before the adhesive system] and PA5 [5% proanthocyanidin applicated before the adhesive system] classified according to the adapted United States Public Health Service (USPHS) criteria [35], [36].

Table 8 - Restorations acceptable or not acceptable according to the Federation Dental International (FDI) criteria after 24 months [34].

Table 1

Dentin sclerosis scale*	
CATEGORY	**CRITERIA**
1	No sclerosis present; dentin is light yellowish or whitish, with little discoloration; dentin is opaque, with little translucency or transparency
2	More sclerosis than in category 1 but less than halfway between categories 1 and 4
3	Less sclerosis than in category 4 but more than halfway between categories 1 and 4
4	Significant sclerosis present; dentin is dark yellow or even discolored (brownish); glassy appearance, with significant translucency or transparency evident

* Adapted from Swift and co

lleagues[31] with permission from Elsevier.

Table 2 - Materials, composition and application mode.

Materials	Composition (*)	Application Mode (**)
Condac 37 phosphoric acid (FGM,Joinville, Santa Catarina, Brazil)	Phosphoric acid 37% wt%, thickening agents and pigments.	1. Prepare the region to be etched by cleaning 2. Drying it 3. Apply Condac 37 to the area to be etched and wait for a period of 15 seconds 4. Wash the surface with plenty of water 5. Dry the cavity in such a manner that the dentin does not become dehydrated.
ExciTE F adhesive systems (Ivoclar Vivadent, Schaan, Liechnstein)	Contains HEMA, dimethacrylate, Bis-GMA, UDMA, phosphonic acid acrylate, highly dispersed silicone dioxide, initiators, stabilizers and potassium fluoride in an ethanol solution.	6. Apply to the enamel and dentin and agitate the adhesive on the prepared surfaces for at least 10 seconds. Make sure that all the cavity walls are completely covered 7. Disperse to a thin layer with a weak stream of air, thereby removing any excess. 8. Polymerize for 10 seconds at a light intensity of more than 500 mW/cm2
IPS Empress Direct resin composite (Ivoclar Vivadent, Schaan, Liechnstein)	Dimethacrylates (20-21.5 wt%, opalescent shade 17 wt%). The fillers contain barium glass, ytterbium trifluoride, mixed oxide, silicon dioxide and copolymer (77.5-79 wt%, opalescent shade 83 wt%). Additional contents: additives, initiators, stabilizers and pigments (<1.0 wt%). The total content of inorganic fillers is 75-79 wt% or 52-59 vol% (opalescent shade 60.5 wt% or 45 vol%). The particle size of the inorganic fillers is between 40 nm and 3 μm with a mean particle size of 550 nm.	9. Apply IPS Empress Direct Effect in layers of max. 2 mm thickness. 10. Polymerize each layer for 20 s and keep the light emission window as close as possible to the surface of the restorative material

(*) HEMA = 2-hydroxyethyl methacrylate Bis-GMA = bisphenol glycidyl methacrylate; UDMA = urethane dimethacrylate

(**) According to the manufacturer's instructions

Table 3

	Esthetic Property	**Functional Properties**		**Biological Properties**	
	1. Staining margin	**2. Fractures and retention**	**3. Marginal adaptation**	**4. Postoperative (hyper-) sensitivity**	**5. Recurrence of caries**
1. Clinically very good	1.1 No marginal staining	2.1 Restoration retained, no fractures / cracks	3.1 Harmonious outline, no gaps, no discoloration.	4.1 No hypersensitivity.	5.1 No secondary or primary caries
2. Clinically good (after correction very good	1.2 Minor marginal staining, easily removable by polishing.	2.2 Small hairline crack.	3.2.1 Marginal gap (50 μm). 3.2.2 Small marginal fracture removable by polishing.	4.2 Low hypersensitivity for a limited period of time	5.2 Very small and localized demineralization. No operative treatment required
3.Clinically sufficient / satisfactory (minor shortcomings with no adverse effects but not adjustable without damage to the tooth)	1.3 Moderate marginal staining, not esthetically unacceptable.	2.3 Two or more or larger hairline cracks and/or chipping (not affecting the marginal integrity).	3.3.1 Gap < 150 μm not removable 3.3.2. Several small enamel or dentin fractures	4.3.1 Premature / slightly more intense 4.3.2 Delayed/weak sensitivity; no subjective complaints, no treatment needed.	5.3 Larger areas of demineralization, but only preventive measures necessary (dentine not exposed)
4. Clinically unsatisfactory (repair for prophylactic reasons)	1.4 Pronounced marginal staining; major intervention necessary for improvement	2.4 Chipping fractures which damage marginal quality; bulk fractures with or without partial loss (less than half of the restoration).	3.4.1 Gap > 250 μm or dentine/base exposed. 3.4.2. chip fracture damaging margins 3.4.3 Notable enamel or dentine wall fracture	4.4.1 Premature/ very intense 4.4.2 Extremely delayed/weak with subjective complaints 4.4.3 Negative Sensitivity Intervention necessary but not replacement.	5. 4 Caries with cavitation (localized and accessible and can be repaired
5. Clinically poor (replacement necessary)	1.5 Deep marginal staining not accessible for intervention.	2.5 (Partial or complete) loss of restoration.	3.5 Filling is loose but in situ.	4.5 Very intense, acute pulpitis or non vital. Endodontic treatment is necessary and restoration has to be replaced.	5.5 Deep secondary caries or exposed dentine that is not accessible for repair of restoration.
Acceptable or not acceptable (n, % and reasons	Aesthetic criteria	Functional criteria		Biological criteria	

Table 4

	Marginal staining	**Retention**	**Fracture**	**Marginal adaptation**	**Postoperative sensitivity**	**Recurrence of caries**
Alfa	No discoloration along the margin	Retained	None	Restoration is continuous with existing anatomic form.	No postoperative sensitivity directly after the restorative process and during the study period	None evidence of caries contiguous with the margin
Bravo	Slight and superficial staining (removable, usually localized)	Partially retained	Small chip, but clinically acceptable	Detectable V-shaped defect in enamel only. Catches explorer going both ways.	--	--
Charlie	Deep staining cannot be polished away	Missing	Failure due to bulk restorative fracture	Detectable V-shaped defect to dentin-enamel junction	Sensitivity present at any time during the study period	Evidence of presence of caries

Table 5

Characteristics of research subjects			Number of
Gender distribution			
Male			28
Female			17
Age distribution (years)			
20-29			06
30-39			11
40-49			9
> 49			19
Characteristics of Class-V lesions			**Number of**
	PA0	PA2	PA5
Shape (degree of angle)			
< 45	1	2	2
45-90	10	12	15
90-135	19	18	16
> 135	15	13	12
Cervico-incisal height (mm)			
< 1.5	2	7	7
1.5-2.5	28	22	25
> 2.5	15	16	13
Degree of sclerotic dentin			
1	22	19	22
2	13	16	15
3	9	9	6
4	1	1	2
Presence of antagonist			
Yes	45	45	45
No	00	00	00
Attrition facet			
Yes	43	41	42
No	2	4	3
Pre-operative sensitivity (spontaneous)			
Yes	00	00	00
No	45	45	45
Pre-operative sensitivity (air dry)			
Yes	24	21	24
No	21	24	21
Tooth distribution			
Anterior			
Incisor	6	5	9
Canines	9	14	5
Posterior			
Premolar	28	23	29
Molar	2	3	2
Arc distribution			
Maxillary	20	19	20
Mandibular	25	26	25

Table 6

Time		Baseline			6 months			24 months		
FDI Criteria	***(*)***	PA0	PA2	PA5	PA0	PA2	PA5	PA0	PA2	PA5
Marginal adaptation	**VG**	45	45	45	13	09	09	04	01	--
	GO	--	--	--	28	34	24	33	35	27
	SS	--	--	--	03	01	04	03	02	03
	UN	--	--	--	--	--	--	--	--	--
	PO	--	--	--	--	--	--	--	--	--
Marginal staining	**VG**	45	45	45	40	37	36	26	23	17
	GO	--	--	--	02	02	01	07	04	10
	SS	--	--	--	02	05	--	07	11	03
	UN	--	--	--	--	--	--	--	--	--
	PO	--	--	--	--	--	--	--	--	--
Fractures and retention	**VG**	45	45	45	44	44	37	40	38	30
	GO	--	--	--	--	--	--	--	--	--
	SS	--	--	--	--	--	--	--	--	--
	UN	--	--	--	--	--	--	--	--	--
	PO	--	--	--	01	01	08	03	05	13
Post-operative sensitivity	**VG**	45	45	45	44	44	37	40	38	30
	GO	--	--	--	--	--	--	--	--	--
	SS	--	--	--	--	--	--	--	--	--
	UN	--	--	--	--	--	--	--	--	--
	PO	--	--	--	--	--	--	--	--	--
Recurrence of caries	**VG**	45	45	45	44	44	37	40	38	30
	GO	--	--	--	--	--	--	--	--	--
	SS	--	--	--	--	--	--	--	--	--
	UN	--	--	--	--	--	--	--	--	--
	PO	--	--	--	--	--	--	--	--	--

(*) VG for clinically very good; GO for clinically good; SS for clinically sufficient/satisfactory; UN for clinically unsatisfactory and; PO for clinically poor.

Table 7

Time		Baseline			6 months			24 months		
USPHS Criteria		PA0	PA2	PA5	PA0	PA2	PA5	PA0	PA2	PA5
Marginal adaptation	**Alfa**	45	45	45	41	43	33	37	35	28
	Bravo	--	--	--	03	01	04	03	02	03
	Charlie	--	--	--	--	--	--	--	--	--
Retention	**Alfa**	45	45	45	44	44	37	40	37	31
	Charlie	--	--	--	01	01	08	03	06	12
Marginal staining	**Alfa**	45	45	45	42	69	37	33	26	28
	Bravo	--	--	--	02	05	--	07	11	03
	Charlie	--	--	--	--	--	--	--	--	--
Post-operative sensitivity	**Alfa**	45	45	45	44	44	37	40	37	31
	Charlie	--	--	--	--	--	--	--	--	--
Recurrence of caries	**Alfa**	45	45	45	44	44	37	40	37	31
	Charlie	--	--	--	--	--	--	--	--	--

Table 8

Properties	**Aesthetic**			**Functional**						**Biological**					
	Marginal staining			**Fractures and retention**			**Marginal adaptation**			**Postoperative (hyper-) sensitivity**			**Recurrence of caries**		
	PA0	**PA2**	**PA5**	**PA0**	**PA2**	**PA5**	**PA0**	**PA2**	**PA5**	**PA0**	**PA2**	**PA5**	**PA0**	**PA2**	**PA5**
Acceptable	40	38	30	40	38	30	40	38	30	40	38	30	40	38	30
Not acceptable	-	-	-	-	-	-	3	5	13	-	-	-	-	-	-
Reasons				Total loss of the restorations: 21											

CAPÍTULO 2

Two-year clinical evaluation of proanthocyanidin incorporation in two-step etch-and-rinse adhesive system

Short Title: Proanthocyanidin incorporation in adhesive system – clinical trial

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Keywords: Dentin adhesive; Non-carious cervical lesions; Proanthocyanidin; Longevity; Clinical trial.

