

Chlorhexidine added to different adhesive systems - Assessment of antibacterial activity and bond strength

Adição de clorexidina a diferentes sistemas adesivos - Avaliação da atividade antibacteriana e resistência de união

Adición de clorhexidina a diferentes sistemas adhesivos - Evaluación de la actividad antibacteriana y fuerza de union

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Abstract

This study aimed to assess the effects of adding chlorhexidine (CHX) to different adhesive systems on antibacterial activity and bond strength after 24 hours and 30 days under *in vitro* conditions. A total of 210 bovine incisor crowns and divided into three groups, based on the adhesive system: Single Bond Plus (SB), Clearfil SE Bond (CL), and AdheSE Primer and Bond (AD). All groups were subdivided by CHX concentration (n=7): no CHX, CHX applied before the adhesive (CPA), and the adhesive incorporated with 0.5% CHX, 1.0% CHX, and 2.0% CHX. The micro shear test was conducted on one-half of the specimens after 24 hours, and on the other half after 30 days. Antibacterial activity was evaluated using the agar diffusion test with *Streptococcus mutans*. The bond strength variable was assessed using the factorial analysis of variance for repeated measurements, whereas the variable of the bacterial halo size was subjected to the Kruskal–Wallis nonparametric test. The significance level was 5% for both tests. The qualitative variable of fracture pattern was assessed by descriptive statistical analysis. Thus, CHX concentration of 2% (10.80 ±2.83) and CPA (9.95 ±1.47) (p >0.05) helped to increase the bond strength values for most adhesive systems, whereas CL had the highest bond strength values. The CL adhesive system also presented the best results in the antibacterial test with *S.mutans*.

Keywords: Chlorhexidine; Dentin-bonding agents; Anti-bacterial agents.

Resumo

Este estudo teve como objetivo avaliar os efeitos da adição de clorexidina (CHX) a diferentes sistemas adesivos sobre a atividade antibacteriana e a resistência de união após 24 horas e após 30 dias em condições *in vitro*. Foram selecionadas 210 coroas de incisivos bovinos e divididas em três grupos, com base no sistema adesivo: Single Bond

Plus (SB), Clearfil SE Bond (CL) e AdheSE Primer and Bond (AD). Todos os grupos foram subdivididos pela concentração de CHX (n=7): sem CHX, CHX aplicado antes do adesivo (CPA) e o adesivo incorporado com 0,5% de CHX, 1,0% de CHX e 2,0% de CHX. O teste de microcissalhamento foi realizado em metade das amostras após 24 horas e na outra metade após 30 dias. A atividade antibacteriana foi avaliada pelo teste de difusão em ágar com *Streptococcus mutans*. A análise da resistência de união foi feita pela análise fatorial de variância para medidas repetidas e o crescimento do halo bacteriano pelo teste não paramétrico de Kruskal-Wallis. O nível de significância foi de 5% para ambos os testes. A variável qualitativa do padrão de fratura foi avaliada por meio de análise estatística descritiva. Assim, a concentração de CHX de 2% ($10,80 \pm 2,83$) e de CPA ($9,95 \pm 1,47$) ($p > 0,05$) ajudaram a aumentar os valores de resistência de união para a maioria dos sistemas adesivos, onde o CL apresentou os maiores valores de resistência de união. O sistema adesivo CL também apresentou os melhores resultados no teste antibacteriano com *S.mutans*.

Palavras-chave: Clorexidina; Adesivos dentinários; Agente antibacteriano.

