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FACULDADE DE ODONTOLOGIA DE PIRACICABA

BEATRIZ OMETTO SAHADI

EFEITO DE SOLUÇÕES DE PRIMERS EXPERIMENTAIS À BASE DE FLAVONÓIDES NA RESISTÊNCIA DE UNIÃO À DENTINA E NA MORFOLOGIA DA ÁREA DE UNIÃO DENTINA-ADESIVO

EFFECT OF FLAVONOID-BASED EXPERIMENTAL PRIMER SOLUTIONS ON DENTIN BOND STRENGTH AND DENTIN-ADHESIVE INTERFACE MORPHOLOGY

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Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Mestra em Clínica Odontológica, na Área de Dentística.

Dissertation presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Clinical Dentistry, in Operative Dentistry area.

Orientador: Prof. Dr. Marcelo Giannini

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RESUMO

A degradação das fibrilas colágenas é uma das causas de falhas da adesão. Alguns compostos têm sido sugeridos para serem aplicados previamente aos adesivos ou incorporados a eles. O objetivo desse estudo *in vitro* foi avaliar o efeito de quatro soluções de *primers* experimentais na resistência de união à dentina por microtração, após 24 horas e um ano de armazenamento em saliva artificial, e na morfologia da área de união dentina-adesivo. Dois diferentes flavonóides foram diluídos em álcool absoluto: Kaempferol (Ka) e Naringina (Na) em diferentes concentrações (10 mM e 20 mM), obtendo quatro soluções de *primers*. Os *primers* foram aplicados por 30 ou 60 segundos em dentina previamente condicionada com ácido fosfórico a 35%, seguido à aplicação de um sistema adesivo convencional de dois passos (Optibond S, Kerr). No grupo Controle Negativo (CN) não houve aplicação de nenhum *primer* previamente à aplicação do adesivo e no grupo Controle Positivo (CP) foi usado solução de Digluconato de Clorexidina 0,2% por 30 ou 60 segundos. Oitenta e oito terceiros molares humanos foram distribuídos, aleatoriamente, em onze grupos (n=8), de acordo com o tipo, concentração e tempo de aplicação dos *primers* para o ensaio de resistência à união (RU). Os dentes foram restaurados de acordo com cada grupo e seccionados nas direções mesial-distal e vestíbulo-lingual, obtendo-se espécimes no formato de "paralelepípedos". Metade dos espécimes foram submetidos ao ensaio de RU após 24 horas, enquanto a outra metade foi armazenada a 37º em solução de saliva artificial por um tempo de um ano e testada. Para a análise da morfologia da área de união dentina-adesivo, quarenta e quatro dentes (n=4) foram restaurados seguindo a mesma metodologia do ensaio de RU, porém, no adesivo foi adicionado o corante Rodamina B na concentração 0,1% em peso. Após 24 horas, os dentes foram seccionados em fatias de 1 mm de espessura. As fatias foram polidas e analisadas em Microscopia Confocal de Varredura a Laser (MCVL). Os dados de RU foram avaliados quanto à distribuição e homoscedasticidade, seguidas por análises estatísticas apropriadas (α=0,05). Em relação aos resultados do ensaio de RU, não foi encontrada diferença estatística entre os grupos experimentais e o CN em 24 horas. No entanto, os primers experimentais contendo 20 mM Na, 10 e 20 mM Ka, aplicados por 60 segundos na dentina, apresentaram maiores valores de RU em relação ao CN e CP após um ano. Formação de camada híbrida e penetração do adesivo nos túbulos dentinários foram observadas em todos os grupos. A aplicação de todos os *primers* experimentais à base de flavonóides em dentina por 60 segundos

foi capaz de aumentar a RU em comparação ao CN após um ano de envelhecimento artificial.

Palavras-Chave: Flavonoides, Flavanonas, Adesivos Dentinários, Dentina, Condicionamento ácido do dente.

ABSTRACT

The degradation of collagen fibrils is one of the causes of adhesion failures. Somo compounds have been suggested to be applied before adhesives or incorporated into them. The objective of this *in vitro* study was to evaluate the effect of four experimental *primer* solutions on dentin bond strength (μTBS), after 24 hours and one year of storage in artificial saliva, and the dentin-resin bonding interface morphology. Two different flavonoids were diluted in absolute alcohol: Kaempferol (Ka) e Naringin (Na) at different concentrations (10 mM and 20 mM), obtaining four *primer* solutions. P*rimers* were applied for 30 or 60 seconds in dentin previously conditioned with 35% phosphoric acid, followed by the application of a two-steps etch-and-rinse adhesive system (Optibond S, Kerr). The Negative Control (NC) consisted of no *primer* application prior to adhesive, while the Positive Control (PC) comprised 0.2% Chlorhexidine Digluconate solution used for 30 seconds or 60 seconds. Eighty-eight third human molars were randomly assigned into eleven groups (n=8), according to the type, concentration and time of application of the *primer* for the μTBS test. The teeth were restored according to each group and were cut into lingual-buccal and mesial-distal directions sections, obtaining stick-shaped specimens. Half of the specimens were submitted to the μTBS test after 24 hours, while the other half was stored at 37ºC in an artificial saliva solution for one year and tested. For the analysis of the morphology of the bonding interface morphology, forty-four teeth (n=4) were restored following the same methodology of the μTBS methodology. However, the Rhodamine B dye was added to the adhesive at 0.1% weight concentration. After 24 hours, the teeth were sectioned into 1 mm thick slices. The slices were polished and analyzed in Confocal Laser Scanning Microscopy (CLSM). All methodological analyses were evaluated for distribution and homoscedasticity, followed by appropriate statistical analyses (α=0.05). Regarding μTBS, no statistical difference was found among the experimental groups and NC at 24 hours. However, experimental primers containing 20 mM Na, 10 and 20 mM Ka applied for 60 s presented higher means than that obtained for NC and PC at one year. Hybrid layer formation and adhesive penetration into dentinal tubules were observed in all groups. The application of all flavonoid-based experimental primers for 60 s was able to produce higher μTBS than those obtained with NC after one-year of artificial aging.

Key words: Flavonoids, Flavanones, Dentin-bonding agents, Dental acid etching, Dentin.

SUMÁRIO

1 INTRODUÇÃO

Embora os avanços nas técnicas restauradoras adesivas, na química dos materiais adesivos e no conhecimento sobre os mecanismos de união à dentina (Bedran-Russo AK. et al., 2013) ainda há problemas associados à estabilidade da união dentina-resina ao longo do tempo, com relatos de diminuição significativa na resistência de união dos adesivos à dentina (Breschi et Al., 2008; Pashley et al., 2011; Tjaderhane L, 2015). Os adesivos sofrem sorção dos fluidos orais, degradação polimérica e, consequentemente, a lixiviação de monômeros e oligômeros (De Munck et al., 2003; Tay FR et al., 2003).

A longevidade de uma restauração adesiva também está intimamente ligada à formação de retenções micromecânicas de um infiltrado resinoso no esmalte e na dentina (Leme-Kraus AA. et al., 2020). O condicionamento ácido dentinário tem a função de remover a *smear layer* e a porção inorgânica superficial da dentina, para facilitar a penetração dos monômeros adesivos entre as fibrilas colágenas (Nakabayashi et al., 1982). Este processo é conhecido como hibridização (Nakabayashi & Pashley, 1998), e resulta na formação de uma camada ácido-resistente de dentina “reforçada” por resina adesiva fluida (camada híbrida), a qual é responsável pela retenção do material restaurador ao substrato dentinário (Martins et al., 2008).

Entretanto, como as fibrilas de colágeno não são completamente encapsuladas pelos monômeros resinosos, principalmente na base da camada híbrida (Wang Y. et al., 2003), as metaloproteinases da matriz dentinária (MMPs) podem degradar esse colágeno, o qual perdeu o conteúdo mineral intra e extra-fibrilar (Perdigão J. et al., 2013). A perda mineral torna as fibrilas desprotegidas e limita a longevidade da união dentina-adesivo. A difusão prejudicada da resina adesiva na parte inferior da camada híbrida também pode ser causada por alterações de fase da solução do adesivo, devido ao seu contato com água residual deixada entre as fibras colágenas (Sebold M. et al., 2019). Desta forma, com a deficiente infiltração da resina adesiva, principalmente na base da camada híbrida, as fibrilas de colágeno desprotegidas são propensas à degradação enzimática (Tay et al., 2002).

O colágeno é uma unidade elemental estrutural da matriz extracelular. As fibrilas colágenas apresentam estabilidade química pelas ligações cruzadas enzimáticas inter- e intra-moleculares (Bedran-Russo AK. et al., 2014). Assim, a

estrutura e composição da matriz extracelular dentinária desempenham um papel fundamental nos procedimentos restauradores adesivos e na longevidade das restaurações adesivas (Bertassoni LE. et al., 2017). Aumentar a resistência do colágeno contra à hidrólise por meio da biomodificação de proteínas é uma alternativa para melhorar a estabilidade da área de união dentina-adesivo. Substâncias naturais e sintéticas são capazes de aumentar o número de ligações peptídicas intra e intermoleculares, além das microfibrilares do colágeno (Bedran-Russo AK. et al., 2013). O resultado é a melhoria das propriedades mecânicas da matriz de colágeno desmineralizada (Scheffel et al., 2014).