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Two-year clinical evaluation of proanthocyanidin incorporation in two-step etch-and-rinse adhesive system

ABSTRACT

Objective: The aim of this double-blind randomized clinical trial was to compare the retention rates of Proanthocyanidin (PA)-free and PA-containing etch-and-rinse simplified adhesive systems on the clinical behavior of resin composite restorations in non-carious cervical lesions (NCCLs) over a 6- and 24-month period.

***Methods*:** A total of 135 restorations were randomly placed in 45 patients. The NCCLs were conditioned (37% phosphoric acid for 15 s) and distributed into 3 groups: Control (EX0) - adhesive ExciTE F (Ivoclar Vivadent) applied following the manufacturer's recommendations; EX2 and EX5 – 2wt% and 5wt% of PA from grape-seed extract was added to adhesive ExciTE F, respectively, and applied according to the EX0. The resin composite was placed incrementally and light-cured. After one-week, each one was finished and polished. The restorations were evaluated at baseline, 6 and 24 months, using FDI and USPHS criteria. Statistical analyses were performed using Friedman and Wilcoxon tests (α=0.05).

Results: The retention rates were 98% (EX0), 92% (EX2) and 85% (EX5) after 6 months. In this period, a significant difference was found only for EX5 when compared with their baseline findings ($p = 0.03$) and when compared with EX0 and EX2 ($p = 0.001$). After 24 months, the retention rates were 98% (EX0), 73% (EX2) and 71% (EX5). Only EX0 did not show a significant difference when compared with their baseline and showed a significant higher retention when compared with EX2 and EX5 in this period ($p=0.001$).

Conclusion: The incorporation of proanthocyanidin into adhesive did not present clinical advantages after 24 months of clinical evaluation.

Clinical relevance: The use of proanthocyanidin incorporated into adhesive system, while more acceptable to clinicians, impairs the longevity of restorations, probably because it causes changes in the degree of conversion of adhesive systems.

Keywords: Dentine adhesive; Non-carious cervical lesions; Proanthocyanidin; Longevity; Clinical trial.

1. Introduction

A resistant long-term resin-dentin bond is fundamental for the success of adhesive restorations. In the ideal adhesion, adhesive monomers must thoroughly infiltrate and encapsulate exposed collagen fibrils after etching, creating the hybrid layer, however, in general, this does not occur. [1] As a result, collagen fibrils in the hybrid layer are partially exposed and susceptible to deterioration. [2] The most often degradation of this collagen, as well as adhesive resin, is due to a variety of physical and chemical factors, including hydrolysis and enzymatic action such as host-derived matrix metalloproteinases and cathepsins. [3-7]

So, the strengthening of collagen fibrils could increase the resistance of the resin-dentin interface. The use of crosslinking agents to increase mechanical properties and decrease enzymatic degradation has been an important application in restorative dentistry. [8-10]

Several strategies were developed to decrease the collagen degradation using enzymatic inhibitors, as well as, increasing the collagen's resistance against the degradation process. [10-12] These two associated treatments may improve the stability of the resin–dentin bonded interface and this was the main purpose of incorporating collagen cross-linkers into the bonding process. [10]

The use of collagen cross-linking agent stabilizes and strengthen collagen fibrils of dentin matrix, reduce biodegradation rates of collagen and improve the mechanical stability, extending the longevity of adhesive restorations. The most evaluated substance with this purpose is the proanthocyanidin. [10,13,14]

Several studies have shown that PA as primer improves the durability of the resine–dentine bonds. [13-16] However, PA as primer adds an extra step to the bonding

protocol, contradicting the clinician's preference for simplification. Thus, the addition of PA into adhesive system seems more clinically acceptable. [17]

Incorporating PA into dentin adhesives may provide a new delivery method that allows the substance to remain in the hybrid layer for an extended period of time, enhancing the degree of collagen cross-linking. Some studies have incorporated proanthocyanidin in the adhesive resins. [17-20] Green et al. (2010) [20] evaluated models of adhesives formulated with and without 5% PA and concluded that the presence of grape seed extract PA in dental adhesives may inhibit the biodegradation of unprotected collagen fibrils within the hybrid layer. Epasinghe et al. (2012) [17] incorporated PA 1%, 2% and 3% into experimental etch-and-rinse adhesives to evaluate the effect on dentine bond strength fibrils and showed that incorporation of 2% proanthocyanidin into dental adhesives has no adverse effect on dentine bond strength.

Unfortunately, to extent of our knowledge, no clinical trials were conducted to predict the effect of PA into the adhesive system on clinical performance of adhesive restorations in non-carious cervical lesions (NCCLs). Therefore, the aims of this double-blind, randomized equivalence clinical trial were to compare the retention rates of PA-free and 2% and 5% PA-containing etch-and-rinse adhesive systems (ExiciTE F) on the clinical behavior of composite restorations in NCCLs over a 6 and 24 months period, using two evaluation criteria: World Dental Federation (FDI) and United States Public Health Service (USPHS) criteria. The null hypothesis tested was that bonding to NCCLs with PA-free or 2% and 5% PA-containing etch-and-rinse adhesive systems yield similar clinical performance over 6- and 24-month of clinical service.

2. Materials and methods

The description of the experimental design followed the Consolidated Standards of Reporting Trials (CONSORT) statement. [21]

2.1 Ethics approval

The local Ethics Committee on Investigations Involving Human Subjects reviewed and approved the protocol and issued a consent form for this study (protocol 640.695). Written informed consent was obtained from all patients prior to starting the treatment.

2.2 Protocol registration

This clinical trial was registered in http://www.ensaiosclinicos.gov.br/ clinical registry under protocol RBR-366MBJ. All participants were informed about the nature and objectives of the study.

2.3 Trial design, settings and location of data collection

This was a double-blind, equal allocation rate, split-mouth randomized clinical trial. The study was carried out in the clinics of the School of Dentistry at the local University from November 2014 to January 2017.

Recruitment

Patients were recruited as they seek for treatment in the clinics of Dentistry of the local university. No advertisement was made for participant recruitment. Patients were recruited in the order in which they reported for the screening session, thus forming a sample of convenience.

Eligibility criteria

A total of 69 participants were examined by two calibrated dental postgraduate students to check if they met the inclusion and exclusion criteria (Figure 1). The

evaluations were performed using a mouth mirror, an explorer, and a periodontal probe. Participants had to be in good general health, older than 18 years old, have an acceptable oral hygiene level, and present at least 20 teeth under occlusion.

Participants were required to have at least three NCCLs to be restored in three different teeth. These lesions had to be non-retentive, deeper than 1 mm, and involving both the enamel and dentin of vital teeth without mobility. The cavosurface margin could not involve more that 50% of enamel.[22] Patients with extremely poor oral hygiene, severe or chronic periodontitis, or heavy bruxism habits were excluded from the study as they would receive other treatments before restorative intervention.

2.4 Sample size calculation

The two-step etch-and-rinse simplified ExciTE F adhesive system (Ivoclar Vivadent AG, Schaan, Liechtenstein) was used in the present study. The percentage of retention of the ExciTE adhesive system is 73% after 5 years of clinical evaluation.[23] Considering a 5% alpha, an 80% power and a two-sided test, the minimum sample size was 43 restorations per group to find a 22% difference between the tested groups

2.5 Random sequence generation and allocation concealment

The randomization was done on an intra-individual basis so that each subject ended up with three restorations. These randomization schemes were performed using software available at http://www. sealedenvelope.com.

A staff member not involved in the research protocol performed the randomization process with computer-generated tables. Details of the allocated groups were recorded on cards contained in sequentially numbered, opaque, sealed envelopes. Opening the envelope only on the day of the restorative procedure

guaranteed the concealment of the random sequence. In all cases, the tooth with the highest tooth number received the treatment described first, while the tooth with the next number in sequence received the treatment mentioned second and the next tooth received the treatment mentioned third.

2.6 Interventions: restorative procedure

Forty-five patients were selected for this study and all received dental prophylaxis with a suspension of pumice and water in a rubber cup and signed an informed consent before the restorative procedures.

The degree of sclerotic dentin from the NCCLs was measured according to the criteria described by Swift and others[24] (Table 1). The cavity dimensions in millimeters (height, width, and depth), the geometry of the cavity (evaluated by profile photograph and labeled at <45º, 45º-90º, 90º<135º, and >135º), [25] the presence of an antagonist, and the presence of attrition facets were observed and recorded. Pre-operative sensitivity was also evaluated by applying an air-blast for 10 s from a dental syringe placed 2 cm from the tooth surface and with an explorer. These features were recorded to allow comparison of the baseline features of the dentin cavities among experimental groups.

To calibrate the restorative procedure, the study director placed one restoration of each group to identify all steps involved in the application technique. Then, one operator, who has more than five years of clinical experience in operative dentistry, placed three restorations, one of each group, under the supervision of the study director in a clinical setting. The restoration failures were shown to the operator prior to starting the study. At this point, the operator was considered calibrated to perform the restorative procedures.

One operator restored all teeth. All participants received three restorations, one of each experimental group in different lesions previously selected according to the inclusion criteria.

Before restorative procedures, the operator cleaned all lesions with pumice and water in a rubber cup, followed by rinsing and drying. Then, shade selection was made using a shade guide (Ivoclar Vivadent AG, shade guide, Schaan, Liechtenstein). The tooth to be restored was isolated with cotton rolls and a light cured gingival barrier (Top Dam, FGM, Joinville, Santa Catarina, Brazil). The operator did not prepare any additional retention or bevel in the class V cavity.

The adhesive (ExciTE F, Ivoclar Vivadent, Schaan, Liechtenstein) (Table 2) was used as control. For experimental groups, the same adhesive was modified by the incorporation of 2 mg of proanthocyanidin (PA) (V. vinifera,Meganatural Gold, Madera, CA, USA) to 98 mg of the adhesive or the incorporation of 5 mg PA (V. vinifera, Meganatural Gold, Madera, CA, USA) to 95 mg of the adhesive to form a mixture with PA concentration of 2.0 wt% or 5.0 wt%, respectively. The teeth were distributed in these 3 groups and the adhesives were applied as described below. The materials, compositions and application modes are described in Table 2.

- *EXO (Control)*– The 37% phosphoric acid (Condac acid, FGM, Brazil) was applied for 15 s. Then, cavities were rinsed thoroughly for 15 s, keeping dentin visible moist slightly with absorbent paper-dried, One coat of adhesive was gently scrubbed on the entire enamel and dentin surface for approximately 10 s, according to the manufacturer's recommendations (Table 2). Then, the solvent was evaporated by gentle air stream for 5 s and light cured for 10 s at 1250 mW/cm^2 (Emitter A Schuster, Santa Maria, RS, Brazil).

- EX*2* – The 37% phosphoric acid (Condac 37% acid, FGM, Brazil) was applied for 15 s. Then, cavities were rinsed thoroughly for 15 s, and slightly with absorbent paper, keeping dentin visible moist. A modified adhesive ExciTE F with 2% proanthocyanidin was applied according to the control group.

