UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

CLAUDIO AUGUSTO CERVINI TRIGO

EFEITO DO ENVELHECIMENTO ARTIFICIAL NA RESISTÊNCIA DE UNIÃO DE UM ADESIVO UNIVERSAL EM RESTAURAÇÕES DIRETAS DE LESÕES CERVICAIS NÃO CARIOSAS

EFFECT OF ARTIFICIAL AGING ON THE BOND STRENGTH OF AN UNIVERSAL ADHESIVE IN DIRECT RESTORATIONS OF NON-CARIOUS CERVICAL LESIONS

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Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Cirurgião Dentista.

Undergraduate final work presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Dental Surgeon

Orientador: MSc. Simone Gomes de Oliveira

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DO TRABALHO DE CONCLUSÃO DE CURSO APRESENTADO PELO ALUNO CLAUDIO AUGUSTO CERVINI TRIGO E ORIENTADO PELA MSC. SIMONE GOMES DE OLIVEIRA

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DEDICATÓRIA

Dedico este trabalho

Ao meu pai Dr. José Antônio Trigo, meu exemplo de caráter, honestidade, integridade e lisura. Compartilha seu amor à profissão, me passa segurança e dedica sua vida à minha. Ao meu primeiro mestre, a gratidão eterna.

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RESUMO

O objetivo deste trabalho foi avaliar os efeitos do envelhecimento artificial e da estratégia adesiva em restaurações diretas de lesões cervicais não cariosas (LCNC). Quarenta e oito dentes bovinos foram selecionados e distribuídos aleatoriamente em grupos experimentais de acordo com a estratégia adesiva (condicionamento total (E&R) e autocondicionante (SE)) e envelhecimento (com ou sem ciclagem mecânica seguida de ciclagem térmica). As amostras utilizadas corresponderam às camadas externa (esmalte/dentina) e interna (dentina) das restaurações. O efeito dos fatores testados foi avaliado pelo teste de resistência à microtração (n=10), modo de falha das regiões de fratura (n=10) e nanoinfiltração (n=2). Testes de normalidade e homocedasticidade precederam a análise estatística das diferenças observadas entre os grupos. Todos os testes foram realizados no software R v3.6.1 com $p<0,05$. Diferenças significativas foram observadas para condicionamento e estratégia de envelhecimento. Os grupos E&R foram mais resistentes ao SE, assim como a região dentinária em relação ao esmalte/dentina. O modo de falha adesiva foi predominante em todos os grupos. Os menores valores de resistência de união foram observados na camada mais externa (esmalte/dentina) das restaurações de ambas as estratégias adesivas. O envelhecimento reduziu a força de união. A camada mais externa da restauração (esmalte/dentina) foi mais suscetível ao efeito do envelhecimento, independente da estratégia adesiva. O envelhecimento aumentou a nanoinfiltração em ambas as camadas de restauração, independentemente da estratégia adesiva. A estratégia de E&R parece ser mais adequada para restaurar as LNCC.

Palavras-chave: Adesivo Dentinário. Envelhecimento. Restauração Dentária Permanente.

ABSTRACT

The aim of this work was to evaluate the effects of artificial aging and the adhesive strategy on direct non-carious cervical lesion (NCCL) restorations. Forty-eight bovine teeth were selected and randomly assigned to experimental groups according to the adhesive strategy (total etching (E&R) and self etching (SE)) and aging (with or without mechanical cycling followed by thermal cycling). The samples used corresponded to the external (enamel/dentin) and internal (dentin) layers of the restorations. The effect of the factors tested was evaluated by microtensile bond stregth test (n=10), failure mode of fracture regions (n=10), and nanoleakage (n=2). Normality and homoscedasticity tests preceded the statistical analysis of the differences observed between the groups. All tests were performed in R software v3.6.1 with $p < 0.05$. Significant differences were observed for conditioning strategy and aging. The E&R groups were more resistant to SE, as well as the dentin region in relation to enamel/dentin. The adhesive failure mode was predominant in all groups. The lowest values of bond strength were observed in the outermost layer (enamel/dentin) of the restorations of both adhesive strategies. Aging reduced the bond strength. The outermost layer of the restoration (enamel/dentin) was more susceptible to the effect of aging, regardless of the adhesive strategy. Aging increased nanoleakage in the two restoration layers, regardless of the adhesive strategy. The E&R strategy seems to be more indicated to restore NCCL.

Keywords: Dentin-Bonding Agents. Aging. Dental Restoration, Permanent.