Resumen

Este estudio tuvo como objetivo evaluar los efectos de la adición de clorhexidina (CHX) a diferentes sistemas adhesivos sobre la actividad antibacteriana y la fuerza de unión después de 24 horas y después de 30 días en condiciones *in vitro*. Seleccionamos 210 coronas de incisivos bovinos y las dividimos en tres grupos, según el sistema adhesivo: Single Bond Plus (SB), Clearfil SE Bond (CL) y AdheSE Primer and Bond (AD). Todos los grupos se subdividieron por concentración de CHX (n=7): sin CHX, CHX aplicado antes del adhesivo (CPA) y el adhesivo incorporado con 0,5% de CHX, 1,0% de CHX y 2,0% de CHX. La prueba de corte transversal se realizó en la mitad de las muestras después de 24 horas y en la otra mitad después de 30 días. La actividad antibacteriana se evaluó mediante la prueba de difusión en agar con *Streptococcus mutans*. El análisis de la fuerza de unión se realizó mediante análisis factorial de varianza para medidas repetidas y el crecimiento del halo bacteriano mediante la prueba no paramétrica de Kruskal-Wallis. El nivel de significancia fue del 5% para ambas pruebas. La variable cualitativa del patrón de fractura se evaluó mediante análisis estadístico descriptivo. Por lo tanto, una concentración de CHX de 2% (10.80 ± 2.83) y CPA (9.95 ± 1.47) ($p > 0.05$) ayudaron a aumentar los valores de fuerza de unión para la mayoría de los sistemas adhesivos, donde CL mostró los valores de fuerza de unión más altos. El sistema adhesivo CL también mostró los mejores resultados en la prueba antibacteriana con *S. mutans*.

Palabras clave: Clorhexidina; Recubrimientos dentinarios; Antibacterianos.

1. Introduction

The total acid etching technique used by etch-and-rinse adhesive systems promotes micromechanical retention between dentin collagen, adhesive monomer, and composite resin. It thereby forms a hybrid layer or a dentin/resin interdiffusion zone. Despite their bonding effectiveness, these adhesives cause discrepancies between the acid demineralization depth and monomer diffusion. Dentin that is demineralized and not infiltrated by the adhesive may drive the action of matrix metalloproteinase (MMP) enzymes responsible for the long-term degradation of the hybrid layer (Breschi *et al.*, 2010; Hajizadeh *et al.*, 2013; Araujo *et al.*, 2015).

The self-etching adhesives use monomers with carboxylic functional groups or phosphates. When in contact with water, these compounds are ionized, become acidic, and cross self-etching adhesives simultaneously demineralize and diffuse in the dentin, eliminating the acid-etching step. (Breschi *et al.*, 2008). Thus, the entire extent of the demineralized dentin is infiltrated by resin monomers and decreases the action of MMPs (Strobel *et al.*, 2015).

Different approaches have been proposed to improve the bond strength and infiltration of monomers in the demineralized dentin to reduce the rate of water absorption and the degradation of exposed collagen fibers (Breschi *et al.*, 2008). The most frequently cited approach in the literature is to use MMP inhibitors is chlorhexidine (CHX). An amphiphilic molecule binds to various proteins by a cation-chelating mechanism. It may inhibit the catalytic activity of MMPs by binding to zinc (Zn²⁺) or calcium (Ca²⁺). Thus, CHX in the bonding protocol after phosphoric acid demineralization in dentin can stabilize the bonding interface and prevent long-term bond strength degradation. (Ricci *et al.*, 2010).

A relationship exists between dentin collagenolytic activity and the role of MMPs in hybrid layer degradation, although the exact mechanism of how CHX inhibits MMPs requires further elucidation. Including CHX as a component of adhesive systems may aid the inhibition of MMPs and provide a practical bactericidal effect (Ou *et al.*, 2018). This factor may

be an alternative to suppress residual contamination after caries removal and increase the longevity of the restored tooth (Montagner *et al.*, 2014; Ou *et al.*, 2018). Moreover, these adhesives may prevent the colonization of bacteria in the gaps formed by the polymerization contraction of the composite resins and the degradation of the adhesive interface. However, the effects of CHX on bonding agents remain unknown (André *et al.*, 2015).

Therefore, the aim of this *in vitro* study was to evaluate the effect of different concentrations of CHX incorporated into adhesives regarding micro shear tensile bond strength and antibacterial activity by adding CHX to different adhesive systems. The hypothesis of this study is to verify if the addition of CHX can inhibit the bacterial growth of *S. Mutans* without interfering in the bond strength of the selected adhesives with the dental structure.