- *EX5* - The 37% phosphoric acid (Condac 37% acid, FGM, Brazil) was applied for 15 s. Then, cavities were rinsed thoroughly for 15 s, and slightly with absorbent paper, keeping dentin visible moist. A modified adhesive ExciTE F with 5% proanthocyanidin was applied according to the control group

The resin composite Empress Direct (Ivoclar Vivadent, Schaan, Liechtenstein) resin composite was used in up to three increments, each one being lightly cured for 20 s at 1250 mW/cm^2 (Emitter A Schuster, Santa Maria, RS, Brazil). The restorations were finished immediately with fine and extra-fine #3195 diamond burs (KG Sorensen, Barueri, SP, Brazil) under constant water-cooling. After one-week, each one was finished and polished with slow-speed polishing points (Jiffy Polishers, Ultradent, South Jordan, UT, USA).

2.7 Calibration procedures for clinical evaluation

For training purposes, two experienced and calibrated examiners observed 10 photographs that were representative of each score for each criterion. They evaluated 10 patients each on two consecutive days. These subjects had cervical restorations but were not part of this project. An intra-examiner and inter-examiner agreement of at least 85% was necessary before beginning the evaluation. [26]

2.7.1 Blinding

The examiners, who were not involved with the restoration procedures and therefore blinded to the group assignment, performed the clinical evaluation. Patient

were also blinded to group assignment in a double-blind randomized clinical trial design.

2.7.2 Clinical evaluation

An individual standardized paper case report form was used for each evaluator at each recall time so that evaluators were kept blinded to earlier evaluations during the follow-up recalls. Intraoral color photographs were collected at baseline and at the recall appointments to aid in the evaluation, if necessary. Clinical photographs consisted of digital images obtained using a Nikon D90X camera with a 105-mm Medical Nikon lens (Nikon Inc., Melville, NY, USA).

The restorations were evaluated by World Federation criteria (FDI) [27] and the classical United States Public Health Service (USPHS) criteria (adapted by Bittencourt and others [28] and Perdigão and others [29] at baseline, after 6 and 24 months of clinical service. Only the clinically relevant measures for evaluation of adhesive performance were used and scored (Tables 3 and 4). The primary clinical endpoint was restoration retention/fracture, but the following secondary endpoints were also evaluated: marginal staining, marginal adaptation, postoperative sensitivity, and recurrence of caries.

These variables were ranked according to FDI criteria into clinically very good, clinically good, clinically sufficient/ satisfactory, clinically unsatisfactory but repairable, and clinically poor (replacement required) [27] and in the USPHS criteria into Alfa, Bravo and Charlie. [28] Both examiners evaluated all the restorations once and independently. When disagreements occurred during the evaluations, they had to reach a consensus before the participant was dismissed. The restoration retention rates were calculated according to the ADA guidelines.[30] Cumulative failure percentage =

[(PF + NF) / (PF + RR)] X 100%, where PF is the number of previous failures before the current recall, NF is the number of new failures during the current recall, and RR is the number of currently recalled restorations.

2.8 Statistical Analysis

The statistical analyses followed the intention-to-treat protocol according to CONSORT (Consolidated Standards of Reporting Trials) suggestion. [21] Descriptive statistics were used to describe the distributions of the evaluated criteria. Statistical analysis for each individual item was performed for each evaluation criteria (FDI and USPHS criteria).

The differences in the ratings of the three groups after 6 and 24 months were tested with the Friedman repeated-measures analysis of variance by rank (α=0.05), and differences in the ratings of each group at baseline and after 6 and 24 months were evaluated using the Wilcoxon test (α=0.05). Cohen's kappa statistics was used to test inter-examiner agreement. In all statistical tests, we pre-set the level of significance to 5%.

3. Results

The restorative procedures were implemented exactly as planned and no modification was performed. Twenty-four out of 69 patients were not enrolled in the study because they did not fulfill the inclusion criteria (Figure 1). Thus, 45 subjects were selected. All baseline details relative to the research subjects and characteristics of the restored lesions are displayed in Table 5. All research subjects were evaluated at the baseline and at 6-months and only one patient with three restorations did not attend the 24-month recall rate (Figure 1), because he moved to another city.

3.1 Retention

Twelve restorations were lost at 6 months. According to FDI and USPHS criteria, the 6-month retention rates (95% confidence interval) were 98% (88 – 99%) for EX0; 92% (80 – 97%) for EX2; and 85% (72 – 93%) for EX5. Twenty-nine restorations were lost at 24 months. According to FDI and USPHS criteria, the 24-month retention rates (95% confidence interval) were 98% (88 – 99%) for EX0; 73% (59 – 84%) for EX2; and 71% (56 – 82%) for EX5. When the data from 6-month results from each group were compared with their baseline findings, a significant difference was found only for EX5 ($p = 0.03$; Tables 6 and 7). When the data from 24-month results from each group were compared with their baseline findings, a significant difference was found for EX2 and EX5 ($p = 0.03$; Tables 6 and 7). After 6-month clinical evaluation, the retention rate of the EX0 and EX2 were significantly different when compared with EX5 ($p = 0.001$; Tables 6 and 7). Also, when the retention rate of the EX0 was compared with EX2 and EX5, significant differences in the retention rates were detected after 24-month ($p = 0.001$; Tables 6 and 7).

3.2 Post-operative sensitivity

No restorations showed post-operative sensitivity immediately after restorative procedures according to the FDI and USPHS criteria. After 6 and 24 months, no restoration showed post-operative sensitivity using both the FDI and USPHS criteria (Tables 6 and 7).

3.3 Marginal adaptation

According to the FDI criteria, 93 restorations at the 6-month recall were considered to have some discrepancies in marginal adaptation. After 24-month recall, 126 restorations were considered to have some discrepancies in marginal adaptation.

No significant difference was detected between any pair of groups at the 6- and 24-month recall for both criteria ($p > 0.05$; Tables 6 and 7).

However, significant worsening of marginal adaptation was observed within all groups over time, mainly after 24-month ($p < 0.05$; Tables 6 and 7). Despite the high number of the restorations with lack of marginal adaptation in the FDI criteria, none of them was considered to have clinically relevant discrepancies (clinically unsatisfactory) in the marginal adaptation even after 24-month of clinical evaluation (Table 6).

When the USPHS criteria were used, only 7 restorations were scored as *Bravo* for marginal adaptation at the 6-month recall ($p > 0.05$). After 24-month recall, 10 restorations were scored as *Bravo* for marginal adaptation ($p > 0.05$). No significant difference was detected between any pair of groups at the 6- and 24-month recalls and between recall times within group ($p > 0.05$).

3.4 Marginal discoloration

For the FDI criteria, 18 restorations at the 6-month recall were considered to have minor discrepancies (clinically good and satisfactory). After 24-month recall, 57 restorations at the 24-month recall were considered to have minor discrepancies (clinically good and satisfactory).

A significant difference between baseline vs. 6-month recall was observed for the group EX5 using FDI criteria ($p < 0.05$; Tables 6). However, a significant difference between baseline vs. 24-month recall was observed for all groups using FDI criteria ($p < 0.05$; Tables 6). It worth to mention that, after 24-month recall, EX5 showed a significant higher marginal staining when compared with EX0 and EX2 ($p = 0.001$; Tables 6 and 7).

When the USPHS criteria were used, only eight restorations at 6-month recall were scored as *Bravo* for marginal staining ($p > 0.05$). After 24-month recall, thirty restorations were scored as *Bravo* for marginal staining. A significant difference between baseline vs. 24-month recall was observed for all groups using USPHS criteria ($p < 0.05$; Tables 6). However, after 24-month recall, only EX5 showed a significant higher marginal staining when compared with EX0 ($p = 0.001$; Tables 6 and 7).

3.5 Recurrence of caries

No restoration showed recurrence of caries at the 6- and 24- month clinical recall using the FDI and the USPHS criteria.

3.6 General Overview

When the FDI criteria for 'acceptable' *vs*. 'not acceptable' restorations were applied, only twenty-nine restorations were ranked as 'not acceptable', the majority from EX2 and EX5 groups (Table 8).

4. Discussion

With the promising results of the use of PA as a crosslink agent in laboratory studies, this study aimed to evaluate the clinical performance, mainly retention rate, of PA-containing etch-and-rinse adhesive. However, there was a decrease in the retention rate for experimental groups, being statistically significant to the EX5 group after six months and to EX2 and EX5 after twenty-four months, which leads to rejection of the null hypothesis.

The use of PA as pre-treatment showed great results, enhancing the degree of collagen cross-linking, protecting the exposed collagen fibrils of the hybrid layer, and biodegradation resistance by collagenase solution and increasing their associated bond

strength with time.[17,20,31] However, the use of PA as primer requires an application protocol with longer clinical time, which is not desirable. To simplify the use of PA in clinical situations, some studies has incorporated the PA directly into dental adhesives, which might be a new delivery PA method, [15,17] reducing the number of bonding steps and, if this to improves the durability of resin–dentine adhesive restorations, it would be quite appealing. [32]

The idea of adding PA in an adhesive system is to allow a sustained release of PA from the cured resin into surrounding collagen fibrils to exert its collagen cross-linking and protease inhibitory effects over time. Epashinge et al (2017) showed that quantities of PA release increased with the increased of the concentration of PA in the adhesive resin.[18] Nevertheless, these studies were performed in a laboratory setting. No clinical trials were conducted to predict the effect of PA on clinical performance, which motivated this study.

Although all advantages were observed in laboratory studies, PA-containing adhesives showed a clinical worsening for some clinical parameters evaluated in this study. A reduction in the retention rate of 15% in only 6 months as observed for the EX5 group and, after 24 months, an even greater reduction, from 27% for EX2 and 29% for EX5, is quite significant for a dental adhesive. The ADA guidelines require full acceptance of a 90% retention rate after 24 months.[30] The addition of a therapeutic material into dental adhesive resin can disturb its polymerization and affect the mechanical properties of the polymerized resin. [33]

In a recent study that evaluated the incorporation of different concentrations of PA (0.5, 1.0 1.5 and 2.0 wt%) into adhesive resin was observed that flexural strength, modulus of elasticity and microhardness of PA-incorporated adhesive decreased significantly with higher concentrations of PA (1.5% and 2.0%).[18]

PA has a free radical-scavenging ability. [34,35] When involved in the radical polymerization, PA donates hydrogen atoms to the free radicals and inhibits the initiation and propagation of the chain reaction of polymerization.[36] The incorporation of higher PA-concentration (above to 2%) may reach a threshold radical-scavenging and inhibit the polymerization chain, consequently jeopardizing the mechanical properties of the adhesive resin, which could justify the lower retention rates of the experimental groups (EX2 and EX5). [18]

Moreover, PA presents a dark brown color which might affect the penetration of light in the resin adhesive and reduce the depth of cure incrementally, been another mechanism that affects the resin polymerization. [18]

In terms of marginal adaptation, ninety-three restorations exhibited some marginal adaptive discrepancies in the 6-month recall and a hundred-twenty-six in the 24-month. All the groups showed a significant worsening in this criteria over time, mainly after 24-month. However, none restauration was considered to have a clinically unsatisfactory marginal adaptation. The marginal discrepancies of a composite restoration are common and develop rather rapidly. [37-40] However, this appear to cause no important clinical change, because most of the marginal defects were small and clinically acceptable[41] and the simple procedure of restoration re-polishing can improve these discrepancies without causing any damage to the integrity of the restoration. [42] No significant difference was detected between the groups at the 6- and 24-month recall for FDI and USPHS criteria, but this can be attributed to the large number of restorations lost in the experimental groups, which could not be evaluated in this criterion.