LISTA DE ABREVIATURAS E SIGLAS

E&R – condicionamento total (etch-and-rinse)

HAp – hidroxiapatita (hydroxyapatite)

HEMA – hidroxietil metacrilato (hydroxyethyl methacrylate)

JAC (CEJ) – junção amelocementária (cement-enamel juntion)

LCNC (NCCL) – lesão cervical não cariosa (non-carious cervical lesion)

MDP-Ca salts – sais de cálcio de MDP (calcium salts of MDP)

MTC – ciclagem mecânica e térmica (mechanical and thermical cycling)

PVC – policloreto de polivinila ou policloreto de vinil (polyvinyl chloride)

SE – autocondicionante (self-etch)

SEM – microscopia eletrônica de varredura (scanning electron microscopy)

SA – grupo experimental com tratamento SE com envelhecimento por MTC

SN – grupo experimental com tratamento SE sem envelhecimento

TA – grupo experimental com tratamento E&R com envelhecimento por MTC

TN – grupo experimental com tratamento E&R sem envelhecimento

10-MDP – 10-metacriloiloxidecil dihidrogenofosfato (10-methacryloyloxydecyl dihydrogen phosphate)

μTBS – ensaio de microtração (microtensile bond strength test)

SUMÁRIO

1 INTRODUÇÃO

Uma Lesão cervical não cariosa (LCNC) é formada pela perda de tecido mineralizado na região da junção amelocementária (JAC). Sua etiologia é multifatorial e não está associada à ação bacteriana. As características morfológicas, estruturais e microecológicas específicas da região cervical contribuem para sua formação. Nesta região o esmalte é mais fino e aprismático, o que o torna menos solúvel ao efeito ácido e consequentemente mais resistente à cárie. Por outro lado, a falta de sulcos na junção do esmalte à dentina torna a região de união entre estes tecidos menos resistente à capacidade de dissipação da tensão de contato na superfície, resultando na vulnerabilidade da região à ação de processos mecânicos (Grippo 1992; Perdigão 2010; Peumans et al. 2020).

Agentes mecânicos, físicos e químicos atuam isolada ou conjuntamente para desencadear e agravar a perda de tecido mineralizado (Grippo et al. 2004; Teixeira et al. 2020). Devido a etiologia multifatorial, as LCNCs podem também ser denominadas de acordo com o agente que mais contribui para a formação da lesão (Grippo 1992; Grippo et al. 2004). Clinicamente são observadas como efeitos da fricção (abrasão), da biocorrosão (erosão) e do estresse oclusal (abfração) (Peumans et al. 2020). Os defeitos promovidos pela perda de estrutura dental podem ocorrer nas faces lingual e proximais. Mas é na face vestibular que as LCNCs se apresentam com maior frequência (Borcic et al. 2004). A gravidade e o tipo de agente etiológico envolvido influência na aparência clínica das lesões, que são classificadas de acordo com sua forma em cunha, disco ou achatadas (Bartlett and Shah 2006).

A hipersensibilidade e o comprometimento da função são sintomas associados às LNCCs. Mas elas também podem passar despercebidas pelo paciente quando são assintomáticas (Peumans et al. 2020). A literatura aponta para uma prevalência ampla das LCNCs, podendo variar de 5% a 85% (Levitch et al. 1994). A variação do percentual de prevalência das LCNCs pode estar associada às diferentes denominações, definições e termos encontrados na literatura atribuídos às LCNCs. Mas também a características populacionais e de localização geográfica (Bartlett and Shah 2006). Na população adulta a prevalência das LNCCs é de cerca de 46,7%, aumentando sua prevalência com o envelhecimento e sendo influenciada pelo estilo de vida e hábitos alimentares (Teixeira et al. 2020).

Para restabelecer forma e função, levando em conta a estética e a abordagem minimamente invasiva, o procedimento restaurador adesivo é em geral a opção de escolha. No entanto, o sucesso de restaurações adesivas em LCNC ainda é um desafio (Van Meerbeek et al. 2020).

Os sistemas adesivos universais, também conhecidos como multimodais, surgiram com o intuito de simplificação da técnica adesiva (Sofan et al. 2017), mas podem ser utilizados sob diferentes estratégias de adesão, variando o método de condicionamento (Oliveira et al. 2017), aplicação de dupla camada adesiva (Hirokane et al. 2021), aumento do tempo de fotopolimerização (Cadenaro et al. 2019), aumento do tempo de volatilização de solventes, incorporação de aditivos ao adesivo como inibidores de MMPs (de Moraes et al. 2020), aplicação do adesivo de forma ativa, entre outras com o objetivo de melhorar o resultado adesivo (Hardan et al. 2021; Chen et al. 2022). Na estratégia de condicionamento total, a *smear layer* é previamente removida, utilizando-se em geral o ácido fosfórico a 37%, revelando os prismas no esmalte e, expondo as fibrilas colágenas e abrindo os túbulos da dentina. A adesão promovida por esta estratégia no esmalte é confiável e previsível. Diferentemente do observado na dentina, em que suas características estruturais e de composição comprometem a qualidade da adesão em relação ao observado no esmalte. Por outro lado, a estratégia autocondicionante torna a *smear layer* permeável aos monômeros, em níveis que variam de acordo com a acidez dos adesivos, incorporando-a na camada híbrida formada (Van Meerbeek et al. 2011). Esses adesivos podem conter ainda monômeros funcionais, como o 10-metacriloiloxidecil di-hidrogenofosfato (10-MDP) que proporciona maior interação química com os substratos dentais, evidenciados na formação de sais MDP-Ca, melhorando a qualidade adesiva (Carrilho et al. 2019; Fehrenbach et al. 2021).