O digluconato de clorexidina (CHX) é um agente sintético capaz de prevenir a degradação da camada híbrida (Breschi et al., 2009; Tjaderhane 2013). O CHX pode inativar as proteases endógenas da área de união dentina-adesivo (Tjaderhane L. et al., 2013, 2015) e ainda, pode se ligar a várias proteínas por um mecanismo de quelação (Negrelo Newton AP. et al., 2004). Assim, o CHX pode evitar ligações de íons como Zn^{2+} e Ca^{2+} às estruturas das MMPs, inibindo as suas atividades catalíticas (Sousa ABS et al., 2016). O CHX também apresenta propriedades catiônicas, com alta afinidade por estruturas orgânicas (colágeno) e inorgânicas (hidroxiapatita) da dentina, especialmente quando aplicada após o condicionamento com ácido fosfórico (Iskander M. et al., 2015). Alguns estudos relatam efeito detergente do CHX, que pode aumentar a impregnação dos monômeros resinosos na dentina desmineralizada (Breschi L. et al., 2010), enquanto outros mostram que a aplicação de CHX em dentina desmineralizada aumenta a formação de tags resinosos, que ocluem os túbulos dentinários e favorecem a retenção micromecânica (Gendron R. et al., 1999).

O uso de compostos naturais derivados de plantas para preservar a área de união também é uma alternativa atraente aos agentes sintéticos (Tjaderhane L. et al., 2013). Na literatura, há estudos relatando o uso da proantocianidina (PA), a qual é classificada como um flavonóide e sub-classificada como um flavonol. Este composto é um agente antioxidante e de reticulação de colágeno, além de possuir atividades biológicas e funcionais (Bedran-Russo AK. et al., 2014). A principal fonte de PA está no extrato de semente de uva (Joshi SS. Et al., 2001), que demonstra melhorar as propriedades mecânicas e capaz de reduzir as taxas de biodegradação da dentina desmineralizada (Phansalkar RS. et al., 2015) por multi-interação com componentes da matriz dentinária, incluindo colágeno tipo I, proteoglicanos e proteases endógenas (Bedran-Russo AK. et al., 2014). Sugere-se que o mecanismo de ação de ligação

cruzada entre o colágeno e a PA pode ocorrer por meio de ligações covalentes e pontes de hidrogênio. O isolamento de compostos altamente bioativos, a partir do extrato de semente de uva mostrou recentemente resultados promissores para o planejamento futuro de um material de intervenção clínica (Phansalkar RS. et al., 2015).

O uso de um *primer* elaborado com agentes de ligação cruzada na dentina desmineralizada, como um pré-tratamento e antes da aplicação da resina adesiva parece ser uma abordagem promissora para estender a longevidade das restaurações de compósito (Tjaderhane L. et al., 2013, 2015), uma vez que a esses *primers* experimentais podem aumentar a estabilidade funcional da matriz orgânica da dentina (Hicci HA. et al., 2010). Sabendo-se da afinidade entre flavonóides e proteínas ricas em prolina e da capacidade do grupo fenil-hidroxila em formar pontes de hidrogênio com os grupos carbonila presentes nas fibrilas de colágeno (Hagerman AE. et al., 1981), os compostos Kaempferol, subclassificado como flavonol, e Naringina, subclassificado como flavonona, podem ser utilizados na tentativa de reduzir as taxas de biodegradação da dentina desmineralizada.

Os flavonóides são compostos naturais e possuem propriedades anti-oxidativas, anti-inflamatórias, anti-mutagência e anti-carcinogênica, além disso possuem capacidade de modular a chave da função celular enzimática. Eles também são conhecidos por serem potentes inibidores de várias enzimas, como a xantina oxidase (XO), ciclo-oxigenase (COX), lipo-oxigenase e fosfoinositídeo 3-quinase. Esses flavonóides são uma classe de metabólitos secundários que contêm estruturas fenólicas e a sua subclassificação se dá pela posição da ligação do anel benzênico B no C, e o grau de instauração e oxidação do anel C (Figura 1). Os flavonols como o Kaempferol possuem um grupo cetônico. Já a flavonona (Naringina), possui uma ligação saturada no anel benzênico C (Panche AN et al, 2016).

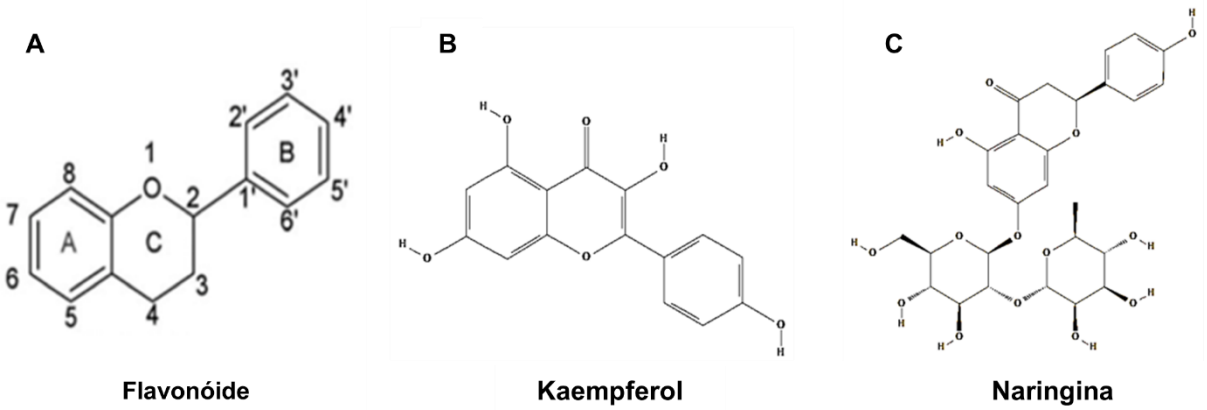

Figura 1. Flavonóides. (A) Esqueleto químico de um flavonóide. (B) Molécula do Kaempferol. (C) Molécula da Naringina. *Adaptado de Panche AN et al, 2016.

Apesar da sua aplicação na Odontologia ainda não ter sido relatada, espera-se que estes compostos tenham ação similar à PA por serem da mesma classe. Porém, a PA, por ser uma molécula oligomérica, apresenta um tamanho molecular maior (592,5 g/mol), podendo apresentar limitada penetração na dentina desmineralizada, além de pigmentar a dentina, provocando problemas clínicos (Epasinghe DJ. et al., 2016). Os flavonóides sugeridos apresentam como vantagem menor tamanho molecular: Kaempferol (286,24 g/mol) e Naringina (580,53 g/mol), que pode facilitar a penetração na dentina desmineralizada e, além disso, podem causar menor ou nenhuma pigmentação à dentina.

Portanto, este trabalho tem como objetivo avaliar o efeito de *primers* experimentais à base de flavonóides, aplicados em dentina desmineralizada previamente ao adesivo, na resistência à união por microtração e na morfologia da área de união dentina-adesivo.

2 ARTIGO: Effect of flavonoid-based experimental primers on dentin microtensile bond strength and interface morphology

Artigo submetido ao periódico Journal of Dentistry **(Anexo 1)**.

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ABSTRACT

Objectives: This study evaluated the effect of four flavonoid-based experimental primers on the dentin bond strength (μTBS) and the bonding interface morphology of an adhesive. Methods: Four experimental primer solutions containing two different flavonoids (Kaempferol - Ka and Naringin - Na) in two concentrations (10 mM and 20 mM) were applied for 30 s or 60 s on dentin after phosphoric acid etching, followed by an etch-and-rinse adhesive system (Optibond S, Kerr). A Negative Control (NC) consisting of dentin not treated with experimental primer solutions and Positive Controls (PC) that used 0.2% chlorhexidine solution for 30 and 60 s were also tested. Eighty-eight teeth were selected for dentin μTBS (n=8). Dentin-adhesive interface morphology and adhesive resin infiltration (n=4) were analyzed by confocal laser scanning microscopy (CLSM). Data were analyzed by mixed three-way ANOVA (μTBS) and two-way ANOVA (CLSM) (α=0.05). Dentin μTBS was analyzed at 24h and one-year, while the bonding interface morphology and adhesive resin infiltration were evaluated at 24 hrs. Results: Regarding μTBS, no statistical difference was found between the experimental groups and NC at 24 h. However, experimental primers containing 20 mM Na, 10 and 20 mM Ka applied for 60 s presented higher values compared to NC at one-year. Hybrid layer formation and adhesive infiltration was observed in all groups by CLSM. Conclusions: The application of flavonoid-based experimental primers for 60 s was able to produce higher μTBS than those obtained with NC and PC after one-year of artificial aging, without interfering with interface morphology.

Clinical significance: Flavonoid-based primers were able to preserve the bond strength of acid-etched dentin after artificial aging. These formulations have the potential to improve the longevity of adhesive restorations.

Keywords: flavonoids; kaempferol; naringin; dental bonding; microtensile bond strength; adhesive.

1. INTRODUCTION

In past decades, dental research has pushed forward remarkable evolution and advancement in the field of adhesive restorative techniques, as well as in the chemistry of adhesive resin-based materials, resulting in broader knowledge about dentin bonding mechanisms [1]. However, there are still concerns regarding the stability of dentin-resin bonding over time, with reports of a significant decrease in dentin bond strength associated with acid etching [2,3]. It is believed the degradation of collagen fibrils due to the activation of dentin matrix metalloproteinases (MMPs), and the hydrolytic degradation of adhesive resin polymer [4] contribute significantly to the failure of composite restorations [5,6].

Within this context, the development of experimental dentin primer solutions might be a promising clinical strategy, with a few protocols already under investigation [7]. For example, increasing collagen resistance to hydrolysis through protein biomodification could be an alternative to improve the stability of dentin bonding. Natural and synthetic substances can increase the number of intra- and intermolecular peptide bonds within collagen fibrils [1], improving the mechanical properties of the collagen matrix that lost the minerals around it [8]. In addition, these substances have been shown to inhibit and reduce MMP activity [8], which could contribute to the longevity of dentin-resin bonding.

Chlorhexidine digluconate (CHX) is one of the synthetic agents capable of inactivating endogenous proteases within the dentin-resin bonding interface [9,10], which might increase the longevity of the hybrid layer [11,12]. CHX binds to several proteins by a chelation mechanism [13], and consequently it avoids ions such as Zn^{2+} and Ca^{2+} from binding to MMPs, inhibiting their proteolytic activity [7]. Also, CHX has cationic properties, presenting strong affinity for organic (collagen) and inorganic (hydroxyapatite) dentin structures, especially when applied after acid etching [14].