When using FDI criteria, just EX5 group showed more marginal discoloration after 6 months compared to baseline. Nevertheless, all groups showed a significant

difference after 24 months when compared with baseline for both criteria (FDI and USPHS). After 24-month recall, only EX5 showed a significant higher marginal discoloration when compared with EX0 (for USPHS criteria) and when compared with EX0 and EX2 (for FDI criteria). FDI criteria were more sensitive than the USPHS criteria modified for identifying small variations in the clinical outcomes when evaluating restorations of NCCLs, [37,38,43] which justifies this difference found after 24 months.

PA presents a dark brown color which might cause an esthetic issue. Dentin treated with PA solutions showed a brownish in color *in vitro*.[44] The presence of high molecular weight polymer polyphenols may justify the color change and the increase of marginal staining for experimental groups. [10,45,46]

The control group (PA-free adhesive) also showed a significant difference in marginal discoloration after 24 months when compared with baseline. Nonetheless, this marginal discoloration was not associated with gap between restauration/tooth, but probably been more associated to the oral habits of patients[47] and usually is solved by re-polishing. [37,48]

For clinical adhesion assessments, NCCL are commonly used. The substrate of these lesions usually presents sclerotic dentine and occlusion of tubules by mineral and may also contain a hypermineralized surface that is resistant to acid etching. [49] Therefore, this substrate is a challenge for adhesion. The most important factors in the retention for restorations of NCCL is the bonding to cavity walls, because these cavities do not have inherent macromechanical retention.[43] The lost retention rate of composite restorations is possibly due to degradation of the adhesive bond. The Excite F is an etch-and-rinse simplified adhesive system and this type of adhesives is

more hydrophilic and most sensitive to water sorption and reduces its mechanical properties after water storage. [44,45]

Over 24-month, the incorporation of PA into adhesive did not prove advantageous for adhesion in the NCCL. Studies have shown the use of PA also incorporated into the acid etch agent with great results, which could be tested in future clinical trials. [50,51] Follow-up of this clinical trial is planned.

5. Conclusion

The incorporation of proanthocyanidin into adhesive did not present clinical advantages after 24 months of clinical evaluation.

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6. References

[1] N. Nakabayashi, K. Kojima, E. Masuhara, The promotion of adhesion by the infiltration of monomers into tooth substrates., J. Biomed. Mater. Res. 16 (1982) 265–73. doi:10.1002/jbm.820160307.

[2] Y. Nishitani, M. Yoshiyama, B. Wadgaonkar, L. Breschi, F. Mannello, A. Mazzoni, R.M. Carvalho, L. Tjäderhane, F.R. Tay, D.H. Pashley, Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives., Eur. J. Oral Sci. 114 (2006) 160–6. doi:10.1111/j.1600-0722.2006.00342.x.

[3] L. Breschi, A. Mazzoni, A. Ruggeri, M. Cadenaro, R. Di Lenarda, E. De Stefano Dorigo, Dental adhesion review: Aging and stability of the bonded interface, Dent.

Mater. 24 (2008) 90–101. doi:10.1016/j.dental.2007.02.009.

[4] E.L. Kostoryz, K. Dharmala, Q. Ye, Y. Wang, J. Huber, J.G. Park, G. Snider, J.L. Katz, P. Spencer, Enzymatic biodegradation of HEMA/BisGMA adhesives formulated with different water content, J. Biomed. Mater. Res. - Part B Appl. Biomater. 88 (2009) 394–401. doi:10.1002/jbm.b.31095.

[5] F.R. Tay, D.H. Pashley, Have dentin adhesives become too hydrophilic?, J. Can. Dent. Assoc. 69 (2003) 726–731. doi:10.1016/S0109-5641(03)00110-6.

[6] F.R. Tay, M. Hashimoto, D.H. Pashley, M.C. Peters, S.C.N. Lai, C.K.Y. Yiu, C. Cheong, Aging affects two modes of nanoleakage expression in bonded dentin., J. Dent. Res. 82 (2003) 537–41. doi:10.1177/154405910308200710.

[7] D.H. Pashley, F.R. Tay, C. Yiu, M. Hashimoto, L. Breschi, R.M. Carvalho, S. Ito, Collagen degradation by host-derived enzymes during aging., J. Dent. Res. 83 (2004) 216–21. doi:10.1177/154405910408300306.

[8] A.K.B. Bedran-Russo, P.N.R. Pereira, W.R. Duarte, J.L. Drummond, M. Yamauchi, Application of crosslinkers to dentin collagen enhances the ultimate tensile strength., J. Biomed. Mater. Res. B. Appl. Biomater. 80 (2007) 268–72. doi:10.1002/jbm.b.30593.

[9] P.H. Dos Santos, S. Karol, A.K. Bedran-Russo, Long-term nano-mechanical properties of biomodified dentin-resin interface components, J. Biomech. 44 (2011) 1691–1694. doi:10.1016/j.jbiomech.2011.03.030.

[10] A.K. Bedran-Russo, G.F. Pauli, S.N. Chen, J. McAlpine, C.S. Castellan, R.S. Phansalkar, T.R. Aguiar, C.M.P. Vidal, J.G. Napotilano, J.W. Nam, A.A. Leme, Dentin biomodification: Strategies, renewable resources and clinical applications, Dent. Mater. 30 (2014) 62–76. doi:10.1016/j.dental.2013.10.012.

[11] L. Tjäderhane, F.D. Nascimento, L. Breschi, A. Mazzoni, I.L.S. Tersariol, S. Geraldeli, A. Tezvergil-Mutluay, M.R. Carrilho, R.M. Carvalho, F.R. Tay, D.H.

Pashley, Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins, Dent. Mater. 29 (2013) 116–135. doi:10.1016/j.dental.2012.08.004.

[12] J. Perdigão, A. Reis, A.D. Loguercio, Dentin adhesion and MMPs: A comprehensive review, J. Esthet. Restor. Dent. 25 (2013) 219–241. doi:10.1111/jerd.12016.

[13] M. Fang, R. Liu, Y. Xiao, F. Li, D. Wang, R. Hou, J. Chen, Biomodification to dentin by a natural crosslinker improved the resin-dentin bonds, J. Dent. 40 (2012) 458–466. doi:10.1016/j.jdent.2012.02.008.

[14] C.S. Castellan, A.K. Bedran-Russo, A. Antunes, P.N.R. Pereira, Effect of dentin biomodification using naturally derived collagen cross-linkers: One-year bond strength study, Int. J. Dent. 2013 (2013). doi:10.1155/2013/918010.

[15] Y. Liu, M. Chen, X. Yao, C. Xu, Y. Zhang, Y. Wang, Enhancement in dentin collagen's biological stability after proanthocyanidins treatment in clinically relevant time periods, Dent. Mater. 29 (2013) 485–492. doi:10.1016/j.dental.2013.01.013.

[16] V. Hass, I.V. Luque-Martinez, M.F. Gutierrez, C.G. Moreira, V.B. Gotti, V.P. Feitosa, G. Koller, M.F. Otuki, A.D. Loguercio, A. Reis, Collagen cross-linkers on dentin bonding: Stability of the adhesive interfaces, degree of conversion of the adhesive, cytotoxicity and in situ MMP inhibition, Dent. Mater. 32 (2016) 732–741. doi:10.1016/j.dental.2016.03.008.

[17] D.J. Epasinghe, C.K.Y. Yiu, M.F. Burrow, F.R. Tay, N.M. King, Effect of proanthocyanidin incorporation into dental adhesive resin on resin-dentine bond strength., J. Dent. 40 (2012) 173–80. doi:10.1016/j.jdent.2011.11.013.

[18] D.J. Epasinghe, C.K.Y. Yiu, M.F. Burrow, Mechanical properties, water sorption characteristics, and compound release of grape seed extract-incorporated resins, J. Appl. Oral Sci. 25 (2017) 412–419. doi:10.1590/1678-7757-2016-0448.

[19] D.J. Epasinghe, C.K.Y. Yiu, M.F. Burrow, Effect of proanthocyanidin incorporation

into dental adhesive resin on resin-dentine bond strength, Int. J. Adhes. Adhes. 63 (2015) 145–151. doi:10.1016/j.ijadhadh.2015.09.006.

[20] B. Green, X. Yao, A. Ganguly, C. Xu, V. Dusevich, M.P. Walker, Y. Wang, Grape seed proanthocyanidins increase collagen biodegradation resistance in the dentin/adhesive interface when included in an adhesive, J. Dent. 38 (2010) 908–915. doi:10.1016/j.jdent.2010.08.004.

[21] K.F. Schulz, D.G. Altman, D. Moher, C. Group, CONSORT 2010 statement : Updated guidelines for reporting parallel group randomised trials, 1 (2010). doi:10.4103/0976-500X.72352.

[22] A.D. Loguercio, A. Reis, A.N. Barbosa, J.F. Roulet, Five-year double-blind randomized clinical evaluation of a resin-modified glass ionomer and a polyacid-modified resin in noncarious cervical lesions., J. Adhes. Dent. 5 (2003) 323–32. http://www.ncbi.nlm.nih.gov/pubmed/15008339.

[23] E.B. Franco, A.R. Benetti, S.K. Ishikiriama, S.L. Santiago, J.R.P. Lauris, M.F.F. Jorge, M.F.L. Navarro, 5-year Clinical Performance of Resin Composite Versus Resin Modified Glass Ionomer Restorative System in Non-carious Cervical Lesions, Oper. Dent. 31 (2006) 403–408. doi:10.2341/05-87.

[24] E.J. Swift, J. Perdigão, H.O. Heymann, A.D. Wilder, S.C. Bayne, K.N. May, J.R. Sturdevant, T.M. Roberson, Eighteen-month clinical evaluation of a filled and unfilled dentin adhesive., J. Dent. 29 (2001) 1–6. http://www.ncbi.nlm.nih.gov/pubmed/11137632.

[25] T.R.F. Da Costa, A.D. Loguercio, A. Reis, Effect of enamel bevel on the clinical performance of resin composite restorations placed in non-carious cervical lesions, J. Esthet. Restor. Dent. 25 (2013) 346–356. doi:10.1111/jerd.12042.

[26] G. Schmalz, G. Ryge, Reprint of Criteria for the clinical evaluation of dental restorative materials, Clin. Oral Investig. 9 (2005) 215–232. doi:10.1007/s00784-005-

0018-z.

[27] R. Hickel, A. Peschke, M. Tyas, I. Mjör, S. Bayne, M. Peters, K.-A. Hiller, R. Randall, G. Vanherle, S.D. Heintze, FDI World Dental Federation - clinical criteria for the evaluation of direct and indirect restorations. Update and clinical examples., J. Adhes. Dent. 12 (2010) 259–72. doi:10.3290/j.jad.a19262.

[28] D. Dalton Bittencourt, I.G. Ezecelevski, A. Reis, J.W. Van Dijken, A.D. Loguercio, An 18-months' evaluation of self-etch and etch & rinse adhesive in non-carious cervical lesions, Acta Odontol. Scand. 63 (2005) 173–178. doi:10.1080/00016350510019874.