2. Methodology

Sample design

The sample was calculated based on probability distributions of the F family with repeated family design and interaction within and among the factors. For bond strength analysis, the effect size was 0.15, and type 1 error (α) of 0.05 and analysis power of 0.88 guaranteed a minimum of 210 sample units (i.e., specimens) with seven samples per experimental subgroup. However, for the analysis of bacterial halo formation, the minimum number of sample units was 130 specimens, with ten samples per subgroup. GPower software (version 3.1.9.2; University of Düsseldorf, Düsseldorf, Germany) was used for the sample calculation. Chart 1 presents the materials used.

Chart 1 - Commercial brand names and compositions of the materials used in the study.

Trademark, Acronym	Manufacturer	Composition
Phosphoric Acid-Condac 37%, AF	FGM (Joinville, SC, Brazil)	37% Phosphoric acid, thickener, dye and deionized water
Single Bond Plus, SB	3M ESPE (Sumaré SP, Brazil)	Alcohol, water, silica treated silica, bis-GMA, HEMA, dimethacrylates, ethanol, water, polyacrylic and polyalkanoic acid methacrylate functional copolymer camphorquinone, dimethacrylate, 10% by weight of silica nanoparticles (5 nanometers).
Clearfil SE, CL	Kuraray (Osaka, Japan)	Primer: 10-MDP, HEMA, camphorquinone, hydrophilic dimethacrylate, water. Adhesive: 10-MDP, Bis-GMA, HEMA, di-camphorquinone, hydrophobic methacrylate.
AdheSE, AD	IvoclarVivadent (Barueri, SP, Brazil)	AdheSE Primer: dimethacrylate, phosphonic acid acrylate, initiators and stabilizers in aqueous solution. AdheSE Bond: HEMA, dimethacrylate, silicon dioxide, initiators and stabilizers.
Composite Resin Opus Bulk FillFlow, RC	FGM (Joinville, SC, Brazil)	Uretanadimetacrylic monomers, stabilizer, camphorquinone and coinhibitor. Inorganic loads of silicon dioxide, silanized, stabilizers and pigments.
20% Chlorhexidine digluconate, CHX	Manipulated Pharmacy-Santa Terezinha (Paraná, Brazil)	20% CHX

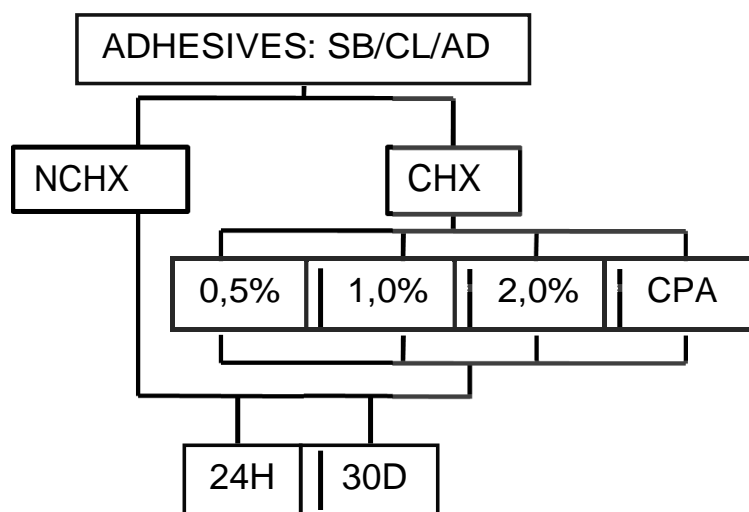
Source: Authors (2022).

Preparation of materials

Different amounts of 20% chlorhexidine digluconate (Manipulated-Farmácia, Santa Terezinha-Laranjeiras do Sul, Paraná, Brazil) were added directly to the adhesives for the preparation of mixtures at CHX concentrations of 0.5%, 1%, and 2%. These concentrations were obtained using high-precision pipettes (Micropipette Monocanal Variable Volume-Ecopipette; Capp, Odense, South Denmark) in a controlled environment (Laboratory of Immunology and Virology at Western Paraná State University, Cascavel, PR, Brazil).