Apart from synthetic agents, the use of natural, plant-derived compounds, such as flavonoids, has been demonstrated to reduce the degradation of collagen fibrils [15]. Previous studies have reported on the application of proanthocyanidin, which is an antioxidant and collagen crosslinker provided with biological and functional activities [16] that can preserve the integrity of the hybrid layer over time [17]. Flavonoids have anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-

carcinogenic properties, while also being able to modulate key processes of cell enzymatic function [18]. They are also known to be potent inhibitors of various enzymes, namely xanthine oxidase, cyclooxygenase, lipooxygenase and phosphonoside 3-kinase [18]. In fact, some flavonoids have shown better performance in reducing the degradation of the hybrid layer when compared to proanthocyanidins [19,20].

Flavonols such as Kaempferol have a ketone group, while Naringin has a saturated bond in the benzene ring C [18], similar to the chemical structure of other flavonoids. Although their application in dentistry has not yet been reported, these compounds are expected to have a similar action mechanism compared to proanthocyanidin, because they come from the same class of biomodifiers, which induce irreversible changes in the catalytic domain or allosteric inhibition of other modular domains of proteases (MMPs) involved in collagen degradation [21]. Considering proanthocyanidin is an oligomeric molecule with a larger molecular size (592.5 g/mol), it might present limited penetration into demineralized dentin. Furthermore, it causes dentin staining, leading to clinical problems [22]. Conversely, flavonoids have the smallest molecular size advantage: Kaempferol (286.24 g/mol) and Naringin (580.53 g/mol). This suggests they would better penetrate demineralized dentin, presenting promising results in the preservation of the dentin-resin bond [23].
Therefore, the aim of this study was to evaluate the effect of the application of flavonoid-based (Kaempferol and Naringin) experimental primers on dentin bonding at different concentrations (10 mM or 20 mM) and application times (30 s or 60 s) after phosphoric acid etching. The null hypotheses of this study were: (1) flavonoid-based primers would not influence the results of dentin microtensile bond strength (μTBS) after 24 h or one-year of storage in artificial saliva when compared to untreated dentin (Negative Control); (2) flavonoid-based primers would not influence the results of μTBS after 24 h or one-year of storage in artificial saliva when compared to dentin treated with CHX (Positive Control); (3) different concentrations and application times would not influence the μTBS results; and (4) flavonoid-based primers would not interfere with the morphology of the bonding interface.

2. MATERIALS AND METHODS

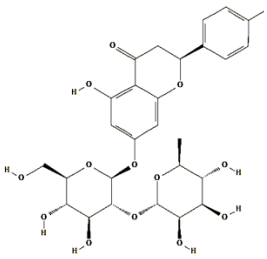
2.1. Teeth Selection and Preparation

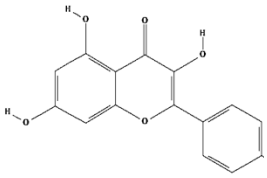
One hundred and thirty-two sound human third molars were obtained after the approval by the Ethics Committee in Research of the Piracicaba Dental School (CAAE #19855119.1.0000.5418). All teeth were cleaned by hand-scaling with a periodontal curette (SS White Duflex, Juiz de Fora, MG, Brazil), and polished with a paste of pumice and water. Afterwards, they were stored in aqueous solution of 0.5% Chloramine-T (Merck KGaA, Darmstadt, Germany) at 4 °C for no longer than three months.

2.2. Experimental Primer Solutions

Naringin (Merck KGaA, Darmstadt, Germany) and Kaempferol (Merck KGaA, Darmstadt, Germany) were diluted at concentrations of 10 mM and 20 mM in absolute ethanol. The physical and chemical properties of these flavonoids are displayed in Table 1. The compounds were diluted and homogenized with the aid of a magnetic stirrer and kept in light-proof containers, with their lids closed to prevent solvent evaporation. The pH of these solutions was determined using a digital pH meter (MS Tecnopon Equipment's, Piracicaba, SP, Brazil) (Table 2). Chlorhexidine 0.2% in aqueous solution was used as Positive Control.

Table 1. Physical and Chemical characteristics of flavonoids used for the experimental primers.

Substance	Empirical formula	2D Chemical structure*	Number of hydroxyfenyl radicals	Molecular Mass	Solubility in water	Solubility in ethanol	Color
Naringin ≥95% HPLC (Merck KGaA, Darmstadt, Germany)	$C_{27}H_{32}O_{14}$		2	580.53 g/mol	1mg/mL at 40º C	0.1 g/mL	White to faint beige

Kaempferol ≥97% HPCL (Merck KGaA, Darmstadt, Germany)	$C_{15}H_{10}O_6$		4	286.24 g/mol	440mg/L at 25º	0.02 g/mL	Yellow very dark yellow

Information provided by the Manufacturer. (*) According to PubChem.

Table 2. Characteristics of experimental primer solutions.

Experimental Primers	pH	Color of the solution
Naringin 10 mM	7.2	Colourless
Naringin 20 mM	7.7	Colourless
Kaempferol 10 mM	7.3	Yellow
Kaempferol 20 mM	7.1	Yellow

2.3 Dentin Microtensile Bond Strength Test (μTBS)

This study followed the methodology described in the guidelines of the Academy of Dental Materials which were published in Dental Materials [24]. The occlusal enamel of each tooth was removed with a diamond saw (Buehler Ltd., Lake Bluff, IL, USA) using a precision cutting machine, and dentin was exposed at medium depth in relation to the pulp. Dentin surfaces were polished with silicon carbide paper (600-grit) for 5 s under water-cooling to create a flat surface and standardize the smear layer.

Eighty-eight teeth were randomly assigned to eleven groups (n=8), according to type of primer, concentration of primer main ingredient, and application time (Table 3). Dentin was etched with 35% phosphoric acid (Ultra-Etch, Ultradent Products, Inc., South Jordan, UT, USA) for 15 s and rinsed with water for 15 s by an oil-free air/water spray. Dentin was kept moist before primer application. An amount of 15 μL of each primer was applied passively to the dentin surfaces, according to the application time

of each group. Excess primer solution was removed with absorbent paper. Then, a 2-step, etch-and-rinse adhesive (Optibond S, Kerr Dental, Orange, CA, USA) was applied for 15 s using light brushing motion, followed by air thinning for 3 s, and light-cured according to the manufacturer's instructions. The Negative Control consisted in the application of adhesive resin without a previous priming step.

Table 3. Treatments, type of flavonoid-based primer, concentrations and primer application time for experimental groups and controls.

Treatment	**Primer/Concentration (abbreviation)**	**Application time (in seconds)**
Positive Controls	Chlorhexidine 0.2% (CHX/30)	30
	Chlorhexidine 0.2% (CHX/60)	60
Flavonoid-based goups	Naringin 10 mM (Na10/30)	30
	Naringin 10mM (Na10/60)	60
	Naringin 20 mM (Na20/30)	30
	Naringin 20 mM (Na20/60)	60
	Kaempferol 10 mM (Ka10/30)	30
	Kaempferol 10mM (Ka10/60)	60
	Kaempferol 20 mM (Ka20/30)	30
	Kaempferol 20 mM (Ka20/60)	60
Negative Control	No primer application	-

A resin composite (Spectra Smart, Dentsply Sirona, Pirassununga, SP, Brazil) build-up of approximately 4-mm height was placed over bonded dentin, using 2 consecutive layers of composite with 2-mm thickness. Each composite layer was light-cured for 20 s (Valo, Ultradent Products Inc., South Jordan, UT, USA). The light-curing unit was verified by a spectroradiometer (MARC-PS, BlueLight Analytics Inc., Halifax, NS, Canada) to ensure the delivery of a radiant exposure of at least 16.8 J/cm^2 (50). All restorative materials used in this study are described in Table 4.

Table 4. Commercial name, composition and batch number of the materials used in the study.

Material	Composition	Batch Number
Ultra-Etch	Phosphoric acid, dimethicone.	BFDJ8
Optibond S	Alkyl dimethacrylate resins, barium aluminoborosilicate glass, fumed silica (silicon dioxide), sodium hexafluorosilicate and ethyl alcohol.	6840312
Spectra Smart	Glass powder, colloidal silica hydrophobic, dimethacrylate, benzophenone-3, ethylamine benzoate, camphorquinone, butylated hydroxytoluene, yellow and red iron oxide, black iron oxide and titanium dioxide.	356416K
SureFil SDR Flow	Modified urethane dimethacrylate resin, triethylene glycol dimethacrylate, polymerizable dimethacrylate resin, polymerizable trimethacrylate resin, camphorquinone, ethyl-4(dimethylamino)benzoate, butylated hydroxy toluene, fluorescent agent, UV stabilizer, barium-alumino-fluoro-borosilicate glass, silanated strontium alumino-fluoro-silicate glass, surface treated fume silicas, ytterbium fluoride, synthetic inorganic iron oxide pigments and titanium dioxide (70.5 wt% / 47.4 vol% filler).	02419

Restored teeth were kept at relative humidity, at 37 °C, for 24 h. Thereafter, teeth were sectioned in lingual-buccal and mesial-distal directions to obtain at least 8 to 16 stick-shaped specimens per tooth with a cross-section area of approximately 1.0 mm^2. Half of the specimens per teeth were tested after 24 h of storage in artificial saliva (12.9 mM KCl, 1.9 mM KSCN, 2.4 mM Na_2SO_4 10 H_2O, 3.3 mM NH_4Cl, 1.5 mM $CaCl_2$ $2H_2O$, 7.5 mM $NaHCO_3$, 0.02 mM $ZnCl_2$ and 5 mM HEPES buffer pH 7.4) [25] at 37°C, while the other half was tested after 12 months of storage under the same conditions. The artificial saliva was replaced monthly.