[29] J. Perdigão, A. Sezinando, P.C. Monteiro, Laboratory bonding ability of a multi-purpose dentin adhesive., Am. J. Dent. 25 (2012) 153–8. http://www.ncbi.nlm.nih.gov/pubmed/22988685.

[30] ADA Council on Scientific Affairs. Revised American Dental Association acceptance program guidelines: dentin and enamel adhesives. American Dental Association, (2001) Chicago, pp 1–9.

[31] B. Hechler, X. Yao, Y. Wang, Proanthocyanidins alter adhesive/dentin bonding strengths when included in a bonding system., Am. J. Dent. 25 (2012) 276–80. http://www.ncbi.nlm.nih.gov/pubmed/23243975.

[32] D.H. Pashley, F.R. Tay, L. Breschi, L. Tjäderhane, R.M. Carvalho, M. Carrilho, A. Tezvergil-Mutluay, State of the art etch-and-rinse adhesives, Dent. Mater. 27 (2011) 1–16. doi:10.1016/J.DENTAL.2010.10.016.

[33] M. Cadenaro, D.H. Pashley, G. Marchesi, M. Carrilho, F. Antoniolli, A. Mazzoni, F.R. Tay, R. Di Lenarda, L. Breschi, Influence of chlorhexidine on the degree of conversion and E-modulus of experimental adhesive blends., Dent. Mater. 25 (2009) 1269–74. doi:10.1016/j.dental.2009.05.008.

[34] D. Bagchi, M. Bagchi, S.J. Stohs, D.K. Das, S.D. Ray, C.A. Kuszynski, S.S. Joshi,

H.G. Pruess, Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention., Toxicology. 148 (2000) 187–97. http://www.ncbi.nlm.nih.gov/pubmed/10962138.

[35] A. Scalbert, S. Déprez, I. Mila, A.M. Albrecht, J.F. Huneau, S. Rabot, Proanthocyanidins and human health: systemic effects and local effects in the gut., Biofactors. 13 (2000) 115–20. http://www.ncbi.nlm.nih.gov/pubmed/11237169.

[36] Y. Liu, Y. Wang, Effect of proanthocyanidins and photo-initiators on photo-polymerization of a dental adhesive, J. Dent. 41 (2013) 71–79. doi:10.1016/j.jdent.2012.10.006.

[37] J. Perdigão, C. Kose, A. Mena-Serrano, E. De Paula, L. Tay, A. Reis, A. Loguercio, A New Universal Simplified Adhesive: 18-Month Clinical Evaluation, Oper. Dent. 39 (2014) 113–127. doi:10.2341/13-045-C.

[38] A. Mena-Serrano, C. Kose, E.A. De Paula, L.Y. Tay, A. Reis, A.D. Loguercio, J. Perdigão, A new universal simplified adhesive: 6-month clinical evaluation, J. Esthet. Restor. Dent. 25 (2013) 55–69. doi:10.1111/jerd.12005.

[39] A.D. Loguercio, E. Andrade, D. Paula, V. Hass, I. Luque-martinez, A. Reis, J. Perdigão, A new universal simpli fi ed adhesive : 36-Month randomized double-blind clinical trial, J. Dent. 43 (2015) 1083–1092. doi:10.1016/j.jdent.2015.07.005.

[40] N.C. Lawson, A. Robles, C. Fu, C. Paul, K. Sawlani, J.O. Burgess, Two-year clinical trial of a universal adhesive in total-etch and self-etch mode in non-carious cervical lesions $, J. Dent. 43 (2015) 1229–1234. doi:10.1016/j.jdent.2015.07.009.

[41] M. Peumans, J. De Munck, A. Mine, B. Van Meerbeek, Clinical effectiveness of contemporary adhesives for the restoration of non-carious cervical lesions ., Dent. Mater. 30 (2014) 1089–1103. doi:10.1016/j.dental.2014.07.007.

[42] R. Hickel, K. Brüshaver, N. Ilie, Repair of restorations – Criteria for decision making and clinical recommendations, Dent. Mater. 29 (2012) 28–50.

doi:10.1016/j.dental.2012.07.006.

[43] C.-S.F. Piva F, A deciduous teeth composite restoration clinical trial using two methods, J. Dent. Res. 88 (2009) abstract 3241.

[44] M.A. Moreira, N.O. Souza, R.S. Sousa, D.Q. Freitas, M. V. Lemos, D.M. De Paula, F.J.N. Maia, D. Lomonaco, S.E. Mazzetto, V.P. Feitosa, Efficacy of new natural biomodification agents from Anacardiaceae extracts on dentin collagen cross-linking, Dent. Mater. 33 (2017) 1103–1109. doi:10.1016/j.dental.2017.07.003.

[45] A.C. Machado, E. Dezan Junior, J.E. Gomes-Filho, L.T.A. Cintra, D.B. Ruviére, R. Zoccal, C.A. Damante, E.G. Jardim Junior, Evaluation of tissue reaction to Aroeira (Myracrodruon urundeuva) extracts: a histologic and edemogenic study, J. Appl. Oral Sci. 20 (2012) 414–418. doi:10.1590/S1678-77572012000400005.

[46] D. Scheffel, J. Hebling, R. Scheffel, K. Agee, G. Turco, C. de Souza Costa, D. Pashley, Inactivation of Matrix-bound Matrix Metalloproteinases by Cross-linking Agents in Acid-etched Dentin, Oper. Dent. 39 (2014) 152–158. doi:10.2341/12-425-L.

[47] N. Akimoto, M. Takamizu, Y. Momoi, 10-year clinical evaluation of a self-etching adhesive system., Oper. Dent. 32 (n.d.) 3–10. doi:10.2341/06-46.

[48] J.W. van Dijken, Clinical evaluation of three adhesive systems in class V non-carious lesions., Dent. Mater. 16 (2000) 285–91. http://www.ncbi.nlm.nih.gov/pubmed/10831784.

[49] F.R. Tay, D.H. Pashley, Resin bonding to cervical sclerotic dentin: a review., J. Dent. 32 (2004) 173–96. doi:10.1016/j.jdent.2003.10.009.

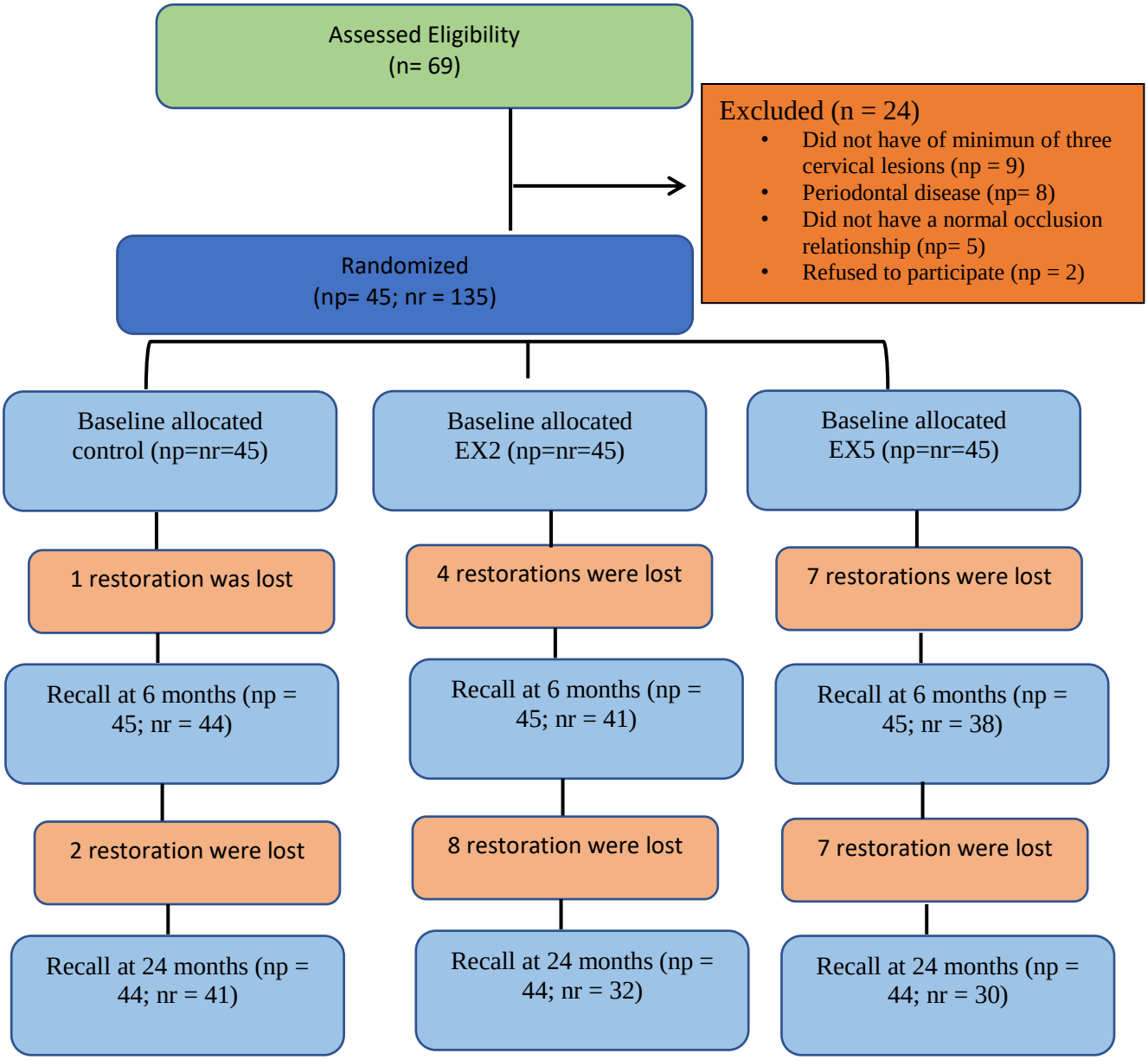
[50] Y. Liu, V. Dusevich, Y. Wang, Addition of Grape Seed Extract Renders Phosphoric Acid a Collagen-stabilizing Etchant., J. Dent. Res. 93 (2014) 821–7. doi:10.1177/0022034514538972.

[51] V. Hass, I. Luque-Martinez, M.A. Muñoz, M.F.G. Reyes, G. Abuna, M.A.C.

Sinhoreti, A.Y. Liu, A.D. Loguercio, Y. Wang, A. Reis, The effect of proanthocyanidin-containing 10% phosphoric acid on bonding properties and MMP inhibition, Dent. Mater. 2 (2015) 468–475. doi:10.1016/j.dental.2015.12.007.

Legends of figure:

Figure 1 – Flow diagram. Np: number of patients, Nr: number of restorations. EX2 = 2% proanthocyanidin incorporated into the adhesive system; EX5 = 5% proanthocyanidin incorporated into the adhesive system.



Legends of Tables:

Table 1 - Dentin sclerosis scale.

Table 2 – Materials, composition and application mode.

Table 3 - World Dental Federation (FDI) criteria used for clinical evaluation. [27]

Table 4 - Modified United States Public Health Service (USPHS) criteria according to Bittencourt and others [28] and Perdigão and others. [29]

Table 5 - Distribution of noncarious cervical lesions according to research subject (gender and age) and characteristics of Class V lesions (shape, cervicoincisal size of the lesion, degree of sclerotic dentin, presence of antagonistic, presence of attrition facets, presence of preoperative sensitivity, and tooth and arch distribution).