The 0.5% concentration was achieved by combining 25µL of CHX in 975 µL of primer or adhesive, depending on the group (Figure_1); the 1% concentration, by combining 50 µL of CHX in 950 µL of primer or adhesive; and the 2% concentration, by combining 100 µL of CHX in 900 µL of primer or adhesive. The solutions were stored in Eppendorf tubes (3810X; Eppendorf do Brasil, São Paulo, SP, Brazil), which were properly sealed, protected from light, and refrigerated in a conventional refrigerator at 7°C until use (Carrilho *et al.*, 2007).

Figure 1 – Distribution of the experimental groups (n = 7).



There are 35 samples in total with n = 7 per subgroup according to the concentration or not of chlorhexidine. Legends - 105 specimens were made for 24 hours and another 105 for 30 days. Source: Authors (2022).

Preparation of the specimens

Two hundred ten bovine incisors were selected, based on the sample calculation. They were freshly extracted, free of periodontal tissue, and stored in 1% thymol solution under refrigeration for thirty days. The teeth were sectioned with high rotation under abundant cooling by using a diamond bur #4138 (KG Sorensen, Cotia, SP, Brazil) to separate the crown from the root. The crowns were then inserted in polyvinyl chloride tubes with acrylic resin. The buccal surface remained free. A polisher (Aropol 2V200; Arotec S.A. Industry and Business, Cotia, SP, Brazil) with sandpaper discs from the highest to the lowest granulation of #600 was used to grind down the tooth enamel on the buccal face until the enamel was completely removed and the dentin was exposed (Suma *et al.*, 2017). The elements were then stored in distilled water at 37°C until the time of use.

The composite resin cylinders were produced using Tygon tubing (TYG-030; Saint-Gobain Performance Plastic, Maimi Lakes, FL, USA) with an internal diameter of 1 mm and height of 2 mm. By using clinical tweezers, the tubing was placed on the tooth surface. The specimens were produced with Bulk Fill Flow composite resin (Opus Bulk Fill Flow; FGM, Joinville, SC, Brazil) in a single photo activated increment for 20 seconds with a light-emitting diode (LED) light device with

an irradiance of 1200 mW/cm² (Bluephase G2; Ivoclar Vivadent, Barueri, SP, Brazil). Three composite resin cylinders were created for each bovine crown to perform a triplicate test, thereby eliminating any bias. The average was calculated for each tooth. One-half of the specimen was stored for 24 hours in relative humidity at 37°C. It then after underwent the microshear test. The other half of the specimen was stored for 30 days in distilled water at 37°C, which was changed every 15 days. Finally, the specimens underwent the microshear test.

Microshear bond strength test and fracture analysis

The composite resin cylinders were tested in a universal testing machine (DL 200–MF; EMIC, São José dos Pinhais, PR, Brazil) with a load cell of 20 kg. The shear load with 0.2-mm orthodontic wire was applied to the base of the cylinders at a speed of 0.5 mm/min until rupture. The microshear bond strength was calculated and expressed in mega-Pascals (MPa). The failure modes were assessed using a stereoscopic magnifying glass with 40× magnification, and the classification followed the criteria recommended in *International Organization for Standardization TR 11405*. An adhesive fracture was a failure at the adhesive/dentin interface; an adhesive-cohesive fracture was failure exclusively within the adhesive; and a mixed fracture was adhesive/dentin/resin interface failures, which included cohesive failures in neighboring substrates.