Specimens were tested on a microtensile device attached to a universal testing machine (EZ Test, Shimadzu, Kyoto, Japan). Each specimen was fixed to the device with cyanoacrylate-based glue (Super Bonder Gel, Henkel/Loctite, Diadema, SP, Brazil) and tested at a constant speed of 1.0 mm/min until failure. The cross-section area of the specimens was measured by means of a digital caliper (Mitutoyo Co., Kanagawa, Japan), and bond strength means were calculated from the average of the specimens per tooth, according to the evaluation time. Data were submitted to normal

distribution and homoscedasticity tests (Shapiro-Wilk and Levene tests) and were analyzed by three-way mixed ANOVA, followed by Bonferroni's test (between-subject factors: "type of primer" and "primer application time"; within-subject factor: "evaluation time"). The Negative Control was analyzed by paired T test and compared to other groups by one-way ANOVA, followed by Dunnett's test [43].

2.4 Failure Mode Analysis by Scanning Electron Microscopy (SEM)

The surfaces involved in the fracture of each specimen after the microtensile bond strength test were analyzed by SEM regarding failure pattern classification. The fractured specimens were fixed on metallic stubs with carbon tape, keeping the areas involved in the fractures facing upwards. Then, these fragments were sputter-coated with gold (SDC 050 Sputter coater, Baltec, Balzers, Liechtenstein) and observed under SEM (JSM 5600LV, Jeol, Tokyo, Japan), using 100x and 400x magnifications. Failure modes were classified according to the structures involved [26]: Type I - cohesive failure within the resin composite; Type II - adhesive failure between resin composite and the bonding agent; Type III - adhesive failure between dentin and the bonding agent; Type IV - mixed failure (dentin, bonding agent, and resin composite can be observed in the same fractured surface); Type V - cohesive failure within the bonding agent layer; Type VI - cohesive failure within the hybrid layer; and Type VII - cohesive failure within dentin. In order for a specimen not to be considered mixed, 70% or more of a single specific failure mode should be present on the evaluated surface. Descriptive analysis was used to report failure modes with their respective percentages of occurrence.

2.5 Adhesive-Dentin Bonding Interface Morphology Analysis by Confocal Laser Scanning Microscopy (CLSM)

Forty-four human third molars (n = 4) were restored following the same methodology described for the microtensile bond strength test. However, Rhodamine B dye (Sigma-Aldrich, St. Louis, MO, USA), which presents a pink-reddish color, was previously added to the adhesive system at a concentration of 0.1 wt% [27]. After acid etching and the application of the tested primers, the adhesive resin containing

Rhodamine B was applied and light-cured. A flowable bulk-fill resin (SureFil SDR Flow (Dentsply Sirona, Konstanz, Germany) was used to cover the adhesive-bonded surfaces and the samples were stored in vegetable oil (Soya, Bunge Brasil, São Paulo, SP, Brazil) to avoid water loss and/or dye dissolution, at 37 °C for 24 h. Then, teeth were sectioned into 1-mm thick slices. These slices were manually polished with 2000-grit silicon carbide paper for 30 s.

Polished samples were analyzed under CLSM (TCS SP5, Leica Microsystems, Mannheim, Baden-Württemberg, Germany). The excitation energy provided by the argon (488 nm) and He-Ne (543 nm) lasers, and the photomultipliers amplification were constant throughout the whole investigation. A layer approximately 10 μm below the surface of the sample was observed and CLSM micrographs were obtained in fluorescent and reflectance modes with oil immersion objective lens (63x magnification, 3x zoom, numeric aperture of 1.3, pinhole of 5.5 μm). At least three sets of micrographs were obtained for each sample, which comprised (1) an image of dye detection in fluorescent mode, (2) an image formed by overlapping the micrographs of fluorescent and reflectance modes, and (3) a gray-scale image of the sample surface in transmission mode. The hybrid layer thickness was measured using the Image J software (Fiji, Version 1.0). Data were submitted to normal distribution and homoscedasticity tests (Shapiro-Wilk and Levene tests) and analyzed by two-way ANOVA and one-way ANOVA (comparing negative controls).

3. RESULTS

3.1. Dentin Microtensile Bond Strength Test

Mean (± standard deviation) μTBS values are presented in Table 5. Three-way mixed ANOVA analysis showed that “type of primer” ($p < 0.001$), “primer application time” ($p = 0.009$), “evaluation time” ($p < 0.001$), and all interactions between each two factors significantly influenced μTBS results.

Table 5. Means (± standard deviation) dentin μTBS (in MPa), comparing the experimental groups with the Negative Control at different evaluation time and primer application time on dentin.

Treatments	24 hours		One year	
	30s	60s	30s	60s
Chlorhexidine 0.2%	69.4 (10.7) Aa	65.1 (11.1) Ab	84.6 (9.5) Aa	76.2 (11.1) Ac
Naringin 10 mM	76.2 (9.4) Aa	80.3 (6.9) Aa	83.9 (10.0) Aa[&]	88.3 (4.8) Abc[&*]
Naringin 20 mM	71.4 (9.3) Aa	81.2 (11.6) Aa	86.0 (7.8) Ba	97.7 (9.3) Aab*
Kaempferol 10 mM	73.0 (12.4) Aa	67.2 (8.2) Aab	91.3 (9.9) Aa*	96.9 (3.5) Aab*
Kaempferol 20 mM	73.2 (5.4) Aa	75.4 (11.6) Aab	94.6 (4.8) Ba*	107.1 (8.6) Aa*
Negative Control	71.1 (10.1)		75.9 (4.4) [&]	

Means followed by different letters indicate significant difference ($p<0.05$). Lowercase letters compare treatments within the same application and evaluation times. Uppercase letters compare application times within the same treatment and evaluation time. ([&]) Indicate no significant difference compared to the 24-hour results for the same treatment and the application time. (*) Indicate significant difference from the Negative Control within the same evaluation time.

When applied for 30 or 60 s, none of the flavonoid-based experimental primers showed higher μTBS than Negative Control (no primer application) at 24 hours. Differences among treatments were observed when experimental primers were applied for 60 s, as naringin-based primers presented higher μTBS means at 24 hours compared to CHX/60, for both concentrations tested (Na10/60 and Na20/60).

After one year of storage, most groups of the tested flavonoid-based experimental primers had higher μTBS means compared to CHX/60. Also, the μTBS of most experimental primer groups increased after one year, while Na10/30 and Na10/60 presented stable μTBS results over time. In general, flavonoids-based primers presented higher μTBS means compared to the Negative Control group at one year, except for Na10/30 and Na20/30. For CHX, the application time did not influence the results, regardless of the evaluation time.

3.2 Failure Mode Analysis

The percentages of occurrence of failure modes for each tested group are presented in Figure 1. Cohesive failures within resin composite (Type I) were predominant (27% or more) for all groups, regardless of evaluation time (Figure 2.1). Cohesive failures within dentin (Type VII) were also observed for all experimental and control groups (10% or more) (Figure 2.7). The sum of both cohesive failures (within resin composite and dentin) was greater than 50%. Adhesive failures between resin composite and bonding agent (Type II) were the most predominant adhesive fracture (Figure 2.2), while adhesive failures between dentin and bonding agent (Type III - Figure 2.3) were not always observed (at 24 hours: Na20/30 and at one year: Na20/30, Na20/60, and Ka10/30) or had a low incidence. Failure modes Type IV (Figure 2.4) and V (Figure 2.5) showed intermediate frequency of occurrence, and Type VI failures were very uncommon (Figure 2.6). Failure modes for the experimental and control groups did not seem to change after one year of storage.