Table 6 - Number of evaluated restorations for each experimental group (EX0 [adhesive without PA], EX2 [2% proanthocyanidin incorporated into the adhesive system] and EX5 [5% proanthocyanidin incorporated into the adhesive system]) classified according to the World Dental Federation (FDI) criteria. [27]

Table 7 - Number of evaluated restorations for each experimental group (EX0 [adhesive without PA], EX2 [2% proanthocyanidin incorporated into the adhesive system] and EX5 [5% proanthocyanidin incorporated into the adhesive system]) classified according to the adapted United States Public Health Service (USPHS) criteria. [28,29]

Table 8 - Restorations acceptable or not acceptable according to the Federation Dental International (FDI) criteria after 24 months. [27]

Table 1

Dentin sclerosis scale*	
CATEGORY	**CRITERIA**
1	No sclerosis present; dentin is light yellowish or whitish, with little discoloration; dentin is opaque, with little translucency or transparency
2	More sclerosis than in category 1 but less than halfway between categories 1 and 4
3	Less sclerosis than in category 4 but more than halfway between categories 1 and 4
4	Significant sclerosis present; dentin is dark yellow or even discolored (brownish); glassy appearance, with significant translucency or transparency evident

* Adapted from Swift and colleagues[24] with permission from Elsevier.

Table 2 - Materials, composition and application mode.

Materials	Composition (*)	Application Mode (**)
Condac 37 phosphoric acid (FGM,Joinville, Santa Catarina, Brazil)	Phosphoric acid 37% wt%, thickening agents and pigments.	11. Prepare the region to be etched by cleaning 12. Drying it 13. Apply Condac 37 to the area to be etched and wait for a period of 15 seconds 14. Wash the surface with plenty of water 15. Dry the cavity in such a manner that the dentin does not become dehydrated.
ExciTE F adhesive systems (Ivoclar Vivadent, Schaan, Liechnstein)	Contains HEMA, dimethacrylate, Bis-GMA, UDMA, phosphonic acid acrylate, highly dispersed silicone dioxide, initiators, stabilizers and potassium fluoride in an ethanol solution.	16. Apply to the enamel and dentin and agitate the adhesive on the prepared surfaces for at least 10 seconds. Make sure that all the cavity walls are completely covered 17. Disperse to a thin layer with a weak stream of air, thereby removing any excess. 18. Polymerize for 10 seconds at a light intensity of more than 500 mW/cm2
IPS Empress Direct resin composite (Ivoclar Vivadent, Schaan, Liechnstein)	Dimethacrylates (20-21.5 wt%, opalescent shade 17 wt%). The fillers contain barium glass, ytterbium trifluoride, mixed oxide, silicon dioxide and copolymer (77.5-79 wt%, opalescent shade 83 wt%). Additional contents: additives, initiators, stabilizers and pigments (<1.0 wt%). The total content of inorganic fillers is 75-79 wt% or 52-59 vol% (opalescent shade 60.5 wt% or 45 vol%). The particle size of the inorganic fillers is between 40 nm and 3 μm with a mean particle size of 550 nm.	19. Apply IPS Empress Direct Effect in layers of max. 2 mm thickness. 20. Polymerize each layer for 20 s and keep the light emission window as close as possible to the surface of the restorative material

(*) HEMA = 2-hydroxyethyl methacrylate Bis-GMA = bisphenol glycidyl methacrylate; UDMA = urethane dimethacrylate

(**) According to the manufacturer's instructions

Table 3

	Esthetic Property	**Functional Properties**		**Biological Properties**	
	1. Staining margin	**2. Fractures and retention**	**3. Marginal adaptation**	**4. Postoperative (hyper-) sensitivity**	**5. Recurrence of caries**
1. Clinically very good	1.1 No marginal staining	2.1 Restoration retained, no fractures / cracks	3.1 Harmonious outline, no gaps, no discoloration.	4.1 No hypersensitivity.	5.1 No secondary or primary caries
2. Clinically good (after correction very good	1.2 Minor marginal staining, easily removable by polishing.	2.2 Small hairline crack.	3.2.1 Marginal gap (50 μm). 3.2.2 Small marginal fracture removable by polishing.	4.2 Low hypersensitivity for a limited period of time	5.2 Very small and localized demineralization. No operative treatment required
3.Clinically sufficient / satisfactory (minor shortcomings with no adverse effects but not adjustable without damage to the tooth)	1.3 Moderate marginal staining, not esthetically unacceptable.	2.3 Two or more or larger hairline cracks and/or chipping (not affecting the marginal integrity).	3.3.1 Gap < 150 μm not removable 3.3.2. Several small enamel or dentin fractures	4.3.1 Premature / slightly more intense 4.3.2 Delayed/weak sensitivity; no subjective complaints, no treatment needed.	5.3 Larger areas of demineralization, but only preventive measures necessary (dentine not exposed)
4. Clinically unsatisfactory (repair for prophylactic reasons)	1.4 Pronounced marginal staining; major intervention necessary for improvement	2.4 Chipping fractures which damage marginal quality; bulk fractures with or without partial loss (less than half of the restoration).	3.4.1 Gap > 250 μm or dentine/base exposed. 3.4.2. chip fracture damaging margins 3.4.3 Notable enamel or dentine wall fracture	4.4.1 Premature/ very intense 4.4.2 Extremely delayed/weak with subjective complaints 4.4.3 Negative Sensitivity Intervention necessary but not replacement.	5. 4 Caries with cavitation (localized and accessible and can be repaired
5. Clinically poor (replacement necessary)	1.5 Deep marginal staining not accessible for intervention.	2.5 (Partial or complete) loss of restoration.	3.5 Filling is loose but in situ.	4.5 Very intense, acute pulpitis or non vital. Endodontic treatment is necessary and restoration has to be replaced.	5.5 Deep secondary caries or exposed dentine that is not accessible for repair of restoration.
Acceptable or not acceptable (n, % and reasons	Aesthetic criteria	Functional criteria		Biological criteria	

Table 4

	Marginal staining	**Retention**	**Fracture**	**Marginal adaptation**	**Postoperative sensitivity**	**Recurrence of caries**
Alfa	No discoloration along the margin	Retained	None	Restoration is continuous with existing anatomic form.	No postoperative sensitivity directly after the restorative process and during the study period	None evidence of caries contiguous with the margin
Bravo	Slight and superficial staining (removable, usually localized)	Partially retained	Small chip, but clinically acceptable	Detectable V-shaped defect in enamel only. Catches explorer going both ways.	--	--
Charlie	Deep staining cannot be polished away	Missing	Failure due to bulk restorative fracture	Detectable V-shaped defect to dentin-enamel junction	Sensitivity present at any time during the study period	Evidence of presence of caries

Table 5

Characteristics of research subjects	Number of lesions		
Gender distribution			
Male	28		
Female	17		
Age distribution (years)			
20-29	06		
30-39	11		
40-49	9		
> 49	19		
Characteristics of Class-V lesions	**Number of lesions**		
	EX0	EX2	EX5
Shape (degree of angle)			
< 45	1	1	1
45-90	11	10	13
90-135	19	20	23
> 135	14	14	8
Cervico-incisal height (mm)			
< 1.5	2	7	5
1.5-2.5	28	20	21
> 2.5	15	18	19
Degree of sclerotic dentin			
1	22	19	18
2	13	15	18
3	9	10	8
4	1	1	1
Presence of antagonist			
Yes	45	45	45
No	00	00	00
Attrition facet			
Yes	43	43	43
No	2	2	2
Pre-operative sensitivity (spontaneous)			
Yes	00	00	00
No	45	45	45
Pre-operative sensitivity (air dry)			
Yes	24	24	25
No	21	21	20
Tooth distribution			
Anterior			
Incisor	4	10	8
Canines	9	6	3
Posterior			
Premolar	30	27	32
Molar	2	2	2
Arc distribution			
Maxillary	20	20	23
Mandibular	25	25	22

Table 6

Time		**Baseline**			**6 months**			**24 months**		
FDI Criteria	***(*)***	EX0	EX2	EX5	EX0	EX2	EX5	EX0	EX2	EX5
Marginal adaptation	**VG**	45	45	45	15	06	07	05	01	--
	GO	--	--	--	27	33	28	33	28	26
	SS	--	--	--	02	02	02	03	03	04
	UN	--	--	--	--	--	01	--	--	--
	PO	--	--	--	--	--	--	--	--	--
Marginal staining	**VG**	45	45	45	41	38	25	27	15	04
	GO	--	--	--	01	02	08	07	07	13
	SS	--	--	--	02	01	04	07	10	13
	UN	--	--	--	--	--	01	--	--	--
	PO	--	--	--	--	--	--	--	--	--
Fractures and retention	**VG**	45	45	45	44	41	38	41	32	30
	GO	--	--	--	--	--	--	--	--	--
	SS	--	--	--	--	--	--	--	--	--
	UN	--	--	--	--	--	--	--	--	--
	PO	--	--	--	01	04	07	03	12	14
Post-operative sensitivity	**VG**	45	45	45	44	41	38	41	32	30
	GO	--	--	--	--	--	--	--	--	--
	SS	--	--	--	--	--	--	--	--	--
	UN	--	--	--	--	--	--	--	--	--
	PO	--	--	--	--	--	--	--	--	--
Recurrence of caries	**VG**	45	45	45	44	41	38	41	32	30
	GO	--	--	--	--	--	--	--	--	--
	SS	--	--	--	--	--	--	--	--	--
	UN	--	--	--	--	--	--	--	--	--
	PO	--	--	--	--	--	--	--	--	--

(*) VG for clinically very good; GO for clinically good; SS for clinically sufficient/satisfactory; UN for clinically unsatisfactory and; PO for clinically poor.

Table 7

Time		Baseline			6 months			24 months		
USPHS Criteria		EX0	EX2	EX5	EX0	EX2	EX5	EX0	EX2	EX5
Marginal adaptation	**Alfa**	45	45	45	42	39	35	38	29	26
	Bravo	--	--	--	02	02	03	03	03	04
	Charlie	--	--	--	--	--	--	--	--	--
Retention	**Alfa**	45	45	45	44	41	38	41	32	30
	Charlie	--	--	--	01	04	07	03	12	14
Marginal staining	**Alfa**	45	45	45	42	40	33	34	22	17
	Bravo	--	--	--	02	01	05	07	10	13
	Charlie	--	--	--	--	--	--	--	--	--
Post-operative sensitivity	**Alfa**	45	45	45	44	41	38	44	41	38
	Charlie	--	--	--	--	--	--	--	--	--
Recurrence of caries	**Alfa**	42	45	45	44	41	38	41	32	30
	Charlie	--	--	--	--	--	--	--	--	--

Table 8

Properties	**Aesthetic**			**Functional**						**Biological**					
	Marginal staining			**Fractures and retention**			**Marginal adaptation**			**Postoperative (hyper-) sensitivity**			**Recurrence of caries**		
	EX0	**EX2**	**EX5**	**EX0**	**EX2**	**EX5**	**EX0**	**EX2**	**EX5**	**EX0**	**EX2**	**EX5**	**EX0**	**EX2**	**EX5**
Acceptable	41	32	30	41	32	30	41	32	30	41	32	30	41	32	30
Not acceptable	--	--	--	03	12	14	--	--	--	--	--	--	--	--	--
Reasons				Total loss of the restorations: 29											

CONCLUSÃO GERAL

4 CONCLUSÃO GERAL

A utilização de proantocianidina, extraídas de semente de uva, nas concentrações de 2% e 5% (em peso), aplicada previamente ou incorporada ao sistema adesivo convencional simplificado ExciTE F, não apresentou vantagens clínicas após 24 meses de avaliação.