Para avaliar o desempenho adesivo os testes in vitro, tradicionalmente utilizados são os testes de microtração e cisalhamento, com a aplicação de forças que desafiam a resistência de adesão de restaurações (Nagarkar et al. 2019). Além disso, a fim de simular o envelhecimento natural e inferir situações clínicas em pesquisas laboratoriais, podem ser utilizados, o armazenamento em água, saliva ou outras soluções por determinado período, além da termociclagem e ciclagem mecânica que submetem as amostras a ciclos de cargas e temperaturas controladas, simulando as condições do ambiente bucal.

Este trabalho tem o objetivo de avaliar os efeitos do envelhecimento artificial na resistência de união de restaurações adesivas realizadas com um sistema adesivo universal em região de lesões cervicais não cariosas, utilizando as estratégias de condicionamento total e autocondicionante.

2 ARTIGO: EFFECT OF ARTIFICIAL AGING ON DIRECT RESTORATIONS OF NON-CARIOUS CERVICAL LESIONS

Submetido no periódico Brazilian Oral Research (Anexo X)

EFFECT OF ARTIFICIAL AGING ON DIRECT RESTORATIONS OF

NON-CARIOUS CERVICAL LESIONS

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ABSTRACT

The aim of this work was to evaluate the effects of artificial aging and the adhesive strategy on direct NCCL restorations. 48 bovine teeth were selected and randomly assigned to experimental groups according to the adhesive strategy (total etching (E&R) and self-etching (SE)) and aging (with or without mechanical cycling followed by thermal cycling). The samples used corresponded to the external (enamel/dentin) and internal (dentin) layers of the restorations. The effect of the factors tested was evaluated by microtensile bond stregth test (n=10), failure mode of fracture regions (n=10), and nanoleakage (n=2). Normality and homoscedasticity tests preceded the statistical analysis of the differences observed between the groups. All tests were performed in R software v3.6.1 with $p < 0.05$. Significant differences were observed for conditioning strategy and aging. The E&R groups were more resistant to SE, as well as the dentin region in relation to enamel/dentin. The adhesive failure mode was predominant in all groups. The lowest values of bond strength were observed in the outermost layer (enamel/dentin) of the restorations of both adhesive strategies. Aging reduced the bond strength. The outermost layer of the restoration (enamel/dentin) was more susceptible to the effect of aging, regardless of the adhesive strategy. Aging increased nanoleakage in the two restoration layers, regardless of the adhesive strategy. The E&R strategy seems to be more indicated to restore NCCL.

KEYWORDS: Dentin-Bonding Agents. Aging. Dental Restoration, Permanent.

1. INTRODUCTION

Non-carious cervical lesions (NCCLs) are caused by the loss of mineralized tissue in the region of the cement-enamel junction (CEJ) whose origin is not associated with bacterial action. The formation of these lesions is the result of morphological, structural and microecological characteristics specific to the cervical region. In this region, the enamel is thinner and non-prismatic, which makes it less soluble to the acid effect and, consequently, more resistant to caries. On the other hand, the lack of grooves at the junction between enamel and dentin makes the region of union between these tissues less resistant to dissipate contact stress, resulting in the vulnerability of the region to the action of mechanical processes [1–3].

Mechanical, physical, and chemical agents can act individually or together to create or increase mineral loss [4]. The formation of NCCLs is generally multifactorial [1,4]. Clinically, they are observed as consequences of friction (abrasion), biocorrosion (erosion), and occlusal stress (abfraction) [3]. The defects in tooth structure can occur on the lingual and proximal surfaces. However, it is on the buccal surface that it appears as a frequency [5]. The severity and

the etiologic agent influence the clinical appearance of the lesions, classified as wedge-shaped, disc, and flattened [6]. Studies point to a wide prevalence of LCNNs, ranging from 5% to 85% [7] The prevalence of NCCLs in the adult population is around 46.7%, increasing with aging and being influenced by contemporany lifestyle and eating habits [8].

Universal adhesive systems, are also known as multimodal, emerged with the aim of simplifying the adhesive technique [9], but they can be used under different adhesion strategies, varying the conditioning method [10], the application of the adhesive actively, among others with the objective of improving the adhesive result [11,12]. In the total etching strategy, the smear layer is previously removed, generally using 37% phosphoric acid, revealing the prisms in the enamel, exposing the collagen fibrils, and opening the dentin tubules. The adhesion promoted by this strategy on enamel is reliable and predictable. Differently from what was observed in dentin, in which its structural and compositional characteristics compromise the quality of adhesion in relation to that observed in enamel. On the other hand, the self-etching strategy makes the smear layer permeable to monomers, at levels that vary according to the acidity of the adhesives, incorporating it into the hybrid layer formed [13]. These adhesives may also contain functional monomers which provides greater chemical interaction with dental substrates improving the adhesive quality [14,15].