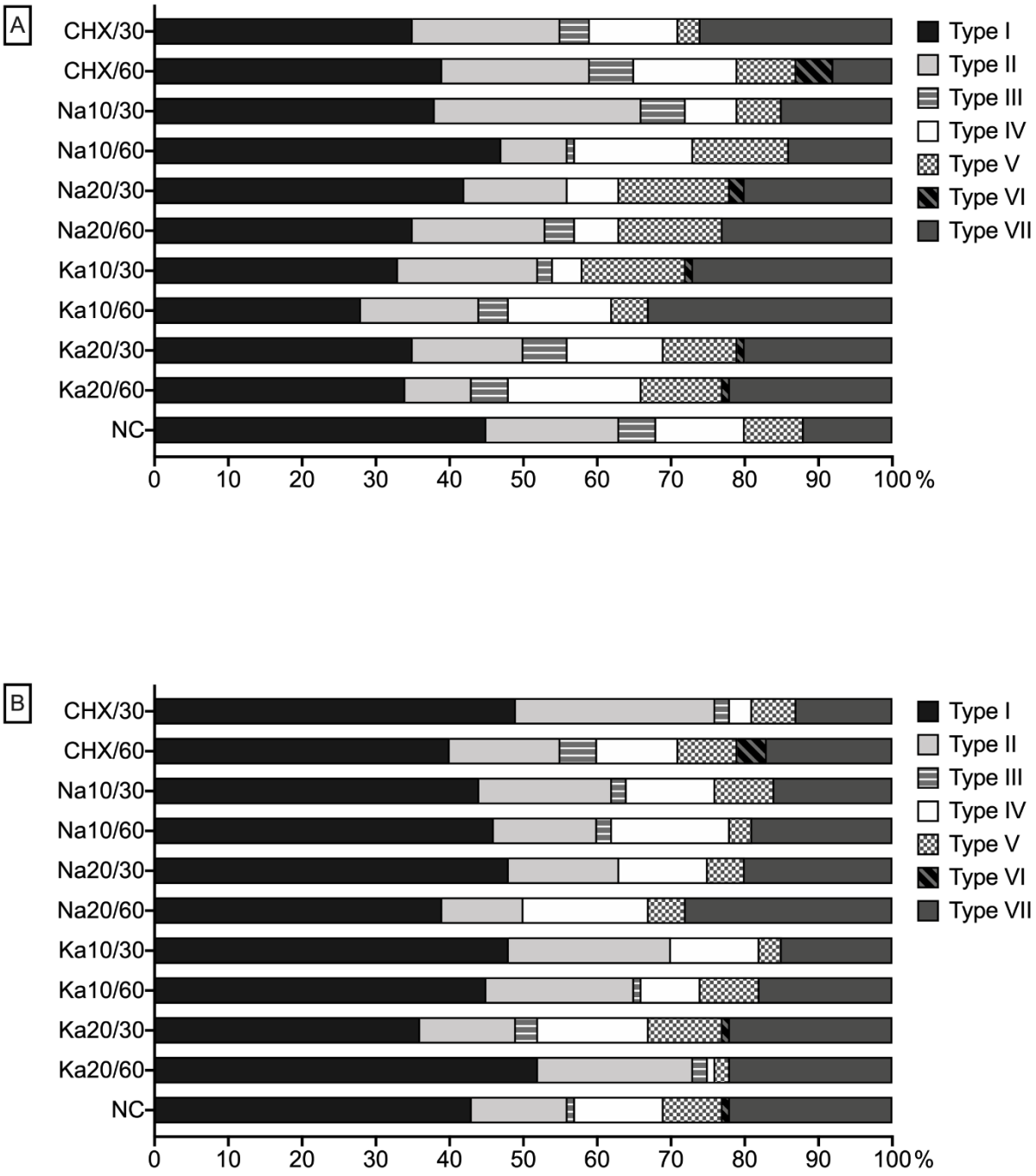
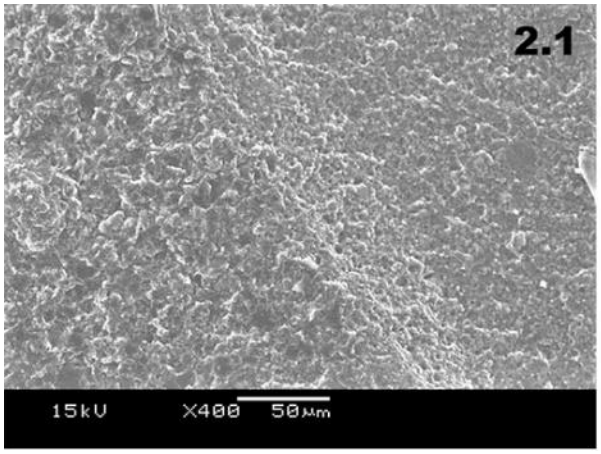
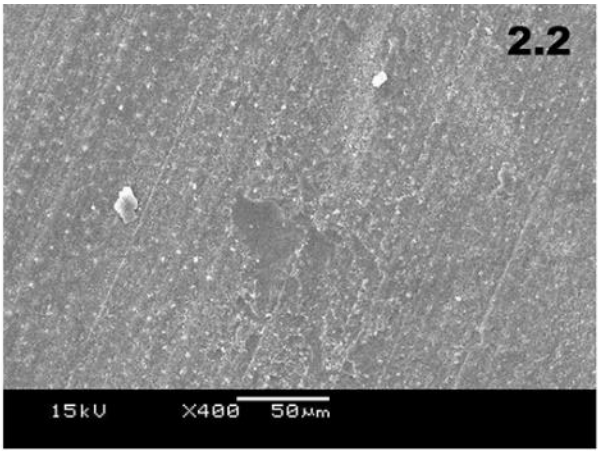


Figure 1. Failure pattern percentage for each group. (A) at 24 hours and (B) at one year. Type I: cohesive failure within resin composite; Type II: adhesive failure between resin composite and bonding agent; Type III: adhesive failure between dentin surface and bonding agent; Type IV: mixed failure; Type V: cohesive failure within adhesive layer; Type VI: cohesive failure in the hybrid layer; Type VII: cohesive failure within dentin.


2.1
15kV X400 50µm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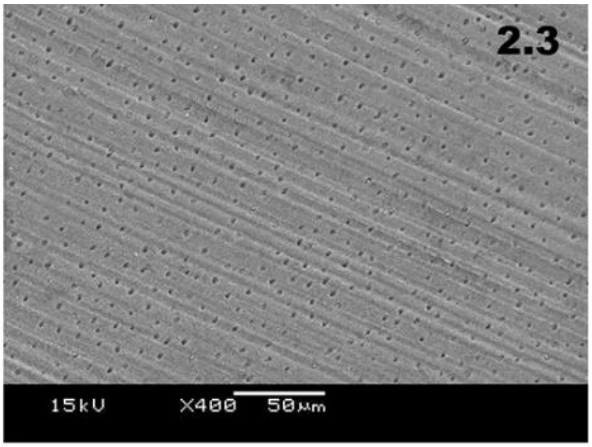
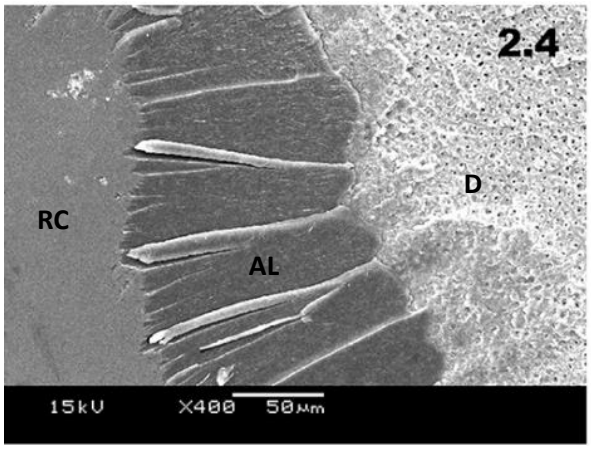
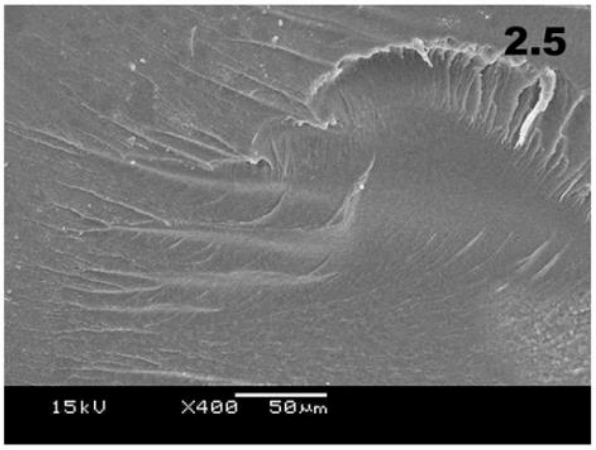
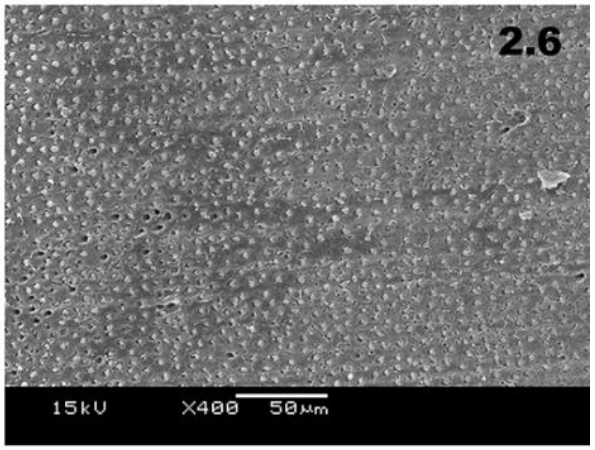
15kV X400 50µm


2.3
15kV X400 50µm


2.4
RC
AL
D
15kV X400 50µm


2.5
15kV X400 50µm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15kV X400 50µm