Antibacterial activity test

The agar diffusion test (ADT) was conducted on blood agar plates in which 200 µL of freshly grown *Streptococcus mutans* were evenly distributed on plates with a sterile swab. In each of nine plates, ten 4-mm diameter circular holes were drilled, one hole for each solution, up to the end of a sterile 4-mm diameter Pasteur pipette. The 10 holes of each plate were immediately filled with 50 µL of the nine adhesive testing solutions and then polymerized using an LED light device with an irradiance of 1200 mW/cm² (Bluephase G2, Ivoclar Vivadent), based on the manufacturer's instructions.

The 10 perforations (4-mm diameter) were filled with solutions containing CHX at concentrations of 0.5%, 1%, and 2% in the primer (Clearfil SE Bond [CL; Kuraray, Osaka, Japan] and AdheSE Primer and Bond [AD; Ivoclar Vivadent, Barueri, São Paulo, SP, Brazil]) or in the adhesive (Single Bond Plus [SB; 3M-ESPE, Sumaré, SP, Brazil]). The agar plates were incubated for 48 hours at 37°C, and then inspected for an inhibition halo. The inhibition halo diameter was measured twice perpendicularly by using a digital caliper (Starret727; Starret, Itu, SP, Brazil) with an accuracy of 0.0001 mm (Penmetsa *et al.*, 2014).

Statistical analysis

The statistical analyses were conducted with Bioestat 5.3 software (Instituto Mamirauá, 2007). The data for quantitative variables (i.e., bond strength and bacterial halo size) were initially assessed by distribution type using the Shapiro–Wilk test. The data did not adhere to the normality curve. Therefore, the Mann–Whitney test ($p < 0.05$) was used to compare the two groups. For other comparisons, the Kruskal–Wallis analysis of variance was used, followed by the Dunn test to analyze the interaction between the groups ($p < 0.05$). The qualitative variable (i.e., fracture pattern) was assessed by descriptive statistical analysis.

3. Results

The CHX concentration used in this experiment after 24 hours produced the lowest statistically significant values at 1% for the SB adhesive. At the concentration of 0.50%, all adhesive systems were statistically similar. At the 2% concentration, the CL adhesive system showed significantly higher values than the other systems.

For CL, applying CHX before the adhesive (i.e., CPA) produced values similar to those of the 2% concentration. Changing the CHX concentration or using CPA after 24 hours did not change the values obtained for the AD adhesive.

The SB adhesive system for CHX concentration of 2% had significantly lower results than those of the other adhesive systems. Applying CPA showed the best values for CL and the lowest values for SB and AD (Table 1).

Table 1 – The mean values and standard deviations of the bond strength variable (in MPa), based on adhesive type and CHX concentration, at 24 hours.

Solutions / Adhesives		SB	CL	AD
24 hours	0%	6,99 (+1,95)ABa	8,24 (+2,73)ABa	9,66 (+3,30)Aa
	0,50%	8,72 (+2,10)Aa	7,99 (+2,26)ABa	7,23 (+2,16)Aa
	[CHX] 1,00%	3,64 (+1,56)Ba	6,01 (+0,90)Aab	7,06 (+1,99)Ab
	2,00%	7,17 (+1,72)ABa	10,80 (+2,83)Bb	7,49 (+0,77)Aab
	CPA	6,07 (+1,41)ABa	9,95 (+1,47)Bb	6,64 (+2,49)Aa

Different letters indicate statistically significant differences, lowercase letters for row and upper-case for the column, $p < 0.05$. Legends: CHX: chlorhexidine; SB = Single Bond Plus; CL = Clearfil SE; AD = AdheSE; CPA = applying CHX before the adhesive. Source: Authors (2022).

SB had the highest frequency of reduced bond strength values (Table 2). The best values for the CHX factor occurred with the CL adhesive at 0,5 and 1%, while the lowest values occurred with the AD and SB adhesives at 1% concentration. The SB adhesive had the lowest bond strength values for CPA, and the CL and AD adhesives had the highest values.

Table 2 – The mean values and standard deviations of the bond strength variable (in MPa), based on the adhesive type and CHX concentration, at 30 days.