Despite the evolution of adhesive systems and restorative techniques, the longevity of adhesive restorations in LCNC is still a challenge. In vitro studies have investigated the performance of universal adhesives on enamel and dentin using different adhesive strategies [16–19] whose location of the lesions does not always correspond to those of the LCNC, failing to consider in their evaluations the composition and structure characteristics of the regions where the LCNC are located. This work, in turn, studies the effect of aging by evaluating two layers of the restoration. One more external and composed of enamel and dentin and another more internal composed only of dentin. Both are located in the most prevalent regions of the NCCL, allowing an even closer assessment of the natural conditions in which these lesions are found.

The objective of this work was to evaluate the effects of aging by mechanical cycling and thermal cycling (MTC) and the effects of etch-and-rinse (E&R) and self-etch (SE) approaches in a universal adhesive used in LCNC restorations. The hypotheses tested were: 1)

the bond strengths for the E&R and SE strategies are similar in both restoration layers after aging; 2) nanoleakage is not affected by aging or by the adhesive strategy in the different layers of the restoration.

2. METHODOLOGY

2.1. EXPERIMENTAL DESIGN

Forty-eight bovine teeth were randomly assigned to 4 groups: E&R (TN); E&R and aging (TA); SE (SN), and SE and aging (SA). The groups were evaluated according to the enamel/dentin (outer) and dentin (inner) layers of the restorations. Aging was performed by mechanical cycling followed by thermal cycling (MTC). The effect of aging and adhesive strategy were evaluated by microtensile test (μTBS) (n=10) and failure mode (n=10) and nanoleakage (n=2), observed by scanning electron microscopy (SEM).

2.2. TOOTH SELECTION AND SAMPLE PREPARATION

The teeth were selected according to closed apex and ratios of cervical-incisal and mesiodistal distances (in the cervical region) and crown-root equal to 1:1. All measurements were performed with a precision instrument (Digital caliper 500 171-30B, Mitutoyo, Brazil). After cleaning the teeth, the teeth were kept in a 0.5% thymol solution for seven days for disinfection. Sample preparation was performed according to Figure 1. For μTBS, the teeth were cut so that the tested regions corresponded to the CEJ region. Teeth sections were made with a diamond disc (IsoMet, Beuhler Ltd, USA) under water cooling. Half of the samples were submitted to the E&R approach (Ultra Etch 35% - Ultradent USA), followed by the active application of Scotchbond Universal adhesive (3M ESPE, USA). The other samples were submitted to the SE approach. All specimens were restored with 2 mm increments of Filtek Z350 XT composite resin (3M ESPE, USA). Description of restorative materials and procedures are presented in Table 1 and 2. Light curing of dental adhesive and composite resin were according to the manufacturer guidelines. The samples were kept in relative humidity at 37^{o}C for 24 hours. Subsequently, half of the samples from each adhesive approach was subjected to artificial aging by MTC.

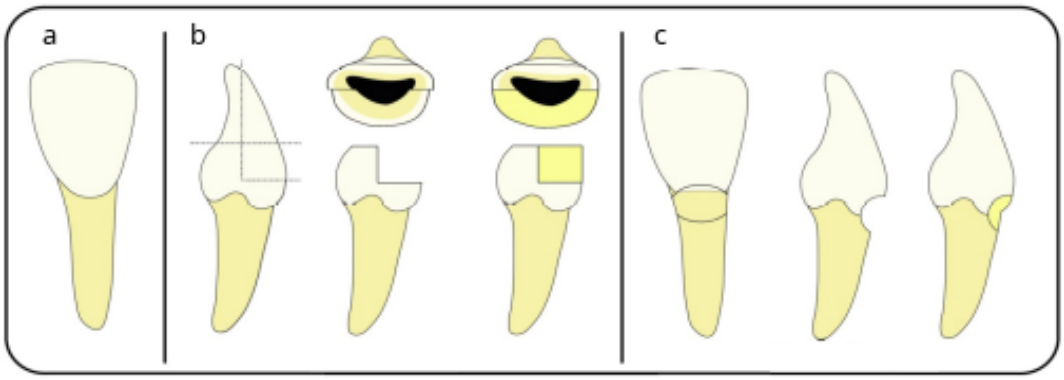

Figure 1: Sample preparation: (a) tooth before preparation; (b) sample preparation for μTBS - occlusal view before and after restoration (top); proximal view before preparation and before and after restoration (below); (c) sample preparation for nanoleakage: vestibular view with preparation demarcation; proximal views: before and after restoration.

Table 1: Dental materials

MATERIAL	COMPOSITION
Scotchbond Universal (3M ESPE, USA)	10-MDP, HEMA, silane, dimethacrylate, VitrebondTM copolymer, fillers, ethanol, water, initiators
Filtek Z350 XT A3B (3M ESPE, USA)	Treated Silanized Ceramic, Silane Treated Silica, Diurethane Dimethacrylate (UDMA), Bisphenol A Polyethylene Glycol Diether Dimethacrylate, Bisphenol A Diglycidyl Ether Dimethacrylate (BisGMA), 3-Methacryloxypropyl Trimethoxysilane Modified Zirconia Ceramic, Polyethylene Glycol Dimethacrylate, Triethylene Glycol Dimethacrylate.
Ultra Etch 35% (Ultradent, USA)	Phosphoric acid