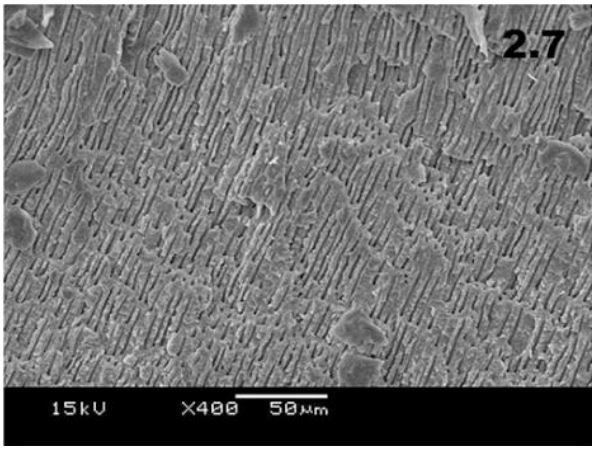
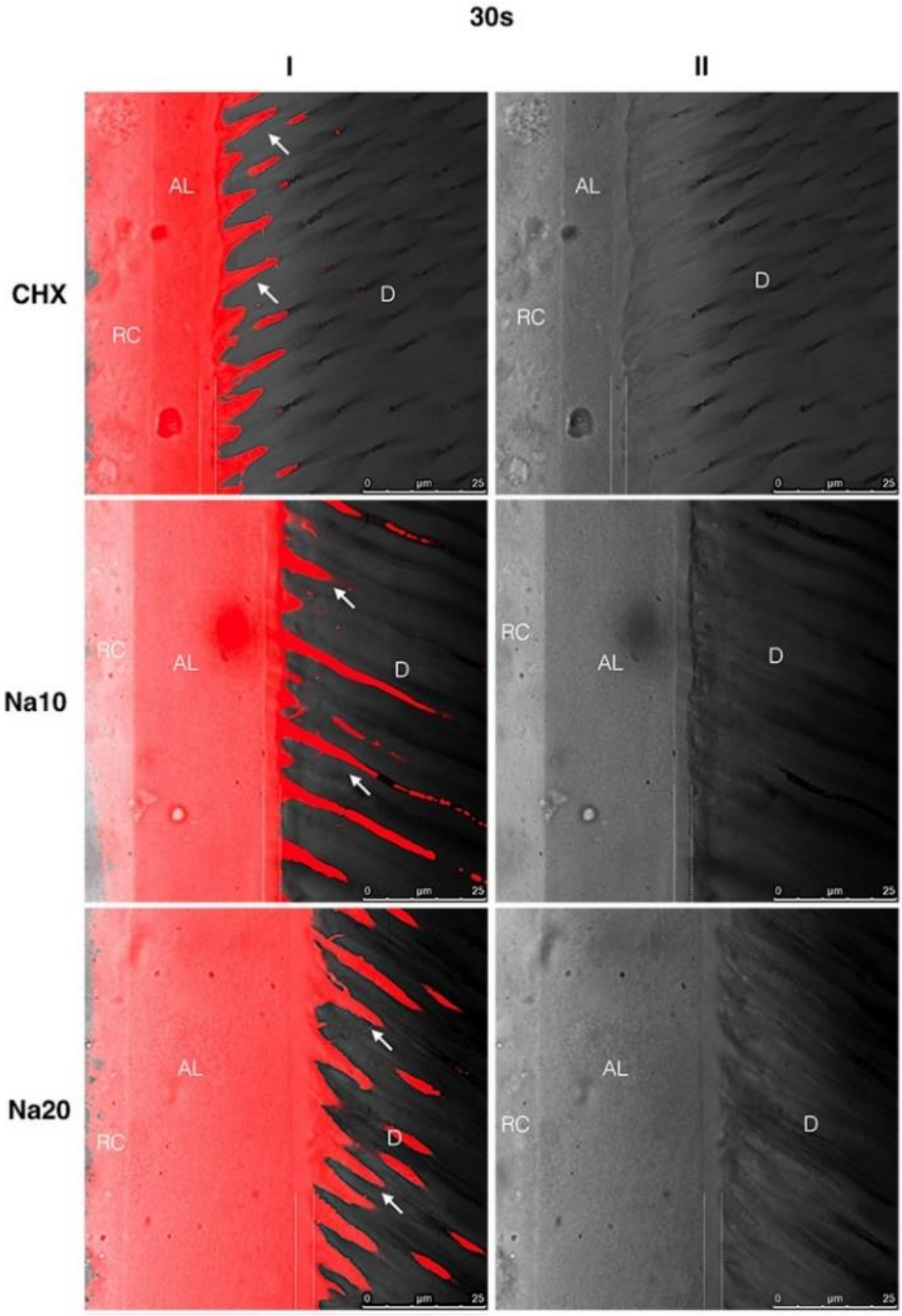
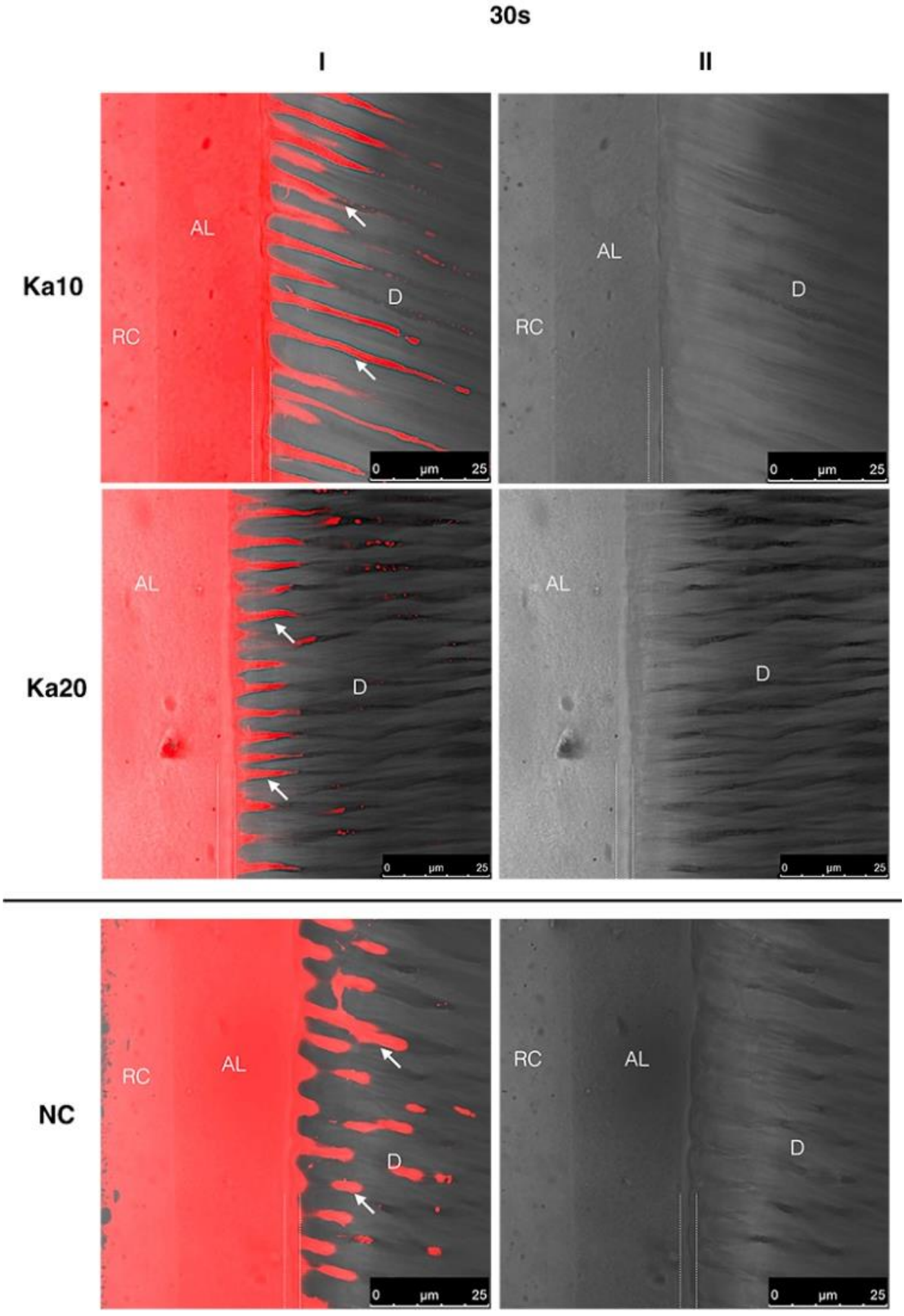

2.7
15kV X400 50µm

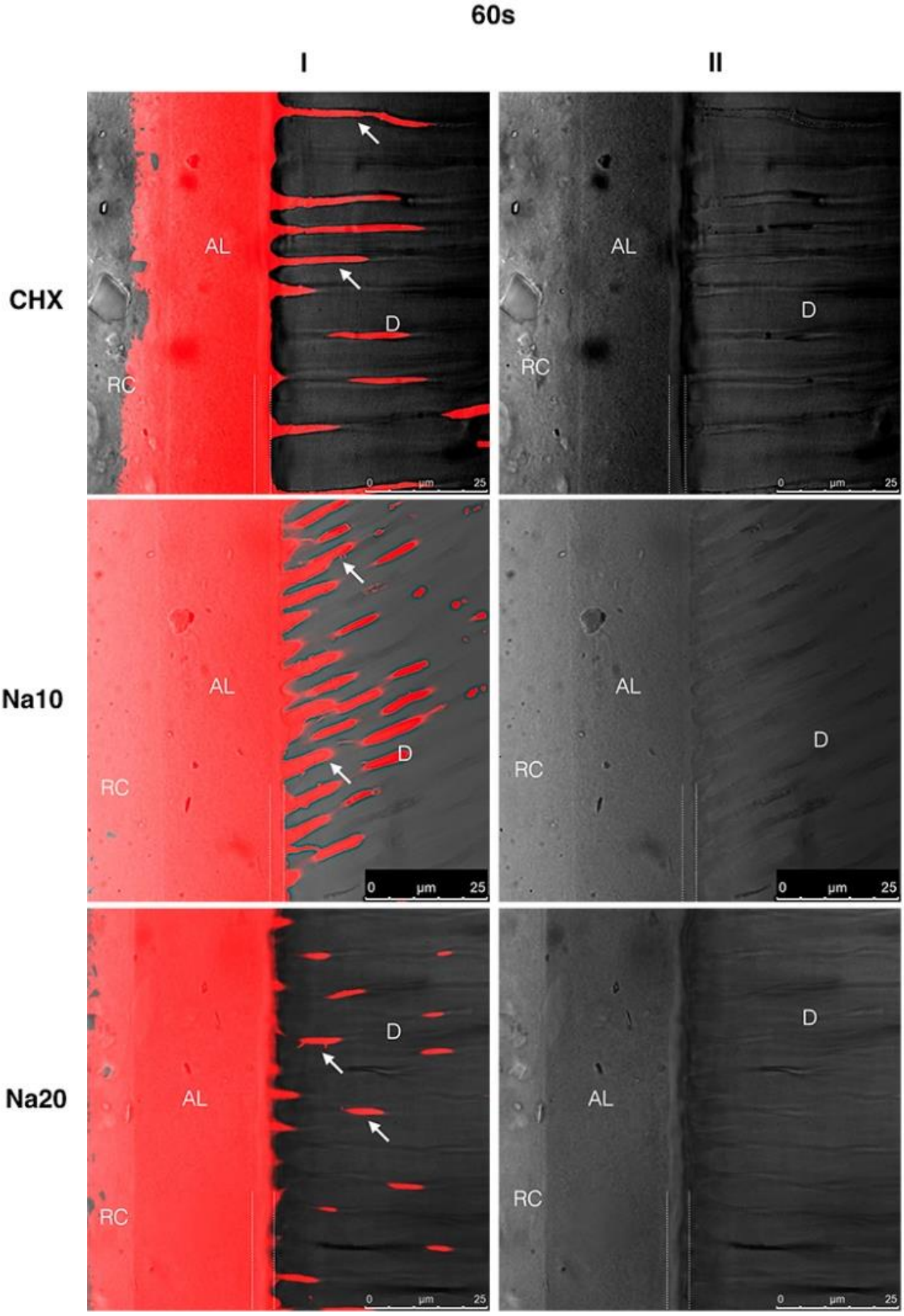
Figure 2. Representative SEM images of each failure pattern at 400x magnification. 2.1: (Type I) cohesive failure within resin composite; 2.2: (Type II) adhesive failure between resin composite and bonding agent; 2.3: (Type III) adhesive failure between dentin surface and bonding agent; 2.4: (Type IV) mixed failure; 2.5: (Type V) cohesive failure within adhesive layer; 2.6: (Type VI) cohesive failure in the hybrid layer; 2.7: (Type VII) cohesive failure within dentin. RC: resin composite; AL: adhesive layer; D: dentin.