A incorporação das PAs diretamente no sistema adesivo, seguindo as metodologias desses estudos, parece ser mais prejudicial à taxa de retenção das restaurações que a aplicação das PAs como um agente de pré-tratamento.

O presente estudo é o primeiro ensaio clínico que utiliza PAs no protocolo de adesão. Portanto, estudos clínicos utilizando outras concentrações de PAs, ou que utilizem PAs com cadeias menores (oligoméricas, diméricas), testando outros sistemas adesivos seriam relevantes para a avaliação do comportamento clínico dessa substância que apresenta resultados tão promissores em estudos *in vitro*.

REFERÊNCIAS

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AFANAS'EV IB, DOROZHKO AI, BRODSKII AV, KOSTYUK VA, POTAPOVITCH AI. **Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation.** Biochem Pharmacol. 1989;38(11):1763-1769.

AGEE K, ZHANG Y, PASHLEY DH. **Effects of acids and additives on the susceptibility of human dentine to denaturation.** J Oral Rehab 2000; 27:136–141.

AL-AMMAR A, DRUMMOND JL, BEDRAN-RUSSO AK. **The use of collagen cross-linking agents to enhance dentin bond strength.** J Biomed Mater Res B: Appl Biomater 2009; 91:419–424.

AMARAL FL, COLUCCI V, PALMA-DIBB RG, CORONA SA. **Assessment of in vitro methods used to promote adhesive interface degradation: a critical review.** J Esthet Restor Dent. 2007; 19(6):340-353.

BEDRAN-RUSSO AK, CASTELLAN CS, SHINOHARA MS, HASSAN L, ANTUNES A. **Characterization of biomodified dentin matrices for potential preventive and reparative therapies.** Acta Biomater. 2011; 7(4):1735-1741.

BEDRAN-RUSSO AK, PASHLEY DH, AGEE K, DRUMMOND JL, MIESCKE KJ. **Changes in stiffness of demineralized dentin following application of collagen crosslinkers**. J Biomed Mater Res B: Appl Biomater 2008; 86B:330–334.

BEDRAN-RUSSO AK, PAULI GF, CHEN SN, MCALPINE J, CASTELLAN CS, PHANSALKAR RS, AGUIAR TR, VIDAL CMP, NAPOTILANO JG, NAM JW, LEME AA. **Dentin biomodification: Strategies, renewable resources and clinical applications.** Dent Mater. 2014; 30: 62–76.

BEDRAN-RUSSO AK, PEREIRA PN, DUARTE WR, DRUMMOND JL, YAMAUCHI M. **Application of crosslinkers to dentin collagen enhances the**

ultimate tensile strength. J Biomed Mater Res B Appl Biomater. 2007; 80(1):268-272.

BEDRAN-RUSSO AK, VIDAL CM, DOS SANTOS PH, CASTELLAN CS. **Long-term effect of carbodiimide on dentin matrix and resin-dentin bonds.** J Biomed Mater Res B Appl Biomater. 2010; 94(1):250-255.

BEDRAN-RUSSO AK, YOO KJ, EMA KC, PASHLEY DH. **Mechanical properties of tannic-acid-treated dentin matrix.** J Dent Res. 2009; 88(9):807-811.

BRESCHI L, MAZZONI A, RUGGERI A, CADENARO M, DI LENARDA R, DE STEFANO DORIGO E. **Dental adhesion review: aging and stability of the bonded interface.** Dent Mater. 2008; 24(1):90-101.

BUENING MK, CHANG RL, HUANG MT, FORTNER JG, WOOD AW, CONNEY AH. **Activation and inhibition of benzo(a)pyrene and aflatoxin B1 metabolism in human liver microsomes by naturally occurring flavonoids.** Cancer Res. 1981; 41(1):67-72.

CASTELLAN CS, BEDRAN-RUSSO AK, ANTUNES A, PEREIRA PN. **Effect of dentin biomodification using naturally derived collagen cross-linkers: one-year bond strength study.** Int J Dent. 2013:918010

CASTELLAN CS, PEREIRA PN, GRANDE RH, BEDRAN-RUSSO AK. **Mechanical characterization of proanthocyanidin-dentin matrix interaction.** Dent Mater. 2010a; 26(10):968-973.

CASTELLAN CS, PEREIRA PN, VIANA G, CHEN SN, PAULI GF, BEDRAN-RUSSO AK. **Solubility study of phytochemical cross-linking agents on dentin stiffness.** J Dent. 2010b; 38(5):431-436.

CHERUKUPALLI RC, REDDY DA. **Resin bond strength to water versus ethanol-saturated human dentin pretreated with three different cross-linking agents.** J Conserv Dent. 2016; 19(6):555-559

COS P, DE BRUYNE T, HERMANS N, APERS S, BERGHE DV, VLIETINCK AJ. **Proanthocyanidins in health care: current and new trends.** Curr Med Chem. 2004; 11(10):1345-1359.

DE MUNCK J, VAN DEN STEEN PE, MINE A, VAN LANDUYT KL, POITEVIN A, OPDENAKKER G, VAN MEERBEEK B. **Inhibition of enzymatic degradation of adhesive–dentin interfaces.** J Dent Res 2009; 88:1101–1106.

DE MUNCK J, VAN LANDUYT K, PEUMANS M, POITEVIN A, LAMBRECHTS P, BRAEM M, VAN MEERBEEK B. **A critical review of the durability of adhesion to tooth tissue: methods and results.** J Dent Res. 2005; 84(2):118-132.

EPASINGHE DJ, YIU CK, BURROW DMF. **Effect of proanthocyanidin incorporation into dental adhesive on durability of resin–dentin bond.** Int. J. Adhes. Adhes. 2015; 63:145–151.

EPASINGHE DJ, YIU CK, BURROW MF, TAY FR, KING NM. **Effect of proanthocyanidin incorporation into dental adhesive resin on resin-dentine bond strength.** J. Dent. 2012; 40(3):173-180.

FANG M, LIU R, XIAO Y, LI F, WANG D, HOU R, CHEN J. **Biomodification to dentin by a natural crosslinker improved the resin-dentin bonds.** J Dent. 2012; 40(6):458-466.

FERREIRA D, SLADE D. **Oligomeric proanthocyanidins: naturally occurring O-heterocycles.** Nat Prod Rep. 2002; 19(5):517-541.

GREEN B, YAO X, GANGULY A, XU C, DUSEVICH V, WALKER MP, WANG Y. **Grape seed proanthocyanidins increase collagen biodegradation resistance in the dentin/adhesive interface when included in an adhesive.** J Dent. 2010; 38(11):908-915.

HAGERMAN AE, KLUCHER KM. **Tannin-protein interactions.** Prog Clin Biol Res. 1986; 213:67–76.

HAN B, JAUREQUI J, TANG BW, NIMNI ME. **Proanthocyanidin: a natural crosslinking reagent for stabilizing collagen matrices.** J Biomed Mater Res. 2003; 65:118–124.

HASS V, LUQUE-MARTINEZ I, MUÑOZ MA, REYES MF, ABUNA G, SINHORETI MA, LIU AY, LOGUERCIO AD, WANG Y, REIS A. **The effect of proanthocyanidin-containing 10% phosphoric acid on bonding properties and MMP inhibition.** Dent Mater. 2016b; 32(3):468-475.

HASS V, LUQUE-MARTINEZ IV, GUTIERREZ MF, MOREIRA CG, GOTTI VB, FEITOSA VP, KOLLER G, OTUKI MF, LOGUERCIO AD, REIS A. **Collagen cross-linkers on dentin bonding: Stability of the adhesive interfaces, degree of conversion of the adhesive, cytotoxicity and in situ MMP inhibition.** Dent Mater. 2016a; 32(6):732-741.

HECHLER B, YAO X, WANG Y. **Proanthocyanidins alter adhesive/dentin bonding strengths when included in a bonding system.** American Journal of Dentistry 2012; 25:276–280.

HEINTZE SD, ROUSSON V. **Pooling of dentin microtensile bond strength data improves clinical correlation.** J Adhes Dent 2011; 13(2) 107-110.

KOLODZIEJ H, HABERLAND C, WOERDENBAG HJ, KONINGS AWT. **Moderate cytotoxicity of proanthocyanidins to human tumour cell lines.** Phytother Res. 1995; 9:410–415.

LEVITCH LC, BADER JD, SHUGARS DA, HEYMANN HO. **Non-carious cervical lesions.** J Dent. 1994; 22(4):195-207.

LIU Y, LIU W, SUN G, WEI X, YI D. **Calcification resistance of procyanidin-treated decellularized porcine aortic valves in vivo.** Heart Surgery Forum 2009; 12:(E)24–29.

LIU Y, WANG Y. **Effect of proanthocyanidins and photoinitiators on photo-polymerisation of a dental adhesive.** J. Dent 2013; 41:71–79.

LOOMIS WD. **Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles.** Methods Enzymol. 1974; 31:528-544.

MACEDO GV, YAMAUCHI M, BEDRAN-RUSSO AK. **Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res.** 2009; 88(12):1096-1100.

MACIEL KT, CARVALHO RM, RINGLE RD, PRESTON CD, RUSSELL CM, PASHLEY DH. **The effects of acetone, ethanol, HEMA, and air on the stiffness of human decalcified dentin matrix.** J Dent Res. 1996; 75(11):1851-1858.

MAFFEI FACINO R, CARINI M, ALDINI G, BOMBARDELLI E, MORAZZONI P, MORELLI R. **Free radicals scavenging action and anti-enzyme activities of procyanidines from Vitis vinifera. A mechanism for their capillary protective action.** Arzneimittelforschung. 1994; 44(5):592-601.

MARSHALL GW JR, MARSHALL SJ, KINNEY JH, BALOOCH M. **The dentin substrate: structure and properties related to bonding.** J Dent. 1997; 25(6):441-458.

MARSHALL GW JR. **Dentin: microstructure and characterization.** Quintessence Int. 1993; 24(9):606-617.

MOREIRA MA, SOUZA NO, SOUSA RS, FREITAS DQ, LEMOS MV, DE PAULA DM, MAIA FJN, LOMONACO D, MAZZETTO SE, FEITOSA VP. **Efficacy of new natural biomodification agents from Anacardiaceae extracts on dentin collagen cross-linking.** Dent Mater. 2017; 33(10):1103-1109

PIERPOINT WS. **o-Quinones formed in plant extracts. Their reactions with amino acids and peptides.** Biochem J. 1969; 112(5):609-616.