Table 2 - Restorative Procedure

PROCEDURE	SELF-ETCH	TOTAL-ETCH
Surface treatment	-	phosphoric acid (30 sec - enamel and 15 sec dentin); rinsing with water for the same time, and drying with a gentle jet of air, at a distance, for 5s.
Dental adhesive	Active application of the adhesive for 20 seconds, followed by a light jet of air at a distance for 10s and a second application of the adhesive, like the first application, followed by photoactivation of the adhesive for 10s.	Active application of the adhesive for 20 seconds, followed by a light jet of air at a distance for 10s and a second application of the adhesive, like the first application, followed by photoactivation of the adhesive for 10s.
Restoration	2 mm increments of composite resin, followed by light curing for 20 seconds, to a thickness of 4 mm.	2 mm increments of composite resin, followed by light curing for 20 seconds, to a thickness of 4 mm.

For the evaluation of nanoleakage, cervical lesions were simulated on the buccal surfaces with a depth of 3,0 mm and edges 2 mm distant from the zenith point of the CEJ, to obtain a rounded transverse contour shape. A handpiece equipped with an oval-shapped milling cutter was used to obtain cavities with dimensions of 7 mm in width between the proximal faces and 4 mm in height, with 2 mm located above the CEJ. A new milling cutter was used every five preparations. The cavity was washed with water, restored, and treated similarly to the microtensile samples.

2.2. MECHANICAL AND THERMAL CYCLING

The root surfaces were covered with a 1 mm layer of utility wax to simulate the periodontal ligament space. The teeth were isolated with solid vaseline and embedded in epoxy resin, in PVC matrices (2 cm x 3 cm). The apical limit of the restoration was kept 2 mm from the epoxy resin. The wax was replaced by light condensation silicone (Optosil Heraeus Kulzer), which has a modulus of elasticity similar to that of the periodontal ligament. Half of the samples were subjected to 200,000 cycles at 2Hz and then to 5,000 cycles at temperatures

of 5°C and 55°C for 30 seconds each, with an interval of 5 seconds in a cycler (MSCT 3, Marnucci ME, Brazil).

2.3. MICROTENSILE BOND STRENGTH

Samples of both adhesive strategies, aged or not, were sectioned with a diamond disc (IsoMet, Buehler Ltd, USA) under water cooling to produce sticks 0.9 mm x 0.9 mm x 6 mm with its long axis perpendicular to the adhesive interface. The sticks corresponding to the enamel and dentin regions were evaluated in a universal testing machine (EZ-S Test, Shimadzu, Japan) at a speed of 0.5 mm. /min. Surfaces of the fracture regions were observed in SEM (JSM-5600V, JEOL, Japan) to assess the fracture mode.

2.4. NANOINFILTRATION

Nanoleakage evaluation followed the description by Tay et al.[20]. The samples were submitted to successive sagittal cuts (n=3), to highlight the entire internal contour of the restoration. After being embedded in acrylic resin, they were polished with sandpaper (#600, #1200, #2000), in addition to felt with diamond paste. Ultrasonic cleaning for 10 minutes was performed between each polishing step and then prepared for observation in SEM (JSM-5600LV Jeol, Japan), operating at 15KV and under backscattered electrons.

2.5. STATISTICAL ANALYSIS

All data were evaluated for normality and homoscedasticity. The μTBS data were analyzed by the Mann-Whitney U and Tukey tests. All statistical analyzes were performed using the software R v3.6.1 10, with a significance level of $p < 0.05$.

3. RESULTS

Means and standard deviations of μTBS are shown in Table 3. The E&R groups had the highest μTBS values compared to the SE groups, except for the enamel/dentin group after aging. However, there was no significant difference between the approaches in this condition. The highest strength values occurred in the dentin, regardless of the conditioning strategy, even after aging (Table 3).

Table 3: Means and standard deviations of microtensile bond strength (MPa).

ADHESIVE APPROACH	DENTIN		ENAMEL/DENTIN	
	24 h	aging	24 h	aging
Etch-and-Rinse	43,4 ± 15,6 ^{aA*}	36,8 ± 16,3 ^{bA**}	26,1 ± 11,6 ^{aA*}	10,2 ± 6,2 ^{bA**}
Self-Etch	29,4 ± 17,6 ^{cB*}	23,8 ± 13,0 ^{cB**}	18,5 ± 13,2 ^{cB*}	12,7 ± 6,3 ^{cA**}

Means with the same letter are not significantly different ($p < 0.05$). Lowercase letters show comparisons between columns (before and after aging) and capital letters show comparisons between rows (E&R and SE). Significant differences between the layers of restorations are indicated by * (24 hours) and by ** (aging) .

The adhesive failure mode was predominant, followed by the cohesive failure mode in resin, regardless of the etching strategy and aging. In the SE approach, cohesive and mixed failure modes were observed only after aging (Figure 2).