Solutions / Adhesives		SB	CL	AD
30 days	0%	7,48 (+4,17)Aa	11,23 (+2,25)ABa	10,28 (+1,54)Aa
	0,50%	5,73 (+0,95)Aa	10,00 (+1,98)ABb	9,94 (+5,87)ABab
	[CHX] 1,00%	5,64 (+2,40)Aa	12,47 (+3,00)Ab	6,13 (+1,55)Ba
	2,00%	6,84 (+1,94)Aa	9,45 (+1,81)ABa	8,33 (+2,65)ABa
	CPA	4,23 (+0,96)Aa	7,93 (+2,89)Bb	6,95 (+1,11)ABb

Different letters indicate statistically significant differences, lowercase letters for row and upper case for column, $p < 0.05$. Legends: CHX: chlorhexidine; SB = Single Bond Plus; CL = Clearfil SE; AD = AdheSE; CPA: applying CHX before the adhesive. Source: Authors (2022).

On analyzing the influence of storage time (i.e., 24 hours and 30 days) on the experimental groups, the results indicated that time did not affect the bond strength of samples for most combinations. This finding occurred in all groups with the AD adhesive. The bond strength values decreased with the SB adhesive at the concentration of 0.5% and with CPA after the storage period, the concentrations of 0%, 1% and 2% did not present significant differences. By contrast, the CL adhesive values at the 0% and 1% concentrations increased after 30 days (Table 3).

Table 3 – The mean values and standard deviations of the bond strength variable (in MPa), based on adhesive type and CHX concentration, at 24 hours and at 30 days.

Adhesives	Concentration of CHX	24 hours	30 days	p-value
SB	0%	6,99(±1,95)	7,48(±4,17)	0,9491
	0,50%	8,72(±2,10)	5,73(±0,95)	0,0088*
	1,00%	3,64(±1,56)	5,64(±2,40)	0,1417
	2,00%	7,17(±1,72)	6,84(±1,94)	0,5653
	CPA	6,07(±1,41)	4,23(±0,96)	0,0253*
CL	0%	8,24(±2,73)	11,23(±2,25)	0,0253*
	0,50%	7,99(±2,26)	10,00(±1,98)	0,1417
	1,00%	6,01(±0,90)	12,47(±3,00)	0,0017*
	2,00%	10,80(±2,83)	9,45(±1,81)	0,2774
	CPA	9,95(±1,47)	7,93(±2,89)	0,1417
AD	0%	9,66(±3,30)	10,28(±1,54)	0,9491
	0,50%	7,23(±2,16)	9,94(±5,87)	0,3379
	1,00%	7,06(±1,99)	6,13(±1,55)	0,4062
	2,00%	7,49(±0,77)	8,33(±2,65)	0,7494
	CPA	6,64(±2,49)	6,95(±1,11)	0,9490

*: Statistically significant differences, $p < 0.05$. Legends: CHX: chlorhexidine; SB = Single Bond Plus; CL = Clearfil SE; AD = AdheSE; CPA = applying CHX before the adhesive; MPa: mega-Pascal. Source: Authors (2022).

The statistical comparison between the same adhesive with different CHX concentrations revealed higher values for bacterial halo formation, most often at 2% CHX, and the lowest values at 0% CHX. However, the same CHX concentration analysis between different adhesives showed that CL presented the best statistically significant results in most comparisons. In contrast, in most comparisons, SB presented the worst growth values of the bacterial inhibition halo (Table 4).

Table 4 – The mean values and standard deviations (in millimeters) of the bacterial inhibition halo reading in the experimental groups.