SCHEFFEL D, HEBLING J, SCHEFFEL R, AGEE K, TURCO G, DE SOUZA COSTA C, PASHLEY D. **Inactivation of Matrix-bound Matrix Metalloproteinases by Cross-linking Agents in Acid-etched Dentin.** Oper. Dent. 2014; 39:152–158.

STENZEL KH, MIYATA T, RUBIN AL. **Collagen as a biomaterial.** Annu Rev Biophys Bioeng. 1974; 3(0):231-253.

SUNG HW, CHANG WH, YUANG M, LEE MH. **Crosslinking of biological tissues using ginipin and/or carbodiimide.** J Biomed Mater Res A. 2003; 64:427–438.

TAY FR, KWONG SM, ITTHAGARUN A, KING NM, YIP HK, MOULDING KM, PASHLEY DH. **Bonding of a self-etching primer to non-carious cervical sclerotic dentin: interfacial ultrastructure and microtensile bond strength evaluation.** J Adhes Dent 2000; 2:9–28.

VAN MEERBEEK B, PEUMANS M, POITEVIN A, MINE A, VAN ENDE A, NEVES A, DE MUNCK J. **Relationship between bond-strength tests and clinical outcomes.** Dent Mater 2010; 26(2)100-121.

WALTER R, MIGUEZ PA, ARNOLD RR, PEREIRA PN, DUARTE WR, YAMAUCHI M. **Effects of natural cross-linkers on the stability of dentin collagen and the inhibition of root caries in vitro.** Caries Res. 2008; 42(4):263-268.

WANG Y, SPENCER P. **Quantifying adhesive penetration in adhesive/dentin interface using confocal Raman microspectroscopy.** J Biomed Mat Res. 2002; 59:46–55.

WANG Y, SPENCER P**. Hybridisation efficiency of the adhesive/dentin interface with wet bonding.** J Dent Res. 2003; 82:141–145.

WEADOCK K, OLSON RM, SILVER FH. **Evaluation of collagen crosslinking techniques.** Biomater Med Devices Artif Organs 1983; 11:293–318.

XIE Q, BEDRAN-RUSSO AK, WU CD. **In vitro remineralization effects of grape seed extract on artificial root caries.** J Dent. 2008; 36(11):900-906.

APÊNDICES

APÊNDICE A - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

UNIVERSIDADE FEDERAL DO CEARÁ

FACULDADE DE FARMÁCIA, ODONTOLOGIA E ENFERMAGEM

PÓS-GRADUAÇÃO EM CLÍNICA ODONTOLÓGICA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você esta sendo convidado (a) a participar do projeto de pesquisa: **EFEITO DA PROANTOCIANIDINA NA LONGEVIDADE DE RESTAURAÇÕES ADESIVAS: ENSAIO CLÍNICO ALEATORIZADO E DUPLO-CEGO.** Sua participação é importante, porém, você não deve participar contra a sua vontade.

Leia com atenção as informações abaixo e sinta-se livre para fazer qualquer pergunta que desejar para que não haja dúvida alguma sobre os procedimentos a serem realizados.

a) O objetivo da pesquisa é avaliar clinicamente o efeito da proantocianidina (PA) aplicada de forma isolada ou incorporada a um adesivo convencional simplificado de dois passos na durabilidade e estabilidade de restaurações de lesões cervicais não cariosas LCNCs

A participação neste estudo consistirá de:

- Exame dentário prévio, profilaxia e realização das restaurações
- Comparecimento à Clínica nos dias previamente agendados para fazer a restauração e 6, 12, 18, 24, 36, 48 e 60 meses após, para avaliação das restaurações.
- Realização de fotografias digitais no exame inicial e nas visitas de controle

c) Os materiais a serem testados são comercialmente usados e já foram testados e aprovados pela ADA (Associação Dental Ameriacana) sem provocar nenhum dano a sua saúde. A proantocianidina é um composto totalmente natural e extraído de sementes

de uva.

d) Você tem a liberdade de desistir de participar desse estudo no momento que desejar sem nenhum prejuízo de qualquer natureza;

e) Os resultados obtidos durante este estudo serão mantidos em sigilo. A Faculdade de Odontologia, Farmácia e Enfermagem (FFOE) não o identificará por ocasião da exposição e/ou publicação dos mesmos e os dados serão publicados somente em revista científica e/ou congressos científicos não identificando o seu nome.

Ao assinar este termo no qual consta o seu nome, idade, e número do prontuário, você estará declarando que por meio de livre e espontânea vontade participará como voluntário do projeto de pesquisa citado acima, de responsabilidade do pesquisador Vicente de Paulo Aragão Saboia, telefones (85) 8807.4623/33668401, da Faculdade de Odontologia, da Universidade Federal do Ceará, rua Monsenhor Furtado, S/N, Rodolfo Teófilo, CEP 60441-750.

Fortaleza, ____de_____de 20___.

Nome do voluntário _____

Data de nascimento___/____/_____

_____ RG:_____

Assinatura do paciente

digital

Telefone do comitê de ética em pesquisa (COMEPE) da Faculdade de Medicina da UFC: (85) 33668338

APÊNDICE B- FICHA DE CLASSIFICAÇÃO DAS LESÕES

Ficha Clínica – NÚMERO DO PACIENTE

Nome

Endereço

Telefone

Dente	Grau de esclerose	Faceta de desgaste.	Geometria (mm)			Borda em esmalte (%)	Sensibilidade		Ângulo da lesão	Presença de Anta-gonista	Data Rest	Grupo
			Alt	Larg	Prof		esp	Ar				

Grau de esclerose: 1 a 4 (fundamental com a foto)

Faceta de desgaste: sim ou não (fotos podem auxiliar)

Geometria: anotar em milímetros (fotos podem auxiliar)

Borda em esmalte: avaliar percentualmente a quantidade de esmalte na borda

Sensibilidade espontânea e a jato de ar (5s a 1cm): sim ou não

Angulação da lesão: < 45º; entre 45-90º; entre 90-135º e > 135º

Presença de antagonista: sim ou não

APÊNDICE C - FICHA DE AVALIAÇÃO DAS RESTAURAÇÕES

PACIENTE:

DATA: _____/____/____

OBSERVADOR:

Avaliação USPHS

Dentes/Grupo																
CRITÉRIOS	ESCORES	*alfa*	*Bravo*	*Charlie*	*alfa*	*bravo*	*Charlie*	*alfa*	*bravo*	*charlie*	*alfa*	*Bravo*	*charlie*	*Alfa*	*bravo*	*Charlie*
Adaptação marginal																
Pigmentação Marginal																
Retenção																
Sensibilidade pós-operatória																
Cárie secundária																

Avaliação FDI

Critérios	ADAPTAÇÃO MARGINAL					PIGMENTAÇÃO MARGINAL					RETENÇÃO					SENSIBILIDADE PÓS-OPERATÓRIA					CÁRIE SECUNDÁRIA				
Dentes																									
Grupo																									
1. Clinicamente aceitável																									
2. Clinicamente bom (excelente após polimento)																									
3. Clinicamente suficiente ou satisfatório																									
4. Clinicamente insuficiente																									
5. Clinicamente deficiente (necessita de substituição)																									

ANEXOS

ANEXO A – Parecer do Comitê de Ética em pesquisa

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DA PROANTOCIANIDINA NA LONGEVIDADE DE RESTAURAÇÕES ADESIVAS: ENSAIO CLÍNICO ALEATORIZADO E DUPLO-CEGO

Pesquisador: Lidiane Costa de Souza

Área Temática:

Versão: 1

CAAE: 30739114.0.0000.5054

Instituição Proponente: Departamento de Odontologia Restauradora

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 640.695

Data da Relatoria: 08/05/2014

Apresentação do Projeto:

Projeto de doutorado de Lidiane Costa de Souza sobre a utilização de um antioxidante natural, a proantocianidina (PA), nas restaurações de resinas de Lesões cervicais não cariosas (LCNCs). Esta substância é capaz de estabelecer ligações cruzadas com o colágeno dentinário e inibir atividades proteolíticas das colagenases. A PA apresenta baixa citotoxicidade, baixo custo e são encontradas abundantemente na natureza, como no açaí, canela e em sementes de uva e de cacau. Trata-se de um ensaio clínico aleatorizado e duplo- cego que será realizado com 45 pacientes, os quais deverão ter um mínimo de 5 LCNCs, totalizando 225 restaurações. A PA será utilizada como primer ou incorporporada ao adesivo dentário em duas concentrações, 2% ou a 5%. As cavidades serão restauradas com resina composta e as restaurações serão avaliadas após o polimento e nos períodos de 6, 12 e 18 meses. Serão feitas réplicas das restaurações para análise do selamento marginal e percentual de fendas em MEV. Os dados serão tabulados e enviados para análise estatística.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar clinicamente o efeito de uma solução contendo proantocianidina a 2 e 5% ou da inclusão de proantocianidina em adesivo na longevidade de restaurações de lesões cervicais não cariosas.

Endereço: Rua Cel. Nunes de Melo, 1127
Bairro: Rodolfo Teófilo **CEP:** 60.430-270
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

Objetivo Secundário:
Avaliar clinicamente o efeito da aplicação de solução de proantocianidina a 2 % antes da aplicação do sistema adesivo convencional simplificado, imediatamente após a confecção das restaurações e nos períodos de 6, 12 e 18 meses.
Avaliar clinicamente o efeito da aplicação de solução de proantocianidina a 5% antes da aplicação do sistema adesivo convencional simplificado, imediatamente após a confecção das restaurações e nos períodos de 6, 12 e 18 meses.
Avaliar clinicamente o efeito da proantocianidina a 2% (em volume) incorporada a um adesivo convencional simplificado, imediatamente após a confecção das restaurações e nos períodos de 6, 12 e 18 meses.
Avaliar clinicamente o efeito da proantocianidina a 5% (em volume) incorporada a um adesivo convencional simplificado, imediatamente após a confecção das restaurações e nos períodos de 6, 12 e 18 meses.
Avaliar a presença de fendas na interface dente/restauração através de análise em MEV das réplicas feitas imediatamente após a confecção das restaurações e nos períodos de 6, 12 e 18 meses.

Avaliação dos Riscos e Benefícios:
A pesquisa apresenta risco mínimo, sendo representado pela possível quebra da restauração, que se ocorrer deverá ser substituída o quanto antes.
Quanto aos benefícios, ressalta-se que, essa modificação do sistema adesivo poderá levar a um aumento da durabilidade das restaurações adesivas.

Comentários e Considerações sobre a Pesquisa:
A pesquisa está bem delineada e contempla todos os requisitos metodológicos e éticos para sua realização.

Considerações sobre os Termos de apresentação obrigatória:
A pesquisadora apresentou ao COMEPE: folha de rosto devidamente preenchida e assinada pela chefia do Departamento de Odontologia restauradora, TCLE adequado, Orçamento, Cronograma detalhado, Currículo Lattes da pesquisadora principal, Carta de encaminhamento a este comitê, Autorização do Laboratório Multidisplinar de Dentística, Declaração de Concordância dos pesquisadores envolvidos.

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

Continuação do Parecer: 640.695

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

Não há pendência documental

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

FORTALEZA, 08 de Maio de 2014

Assinador por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador)

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UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

ANEXO B – Registro Brasileiro de Ensaios Clínicos