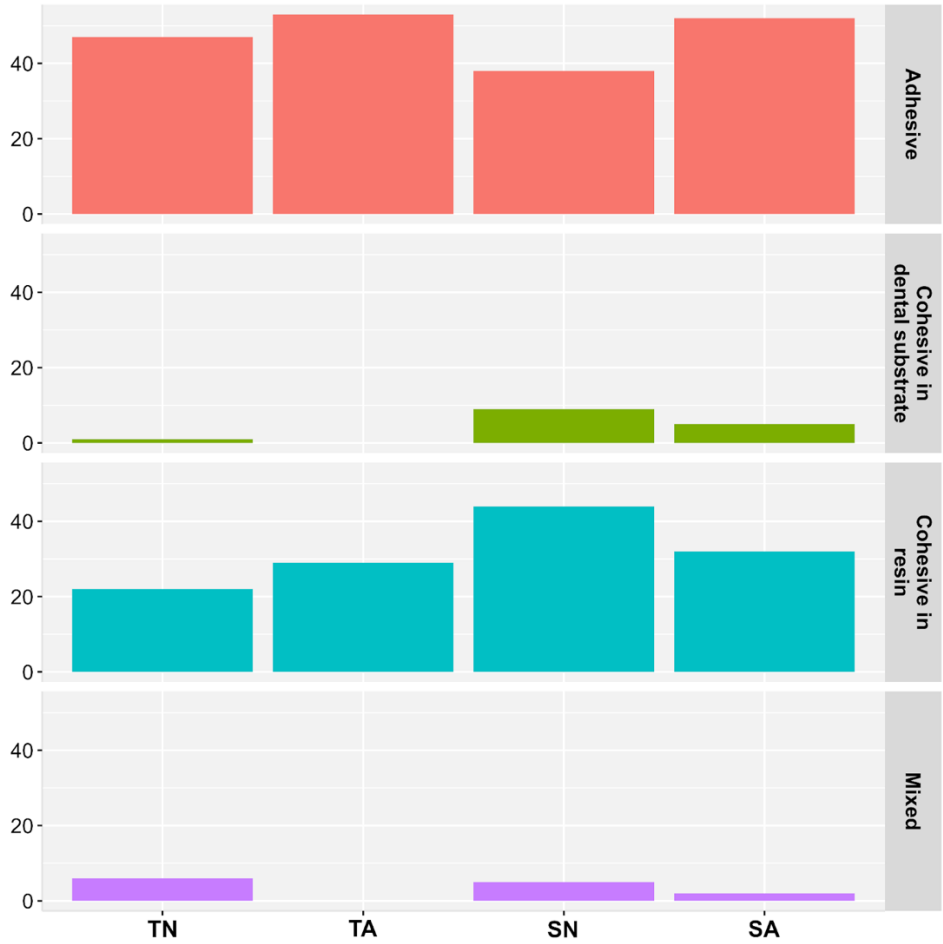

Figure 2: Failure mode distribution (%). (TN): etch-and-rinse; (TA): etch-and-rinse and aging; (SN): self-etching; (SA): self-etch and aging.

Nanoleakage was observed in all groups, being more intense after aging (Figure 3). Similar differences were identified between E&R and SE before and after aging.

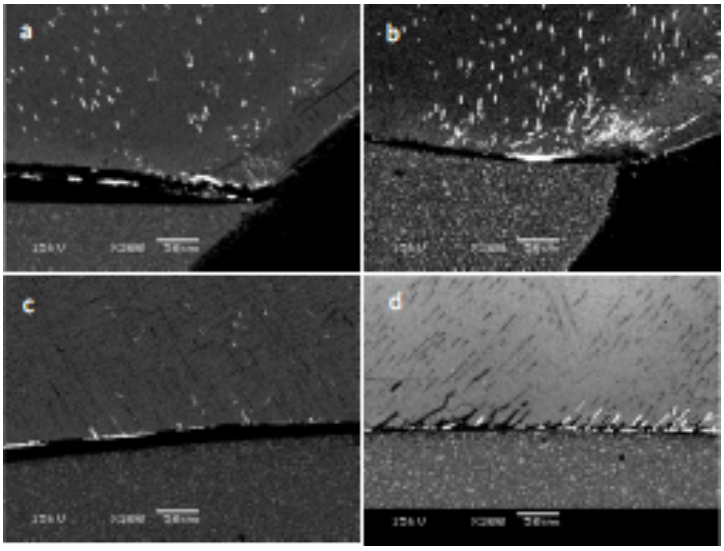

Figure 3: Nanoleakage: no aging (a and c); aging (b and d); enamel/dentin (a and b); dentin (c and d).

4. DISCUSSION

This study evaluated the effect of MTC aging and the effect of the adhesive strategy on the bond strength and nanoleakage of direct LCNC restorations performed with a universal adhesive. According to the results obtained, the hypothesis that the bond strengths for the E&R and SE strategies are similar in both layers of the restoration after aging must be partially rejected, since the reductions in the bond strength between the two layers of restorations were significant only for the E&R strategy. However, the values observed in the E&R groups were higher than those observed for the corresponding groups of the SE strategy, with the exception of the outermost layer (enamel/dentin) of the restoration after aging. The second hypothesis must be rejected, since nanoleakage increased after aging in the E&R and SE strategies in both layers of the restoration (Figure 3).