3.3 Adhesive-Dentin Bonding Interface Morphology Analysis

Representative CLSM images of the dentin bonding interface morphology and measurements of hybrid layer thickness are depicted in Figure 3 and Table 6, respectively. The average hybrid layer thickness of the tested groups ranged from 2.6 to 4.3 μm. Resin tags formation was observed in all groups, despite the application of a dentin primer. Slight differences were observed among groups regarding the number and length of resin tags. However, this feature depends on the dentin cutting direction and on the Z depth of CLSM. The concentration of CHX and flavonoids, as well as the application time did not influence the hybrid layer thickness means (Table 6).


30s
I
II
CHX
Na10
Na20
AL
RC
D
0 µm 25


30s
I
II
Ka10
AL
RC
D
0 µm 25
Ka20
NC


60s
I
II
CHX
Na10
Na20
AL
D
RC
0 µm 25

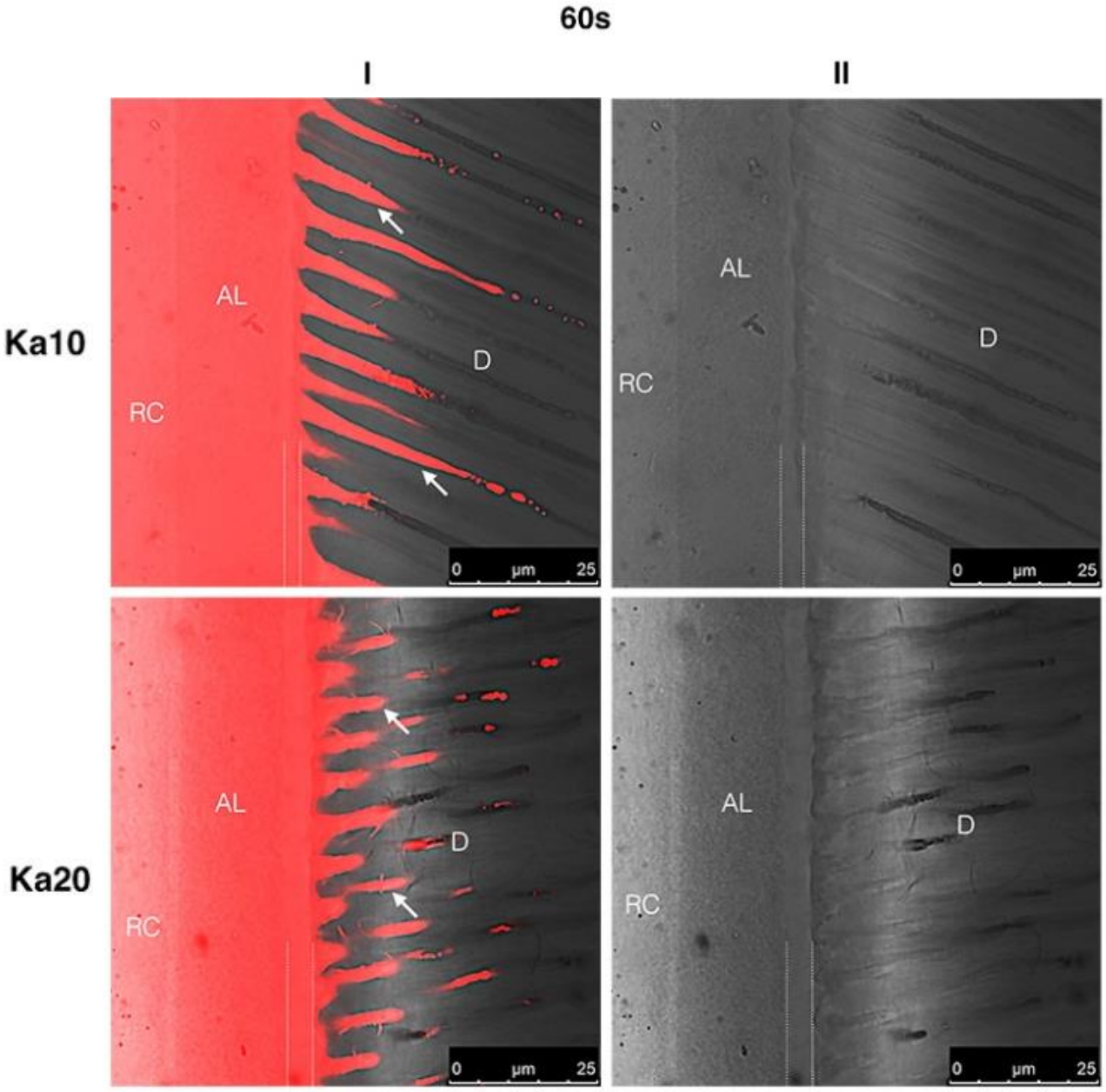


Figure 3. CLSM images of each group tested. (I) Overlapping of the micrographs on fluorescent mode and reflectance mode. (II) Micrographs on reflectance mode. The area between dotted lines indicates the hybridization zone. Arrows indicate resin tags formation. RC: resin composite; AL: Adhesive layer; D: dentin.

Table 6. Hybrid layer thickness means (in µm) for each tested group, according to the primer application time.

Group	30s	60s
CHX	2.7 (0.4)	2.9 (0.3)
Na10	3.2 (0.8)	3.2 (0.6)
Na20	3.6 (0.1)	2.8 (1.1)
Ka10	3.4 (0.6)	2.9 (0.3)
Ka20	3.4 (0.5)	3.9 (0.3)
NC	3.6 (0.8)	

No significant difference was observed among groups.

4. DISCUSSION

Many researchers have investigated and discussed alternative clinical approaches to increase the stability and longevity of dentin-resin bonds [2,18,23]. The use of experimental primers based on flavonoids has been shown to increase collagen resistance against hydrolysis through the biomodification of proteins, which consequently improves the stability of the adhesive interface [7]. Although several flavonoids have already been extensively investigated, the search for different subclasses of these compounds for the development of new techniques and materials remains extremely important in the current scenario of restorative dentistry, especially regarding the longevity of adhesive restorations after dentin acid etching.

This study showed flavonoids used as experimental primer solutions could influence µTBS values when compared with the Negative Control (untreated dentin, i. e. no primer application). Thus, the first null hypothesis was rejected. As observed in Table 5, the tested dentin primers had a positive effect on µTBS results only after one year of aging compared to the Negative Control, unlike what occurred for the 24-hour

evaluation time, when results did not differ statistically from the Negative Control. Although other studies corroborate the data herein presented [28], additional research approaches are needed in order to fully understand the mechanism of action of these compounds, and their long-term effects on dentin bonding.

The present study deals with the use of flavonoids, which are a group of natural substances presenting varied phenolic structures. They can be classified into different subclasses, depending on the position of the carbon element in their molecular ring, the position in which their B ring is attached, and the degree of oxidation of their C ring [18]. Despite the similarity between flavonoids, considerable variations in biological properties can be caused by even minor changes in their molecular structures, such as the number and specific positions of hydroxyl groups and their substitutions [29]. Naringin belongs to the group of flavones, which have a saturated C ring in their structure, while Kaempferol belongs to the subgroup of flavonols, which are characterized by presenting a ketone group and compared to flavones, flavonols have a hydroxyl group at position 3 of the C ring (Table 1). Both subclasses are diverse in methylation and hydroxylation patterns [18].

In addition to the structural difference, the interaction between natural compounds and dentin will depend on the type of existing chemical bonding, following four proposed types: covalent [16], hydrogen [9], ionic [30] or hydrophobic [31] bonds. Thus, a potential positive effect of flavonoids on dentin bonding could be related to the number of hydrogen bonds between their reactive phenolic groups which may react with the carboxyl, amine, hydroxyl, or amide groups in collagen fibrils [32], suggesting these compounds might play an important role in stabilizing these fibrils. Due to its triple-helix structure, collagen allows the binding of hydrogen molecules present in the flavonoids to carbonyl oxygen in the peptide of collagen fibrils [33].

There are also a few factors that might contribute to the cross-linker effect of flavonoids on demineralized dentin: I) the smaller molecular size (weight) of the flavonoids that facilitates their diffusion ; II) the number of molecules available in the experimental primer solution; III) the molecular solubility index and its influence on the miscibility of the vehicle used for dilution of the flavonoids; and IV) the amount and type of reactive sites of the flavonoid molecules (Table 1) [23]. Kaempferol presents a higher amount of hydroxyphenyl sites, lower molecular weight, and better solubility in alcohol

compared to Naringin. These features of Kaempferol might have reflected on the results obtained by this type of flavonoid applied for 60 s, even after artificial aging of the samples for one year.