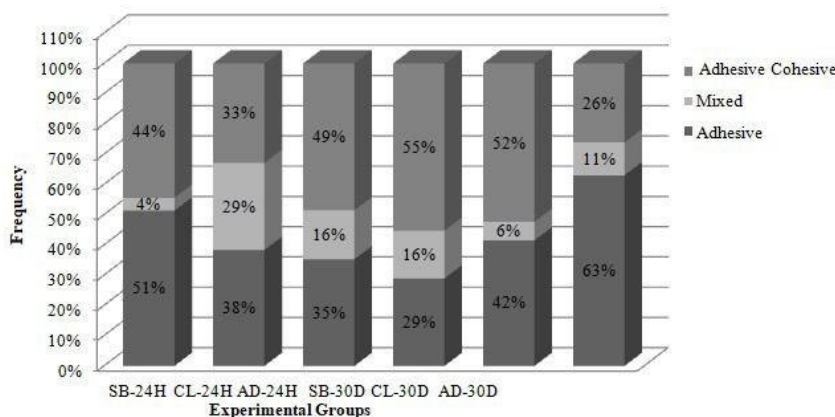
[CHX]/Adhesives	SBL	CL	AD	CHX
0%	0,00 (+0,00)Aa	1,13 (+0,09)Ab	1,85 (+0,21)ABc	-
0,50%	0,76 (+0,19)Aa	2,43 (+0,16)Bb	1,96 (+0,14)Ac	-
1,00%	1,57 (+0,57)Ba	2,71 (+0,18)Bb	1,76 (+0,21)ABa	-
2,00%	2,38 (+0,16)Ba	2,55 (+0,13)Ba	1,64 (+0,26)Bb	2,45 (+0,21)a

Different letters indicate statistically significant differences, lowercase letters for row and upper case for the column, $p < 0.05$. Legends: CHX: chlorhexidine; SB = Single Bond Plus; CL = Clearfil SE; AD = AdheSE; CPA = applying CHX before the adhesive. Source: authors (2022).

Figure 2 presents the absolute frequency of adhesive, mixed, and adhesive-cohesive fractures in SB, CL, and AD at 24 hours and at 30 days. After 30 days of experimentation, the occurrence of adhesive fractures was highest for AD (63%); at 24

hours, it was highest for SB (51%). At 30 days, cohesive fractures were highest for SB (55%), followed by CL (52%). At 24 hours, mixed fractures were lowest (4%) for SB and highest (29%) for CL.

Figure 2 – Frequency distributions of the failure types among the experimental groups at 24 hours (24H) and 30 days (30D).



Legends: CHX: chlorhexidine; SB = Single Bond Plus; CL = Clearfil SE; AD = AdheSE; CPA = applying CHX before the adhesive.
 Source: Authors (2022).

4. Discussion

The present study aimed to contribute to clarifying the role of CHX concentration in inhibiting MMPs because the literature remains controversial (Breschi *et al.*, 2010; Perote *et al.*, 2015; Ou *et al.*, 2018). The findings of this study indicated better bond strength for CL when adding 2% CHX and using CPA within 24 hours, and with 0% and 0.5%, there was no statistical difference between them. These findings agree with literature reports demonstrating significant improvement in bond strength when using 2% CHX; this concentration may be effective in inhibiting MMPs (Carrilho *et al.*, 2007; Breschi *et al.*, 2010; Stanislawczuk *et al.*, 2014).

A 2-year clinical study in which CHX was added to the primer of two adhesives (i.e., AD and CL) did not show clinical benefits at the assessed periods. However, in one study (Araujo *et al.*, 2015), CL presented a better retention rate for restorations, compared with AD. At 24 hours, CL was superior to SB, not different from AD with 2% CHX. However, at 30 days, there was no difference between groups for the same concentration.

The comparative analysis between the experimental groups showed that SB more often had lower bond strength values. This finding may be related to the fact that SB, a conventional adhesive, requires 37% phosphoric acid etching, which removes the smear layer and leaves the collagen fibrils exposed because of incomplete infiltration of the resin monomers. This process then activates MMPs (Hashimoto *et al.*, 2018). By contrast, the CL and AD adhesives are self-etching, the difference between these two self-etching adhesives is in the composition: AD contains the 2-hydroxyethylmethacrylate monomer and CL contains the 10-methacryloyloxydecyl dihydrogen phosphate monomer. The chemical binding promoted by 10-methacryloyloxydecyl dihydrogen phosphate is more effective and more stable in an aqueous environment, compared with that achieved by hydroxyethylmethacrylate, 4-(2-methacryloxyethoxycarbonyl) phthalic anhydride, and phenyl-P monomers (Van Landuyt *et al.*, 2006). This factor may have contributed to increased bond strength after the 30-day period without the addition of CHX and the addition of 1% (Hashimoto *et al.*, 2018), Osorio and collaborators (2008) had similar results.