The versatility of universal adhesives has been highlighted as a favorable aspect, allowing the clinician to use E&R and SE strategies. The E&R technique is a three-step technique that uses phosphoric acid previously to prepare the surface of dental substrates and favor the infiltration of resin monomers applied in subsequent steps. However, differences in the constitution and structure of dental tissues cause phosphoric acid to produce different effects depending on the tissue. In enamel, due to the high amount of hydroxyapatite (Hap), phosphoric acid produces grooves that favor greater bond strength [16].

In dentin, demineralization exposes collagen fibers, which were previously encased in HAp, making them vulnerable to degradation by proteolytic enzymes such as metalloproteinases and cysteine cathepsins [21]. In the SE approach, the etching, primer, and adhesive steps occur all at once. Demineralization and infiltration of resin monomers into the substrate occur simultaneously due to the presence of acidic adhesive co-monomers present in SE dental adhesives [18,19,22].

The adhesive used in this study contains the functional monomers HEMA and 10-MDP. The presence of functional monomers in dental adhesives favors the formation and functionality of polymeric chains due to the reactivity of the functional groups located at the ends of their chains. The amphiphilic nature and low molecular weight of HEMA favor the diffusion of high molecular weight monomers in the moist collagen matrix. On the other hand, its incorporation confers hydrophilicity to dental adhesives, making them more prone to hydrolysis [23], which may explain the reduction in strength observed after aging. Unlike HEMA, 10-MDP has a long spacer chain that gives this functional monomer characteristics of hydrophilicity (greater resistance to hydrolysis), viscosity (promotes wetting and infiltration in the dental substrate), greater flexibility, in addition to establishing ionic bonds with the calcium [24], and the formation of MDP-Ca salts, improving the adhesive quality [14,15], which may have contributed to the higher values of bond strength observed in dentin (innermost layer of restorations). Furthermore, degradation of polymers, leaching of monomers, and degradation of collagen fibers may also contribute to explain the increase in nanoleakage observed in groups subjected to aging [25].

Jacker-Guhr et al.[26] evaluated the bond strength of universal adhesives in enamel and dentin using the E&R and SE strategies. They observed that E&R presented better adhesive performance, as observed in this study. As well as the fracture mode of the adhesive was preponderant for Scotchbond Universal, which was the only one to present mixed failure modes among the other universal adhesives tested. Similar to what was obtained in this study, the difference in bond strength observed between the SE groups for enamel and dentin, before and after aging, was not significant for the universal adhesive studied.

Scotchbond Universal adhesive is classified as an ultra-mild adhesive due to its pH of 2.7, which gives it less able to remove the smear layer and condition enamel in a similar way

to etching obtained with phosphoric acid [16]. The action of phosphoric acid on dentin can promote excessive demineralization and intense calcium removal, inhibiting the formation of ionic bonds between calcium and the phosphate and carboxylate groups of the adhesives [27]. Adhesive infiltration may not be sufficient to reach deeper demineralized regions of dentin in case of excessive demineralization promoted by phosphoric acid. This leaves collagen fibers more vulnerable to hydrolysis and the action of proteolytic enzymes such as metalloproteinases and cysteine-cathepsins.

In vitro studies that evaluate adhesive performance traditionally use microtensile and shear tests, with the application of forces that challenge the bond strength between restorations and dental tissues. The preferred method for evaluating bond strength in enamel is the shear test, and it is possible that the evaluation method may have influenced the reduced values observed in the outer layer of restorations (enamel/dentin) [28]. In this study, the sample preparation and execution of μTBS considered the application of force exerted at the adhesive interface on the samples of the outer layer of the restorations (enamel/dentin) like the shear test, to minimize the effect between tests. However, it should also be considered that the formation of the hybrid layer present in this region (enamel/dentin) is thinner in enamel and that in the μTBS the applied force is opposite and perpendicular to the adhesive interface, which can increase the bond strength challenge and achieve greater fidelity of the results obtained. Controversially, Cecchin et al.[29] showed similar μTBS results between dentin and enamel. In addition, the technique of making the specimens for the μTBS test is sensitive and delicate, considering the characteristics of the cutting disk and the force applied during the tests [30], the conformation and size of the specimens [31,32], in addition to the skill of the operator, suggesting that there are points to be improved in relation to the technique [33]. In this study, the highest μTBS values were observed in the E&R strategy, in the innermost layer of the restoration (dentin). Similar values were observed by Nicoloso et al.[17], who evaluated the bond strength promoted by universal dental adhesives, using the E&R and SE approaches, in sound and caries-affected dentin. They observed that E&R increased bond strength in healthy dentin, as was observed in this study.

The results obtained in this study suggest that structural factors present in the region where the NCCLs are located can influence the bond strength. Non-prismatic and reduced-thickness enamel, in addition to the orientation of the dentinal tubules, may be factors as

important as the presence of sclerotic dentin commonly observed in NCCL, reflecting in a significant reduction in the bond strength of adhesive restorations in this region. However, further studies should be carried out to better characterize the effect of these factors on the success and longevity of LCNC restorations.