Although Naringin has similar characteristics to Kaempferol, they differ regarding molecular weight. Naringin has double the molecular weight of Kaempferol, and it has two hydroxyphenyl radicals less than Kaempferol. These differences may have hindered infiltration and interaction with demineralized dentin for Naringin. Increasing the concentration of Naringin to 20 mM and the application time of its derived primer to 60 s improved the bond strength to dentin when compared to CHX/60. Therefore, the amount of Naringin present in the primer may influence the interaction with demineralized dentin. In fact, the highest results found of μTBS at one year were Ka10/60, Ka20/60, and Na20/60 compared to CHX and NC.

The primer application time on dentin surface was also a factor that influenced the results of μTBS. When studies about the application of natural compounds on dentin were initiated, the recommended application time was of at least 10 min [34]. However, this time would be clinically unfeasible. More recently, studies have shown that application times between 60 and 120 s could be considered acceptable clinical times [35]. These short application times were sufficient to increase the degree of crosslinking and the μTBS results in demineralized dentin [36]. The present study proposed different application times, including a period of time even shorter than those reported in literature so far (30 s).

At 24 hours, the primer application time did not cause any change in μTBS results, which was not observed at one year. After artificial aging, the Na/20 and Ka/20 showed better results when the primers were applied to dentin for 60 s compared to 30 s, rejecting the third null hypothesis. Although the application for 30 s was not comparable with the results of 60 s, it did not demonstrate a poor performance in μTBS after one year of storage. In fact, most of the tested groups were able to increase the μTBS values, except for Na10/30 and Na10/60. Also, the concentration of 10 mM or 20 mM did not affect μTBS comparing within the same flavonoid and application time, for both evaluation times. However, Na10/60 had no statistical difference compared to CHX, while Na20/60 had a statistically significant difference compared to CHX.

A solution of chlorhexidine digluconate at 0.2% was used as a positive control in this study, because it has shown positive effects on bonding interface stability in other studies [11,12]. However, the duration of these effects has been questioned. Albeit CHX has an inhibitory effect on dentin MMPs in the adhesive interface [37], the longevity of CHX-induced effects is a limitation to solve the problem of hybrid layer degradation. Therefore, the second null hypothesis of this study should also be rejected, since some experimental primers based on flavonoids differed from CHX (Na10/6, Na20/6 for 24-hour evaluation; Na20/60, Ka10/60, and Ka20/60 after one-year aging).

Another factor that might be related to the better μTBS performance of the experimental primers after aging compared to CHX is the vehicle that was used to dilute the tested flavonoids. Alcohol, in addition to exerting positive effects on dentin bonding [38], can have a synergistic effect with flavonoids, affecting polyphenolic collagen interactions and the amount of hydrogen bonds formed [39]. These interactions occur due to a decrease in the dielectric constant, which can reduce the interfibrillar spaces filled with water, thus preventing collagen degradation [40]. Moreover, flavonoids may be more reactive when diluted in alcohol, as in the case of Naringin [41]. Despite the described differences in μTBS, no great changes are observed in failure modes among groups. The high prevalence of cohesive failures within resin composite or within dentin demonstrates the bonding area, which was able to withstand tensile testing, might be more resistant that the cohesive strength of the composite or dentin. Additionally, Type III failures (between dentin and adhesive) were very uncommon or not present at all, even for Negative Control, showing the Optibond S adhesive interacted properly with etched dentin.

The analysis of interface morphology by CLSM shows all tested groups had hybrid layer formation and adhesive infiltration into dentin coupled with the formation of resin tags, though none or little influence on hybrid layer thickness was seen. Hence, the fourth null hypothesis was accepted. The thickness of the hybrid layer is not always related to higher μTBS values [42], which can be observed comparing the average thickness of Na20/60 (2.8 μm) and Ka10/60 (2.9 μm) and their respective 24-hour μTBS results with other groups.

Although the μTBS values presented in this study seem promising, especially after artificial aging, the application of the experimental primer solutions on etched dentin is an additional step during the bonding procedure, which can increase chair-side time and technique sensitivity. Moreover, further studies are necessary to investigate the interaction of the tested flavonoids with etched dentin and their effects on MMP activity. However, the beneficial effects produced by flavonoids isolated from natural compounds should be considered for the development of future techniques or adhesive restorative materials, focusing on the reduction of bonding interface degradation.

CONCLUSION

Within the limitations of the present study, the following conclusions can be drawn:

- All the tested flavonoid-based primers seemed to improve dentin bond strength after one year compared to untreated dentin when applied for 60 s, while only Kaempferol improved dentin bond strength after one year when applied for 30 s;

- Kaempferol at both tested concentrations and Naringin at 20 mM concentration presented better results after one year compared to chlorhexidine when applied for 60 s;

- The investigated flavonoid-based primers did not seem to interfere with adhesive interface morphology and failure mode.

- The effectiveness of the experimental primers might depend on the type of flavonoid used and the time of application on dentin;

- Better results were obtained when the flavonoid-based primers were applied for 60 s.

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CrediT authorship contribution statement

Beatriz Ometto Sahadi: Methodology, Investigation, Writing - Original Draft, **Carolina Bosso André:** Conceptualization, Formal analysis, Writing - Review & Editing. **Maicon Sebold:** Methodology, Writing – Review and Editing. **Marcelo Giannini:** Conceptualization, Writing - Review & Editing, Project administration.

FIGURE LEGENDS

FIGURE 1. Failure mode percentages of occurrence for each group at 24 hours (A) and at one year (B). Type I: cohesive failure within resin composite; Type II: adhesive failure between resin composite and bonding agent; Type III: adhesive failure between dentin surface and bonding agent; Type IV: mixed failure; Type V: cohesive failure within adhesive layer; Type VI: cohesive failure within the hybrid layer; Type VII: cohesive failure within dentin.

FIGURE 2. Representative SEM images of each failure pattern at 400x magnification. 2.1: (Type I) cohesive failure within resin composite; 2.2: (Type II) adhesive failure between resin composite and bonding agent; 2.3: (Type III) adhesive failure between dentin surface and bonding agent; 2.4: (Type IV) mixed failure; 2.5: (Type V) cohesive failure within adhesive layer; 2.6: (Type VI) cohesive failure in the hybrid layer; 2.7: (Type VII) cohesive failure within dentin. RC: resin composite; AL: adhesive layer; D: dentin.

FIGURE 3. CLSM images of each group tested. (I) Overlapping of the micrographs in fluorescent and reflectance modes. (II) Micrographs in reflectance mode. The area between dotted lines indicates the hybridization zone. Arrows indicate resin tags. RC: resin composite; AL: adhesive layer; D: dentin.

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3 CONCLUSÃO

Dentro das limitações desse estudo *in vitro*, pode-se concluir que todos os *primers* testados à base de flavonóides pareceram melhorar a resistência à união à dentina após um ano, quando aplicados por 60 s, em comparação com a dentina não tratada. Enquanto, apenas o Kaempferol melhorou a resistência à união à dentina após um ano quando aplicado por 30 s. O Kaempferol nas suas concentrações testadas e a Naringina na concentração de 20 mM apresentaram melhores resultados após um ano em relação à Clorexidina quando aplicado por 60 s. Além disso, os *primers* à base de flavonóides pareceram não interferir na morfologia da interface adesiva e no modo de falha. E, a eficácia dos primers experimentais pode depender do tipo de flavonóide utilizado e do tempo de aplicação na dentina e os melhores resultados foram obtidos quando os *primers* à base de flavonóides foram aplicados por 60 s.

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ANEXO 1 – Comprovação da submissão do artigo

Submission Confirmation for Journal of Dentistry

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Para: Beatriz Ometto Sahadi

Dear Miss Sahadi,

Your submission entitled "Effect of flavonoid-based experimental primers on dentin microtensile bond strength and interface morphology" has been received by the Journal of Dentistry.

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ANEXO 2 - Certificado do Comitê de Ética em Pesquisa

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação in vitro dos efeitos de primers experimentais na união dentina-adesivo e na inibição de metaloproteinases da matriz dentinária

Pesquisador: Marcelo Giannini

Área Temática:

Versão: 1

CAAE: 19855119.1.0000.5418

Instituição Proponente: Faculdade de Odontologia de Piracicaba - Unicamp

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.583.904

Apresentação do Projeto:

Transcrição editada do conteúdo do registro do protocolo e dos arquivos anexados à Plataforma Brasil

Delineamento da pesquisa: Trata-se de um estudo laboratorial, in vitro que irá utilizar cento e setenta e seis terceiros molares humanos hígidos. Para o teste de resistência de união, cada dente será considerado uma unidade experimental, enquanto para as demais metodologias cada espécime será considerado como uma unidade experimental. No caso dos testes de resistência de união e padrão de fratura, as variáveis de estudo serão: (1) tratamento dentinário (6 níveis: controle - sem aplicação de primer; digluconato de clorexidina 0,2%; naringina 10mM; naringina 20mM; kaempferol 10mM e kaempferol 20mM); (2) tempo de aplicação (2 níveis: 30 segundos e 60 segundos); (3) tempo de avaliação (2 níveis: 24 horas e 1 ano). A análise da morfologia da área de união dentina-adesivo e o teste de zimografia in situ terão as mesmas variáveis apresentadas acima, com exceção do tempo de avaliação.

Estima-se que os dentes obtidos sejam de pacientes na faixa etária de 18-30 anos, com base no relato da cirurgiã-dentista responsável em relação ao público-alvo da clínica onde os dentes foram extraídos e nas características dos elementos dentais (por exemplo, tamanho da câmara pulpar, ausência de dentina esclerótica e rizogênese completa). Contudo, como os dentes foram doados

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Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
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PIRACICABA, 18 de Setembro de 2019

Assinado por:
Fernanda Miori Pascon
(Coordenador(a))

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