The use of an MMP inhibitor on the dentin surface before applying the adhesive or incorporating it into the adhesive system may improve the stability and integrity of the adhesive interface over time (De Munck *et al.*, 2010; Li *et al.*, 2015; Tekce *et al.*, 2016). Thus, when comparing the bond strength values relative to the CHX concentration versus time, CL showed a significant improvement after 30 days with 1%; however, SB showed a decrease after 30 days with 0,5%.

For AD, there were no statistically significant differences at 24 hours and at 30 days. Similar results were observed by Gunaydin and collaborators (2016). In their study, pretreatment with 2% CHX produced higher bond strength values than those of samples without pretreatment, which indicated that using CHX produced better adhesive interface stability.

A difference between fracture types is expected when using different adhesive strategies and when using different mechanical tests. In this study, AD showed the highest occurrence of adhesive fractures after 30 days of storage (63%) but showed the highest occurrence of cohesive fractures at 24 hours. This finding suggested satisfactory bond strength after the aging period because adhesive fractures indicate a better force distribution (Rocha *et al.*, 2007).

It has long been believed that cohesive fractures in dentin result from high bond strength because the higher bond strength achieved with adhesive systems tested in large areas by tensile tests promoted an irregular force distribution, and thus led to fractures in the dentin but not at the adhesive interface. Thus, a cohesive fracture occurs. As the dentin fractured, the bond strength in the dentin/resin interface was higher than the cohesive strength of dentin. Therefore, an adhesive fracture would correspond to a low bond strength.

After the introduction of the microshear and microtensile tests (Sano *et al.*, 1994), the current study verified that the lower the adhesive area, the lower was the possibility of defects in the tooth surface that negatively interfere with bond strength. Thus, regardless of the test used - microshear or microtensile - the uniform distribution of forces indicate that fracture patterns should be mixed and adhesive (Rocha *et al.*, 2007).

The ADT also showed significantly superior behavior with the CL adhesive, compared with the other adhesive systems or the control at 2% CHX. This finding showed that, among the CHX adhesive systems, CL had higher antibacterial properties than the other tested adhesives at 72 hours after polymerization. Similar results were achieved in 2014 by Kim & Shin (2014) who tested the CL adhesive and other adhesive system by using 0.12% CHX and 37% phosphoric acid as the positive controls. They found that the antibacterial activity of 0.12% CHX and 37% PA gel was stronger than that of the other groups, except for CL, which had the highest inhibition halo.

The antibacterial activity of adhesive systems depends on several factors such as pH, which for the CL adhesive is approximately 2.0; the presence of antibacterial components in the chemical composition; and the viscosity of the material inhibition (Gondim *et al.*, 2008). Thus, the acidic nature of the primer of self-etching adhesive systems may be a main factor associated with bacterial inhibition (Gondim *et al.*, 2008). Adding CHX or using it before applying an adhesive system at a certain concentration (in this instance, 2%) may improve bond strength and aid inactivating MMPs. However, further clinical studies are required to define the concentration and time for optimum application. However, because efficient *in vitro* performance may improve clinical performance, it needs to be determined whether the growth-inhibiting effects of the dentin adhesives observed in this study would be similar *in vivo*.

5. Conclusion

The findings of this *in vitro* study suggest (1) the concentration of 2% CHX, whether incorporated into the primer or applied separately before the adhesive, aided the increase in the bond strength values and (2) the CL adhesive system showed the best results in the antibacterial test with *Streptococcus mutans*. To complement the findings of this study, future research such as laboratory test of nanoleakage and randomized clinical trials would be of great value.

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