5. CONCLUSION

Aging reduced the bond strength. The outermost layer of the restoration (enamel/dentin) was more susceptible to the effect of aging, regardless of the adhesive strategy. For the SE strategy, aging did not affect the bond strength of both layers of the restoration. Aging increased nanoleakage in the two restoration layers, regardless of the adhesive strategy. The E&R strategy seems to be more indicated to restore NCCL.

6. ACKNOWLEDGMENTS

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3 CONCLUSÃO

O envelhecimento reduziu a força de união. A camada mais externa da restauração (esmalte/dentina) foi mais suscetível ao efeito do envelhecimento, independente da estratégia adesiva. Para a estratégia SE, o envelhecimento não afetou a resistência de união de ambas as camadas da restauração. O envelhecimento aumentou a nanoinfiltração em ambas as camadas de restauração, independentemente da estratégia adesiva. A estratégia de E&R parece ser mais adequada para restaurar as LNCC.

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ANEXOS

Anexo 1 – Verificação de originalidade e prevenção de plágio

EFEITO DO ENVELHECIMENTO ARTIFICIAL NA RESISTÊNCIA DE UNIÃO DE UM ADESIVO UNIVERSAL EM RESTAURAÇÕES DIRETAS DE LESÕES CERVICAIS NÃO CARIOSAS

RELATÓRIO DE ORIGINALIDADE

4%	2%	7%	%
ÍNDICE DE SEMELHANÇA	FONTES DA INTERNET	PUBLICAÇÕES	DOCUMENTOS DOS ALUNOS

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1	"Full Issue PDF", Operative Dentistry, 2015 Publicação	1%
2	"Abstracts of Papers", Journal of Dental Research, 2016 Publicação	1%
3	repositorio.unicamp.br Fonte da Internet	1%
4	Mario Felipe GUTIÉRREZ, Luisa F. ALEGRÍA-ACEVEDO, Alejandra NÚÑEZ, Luján MÉNDEZ-BAUER et al. "In vitro biological and adhesive properties of universal adhesive systems on sound and caries-affected dentine: 18 months", International Journal of Adhesion and Adhesives, 2022 Publicação	1%
5	www.scielo.br Fonte da Internet	1%

Anexo 2 – Iniciação Científica

Universidade Estadual de Campinas
Pró-Reitoria de Pesquisa
Programas de Iniciação Científica e Tecnológica
www.prp.unicamp.br | Tel. 55 19 3521-4891

PARECER SOBRE RELATÓRIO FINAL DE ATIVIDADES

Bolsista: Claudio Augusto Cervini Trigo – RA 135353

Orientador(a): Prof.(a) Dr.(a) FLÁVIO HENRIQUE BAGGIO AGUIAR

Projeto: "O efeito da morfologia de lesões cervicais não cariosas na nanoinfiltração de um adesivo universal sob diferentes formas de condicionamento."

Bolsa: PIBIC/CNPq
Vigência: 01/09/2020 a 31/08/2021
Processo: 147145/2020-1

PARECER

Trata-se de projeto reformulado devido à impossibilidade de uso do serviço de Microscopia Eletrônica de Transmissão em função da pandemia do Covid-19. Foram então avaliados os parâmetros e aspectos envolvidos no envelhecimento artificial de restaurações adesivas e seus efeitos sobre a dentina e esmalte como: resistência adesiva (microtração), modo de fratura e nanoinfiltração, avaliados por Microscopia Eletrônica de Varredura. Em adição, apresentou-se metodologia alternativa para preparo das amostras para a avaliação da resistência adesiva e nanoinfiltração. As amostras de dentes bovino foram preparadas foram divididas em 4 grupos que receberam os tratamentos: condicionamento total + adesivo universal, condicionamento total + adesivo universal + mecânico e térmico, adesivo universal, e adesivo universal + envelhecimento mecânico e térmico. As amostras receberam ciclagens mecânica e térmica e submetidas à microtração e nanoinfitração. Realizada análise estatística, concluiu-se que o envelhecimento artificial envelheceu de fato as restaurações adesivas e reduziu a resistência de união, e a nanoinfiltração tende a aumentar com o envelhecimento das restaurações. Em adição, o teste de microtração em esmalte precisa ser mais investigado. Relatório contempla todas as fases da pesquisa, e em que pese a pandemia, conseguiu-se a reformulação e conclusão do projeto a bom termo.

Conclusão do Parecer:

Aprovado

Pró-Reitoria de Pesquisa, 02 de maio de 2022.

Samuelson Alex Nanini Pereira

PR ASSADMINISTRATIVOS / TÉCNICO EM ADMINISTRAÇÃO

Anexo 3 – Comprovante de submissão do Artigo

Submission Confirmation

Print

Thank you for your submission

Submitted to	Brazilian Oral Research
Manuscript ID	BOR-2022-0297
Title	EFFECT OF ARTIFICIAL AGING ON DIRECT RESTORATIONS OF NON-CARIOUS CERVICAL LESIONS
Authors	Oliveira, Simone Trigo, Claudio Aguiar, Flavio
Date Submitted	06-May-2022