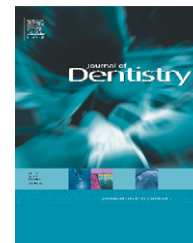


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Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing

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SUMMARY

Objectives: The purpose of this study was to investigate the effects of chlorhexidine (CHX) digluconate at 0.2% and 2% on dentin bonding durability of etch-and-rinse and self-etch adhesive systems.

Methods: In this study were used 24 extracted non-carious human third-molars. The occlusal surfaces of the molar crowns were removed with a low-speed diamond saw to expose flat dentin surfaces. The tested materials were Single-Bond (SB) (two-step etch-and-rinse adhesive) and Clearfil Tri S Bond (CTSB) (all-in-one self-etch adhesive) used in association or not with CHX at 0.2% and 2%. The bonding systems were applied according to manufacturer's instructions and followed by composite application (Z250). For each condition, half of the specimens was immediately submitted to microtensile test and half of them was submitted to long-term storage of 6 months under simulated pulpal pressure and thermo-mechanical stressing before testing. The data were analyzed using Two-Way ANOVA and Tukey post hoc test ($\alpha = 0.05$). Failure patterns of the specimens were observed using scanning electron microscopy.

Results: The falling % in bond strength over the 6-month period was: SB control—43.64%; SB/0.2%CHX—23.79%; SB/2%CHX—26.42%; CTSB control—40.94%; CTSB/0.2%CHX—37.07%; CTSB/2%CHX—22.14%. The fracture modes were predominantly adhesive, mainly in the specimens of terminal groups.

Conclusions: CHX digluconate at 2% was able to diminish loss of microtensile bond strength over time associated to both etch-and-rinse and self-etch adhesives. Lower concentration of CHX (0.2%) was not able to diminish the loss of bond strength over time when associated to the self-etch adhesive CTSB.

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1. Introduction

The longevity of resin restorations is currently an area of interest in adhesive dentistry.¹ The durability of the bond between dentin and resinous adhesives may not be as durable as was

previously assumed.^{2,3} It has been widely stated that resin-dentin bonds obtained with contemporary adhesive systems can deteriorate over time.⁴ Several authors have shown the hydrolytic degradation of collagen matrices in aged dentin-resin bonds,^{1,5} even in the absence of bacterial enzymes.⁴

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Matrix metalloproteinases (MMPs) are a family of host-derived proteolytic enzymes that are capable of degrading the organic matrix of demineralized dentin.⁶ These MMPs can reach the exposed collagen fibrils at the base of the hybrid layer originated from the deficient resin infiltration within demineralized dentin matrix, resulting in hydrolytic degradation and reduction of bond strengths.⁷

The incomplete resin impregnation was also observed for self-etch adhesive systems despite their ability to etch and prime simultaneously.⁸ Nishitani et al.⁹ related that self-etch adhesives may activate latent MMPs and increase their activity to near-maximum levels and contribute to the degradation of resin–dentin bonds over time.

Chlorhexidine (CHX) is widely used as antimicrobial agent and possesses a large spectrum of activity against oral bacteria. Concentrations ranging from 0.05% to 2% are capable to demonstrate antimicrobial activity against *E. faecalis*.¹⁰ It has been stated that the currently accepted disinfection technique applying CHX to acid-etch dentin prior to the use of etch-and-rinse adhesives may prevent the degradation of collagen fibrils, besides its antimicrobial property.⁴ The minimal concentration of CHX capable to totally inhibiting the MMP-9 is 0.002%, while MMP-2 activity is more sensitive, being inhibited at a CHX concentration as low as 0.0001%, and MMP-8 is inhibited by 0.02% CHX.¹¹ Additionally, it was shown that 0.2% CHX for 60 s inhibited the collagenolytic activity to near-zero levels in dentin powder.⁴

The aim of this study was to evaluate the influence of CHX in different concentrations on the microtensile bond strength (μ TBS) before and after long-term storage. The null hypothesis tested was that CHX does not influence the μ TBS results of etch-and-rinse and self-etch adhesive systems after thermo-mechanical stressing and long-term storage under simulated pulpal pressure.

2. Materials and methods

In this study were used 24 extracted non-carious human third-molars. The teeth were stored in tymol solution 0.1% and were used within 3 months after extraction. All the restorative procedures were made under simulated dentinal hydrostatic pressure. A metallic tube was inserted into the pulp chamber of the teeth and sealed with a filled light-cured dentinal adhesive (Optibond FL, Kerr Co., Orange, CA, USA). This tube was connected by a flexible silicon hose to an infusion bottle placed 30 cm vertically above the test tooth. The infusion bottle was filled with saline solution 0.9% to simulate the dentinal fluid under normal hydrostatic pressure of about

22.07 mmHg. The pulp chambers were evacuated with a vacuum pump and subsequently filled with the saline solution. The intra-pulpal pressure was maintained at 22.07 mmHg during tooth preparation, adhesive procedure, insertion of composite and thermo-mechanical stressing. The occlusal surfaces of the molar crowns were removed with a low-speed diamond saw under water lubrication (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) and the dentin surfaces were polished with 320 and 600-grit silicon-carbide paper under running water to create a standardized smear layer. The specimens were randomly divided into 6 groups of 4 teeth each, and submitted to the bonding protocols using two-step etch-and-rinse (Single Bond, 3M ESPE, St. Paul, MN, USA) (SB) or all-in-one self-etch (Clearfil Tri S Bond, Kuraray Co., Osaka, Japan) (CTSB) adhesive system, in combination or not with CHX digluconate solution at 0.2% or 2%. The CHX solution was applied after the acid-etching procedure for the etch-and-rinse adhesive and prior the self-etch primer using a foam pellet saturated with the solution, with a dwell time of 60 s. Excess solution was removed using absorbent paper, leaving the dentin surface visibly moist, and the adhesive systems were applied following the manufacturer's directions. Flat 3 mm thick buildups of resin composite (Z250, 3M Espe, MN, USA) were placed in 1 mm increments and each layer was polymerized for 40 s. Additionally to the peripheral enamel adhesion, an additional layer of composite was applied externally to the entire adhesive interface to prevent the water leakage through this region. All the materials used in this study are listed in Table 1. For each condition, half of the specimens was immediately submitted to μ TBS test and half of them was submitted to long-term storage of 6 months under simulated pulpal pressure and thermo-mechanical stressing before testing.

The aging procedure consisted of masticatory periods associated to thermal cycling. The teeth were submitted to three masticatory cycles per day (every 8 h), during 180 days, always under pulpal pressure simulation. Each masticatory period consisted of 300 load cycles with a frequency of 1.32 Hz and maximal load of 40 N. Thermal cycling was carried out simultaneously with temperatures changing from 5 °C to 50 °C, with a dwelling time of 20 s each. In the periods between two masticatory cycles, the teeth were kept at 37 °C in water that contained 0.5% chloramine to prevent bacterial growth.¹² The mechanical load was applied perpendicularly to the adhesive interface by a natural cusp directed to the center of the flat composite surface. For the thermo-mechanical stressing, was used an computer-assisted experimental apparatus similar to the machine developed by Krejci et al.¹³ (Fig. 1).

Table 1 – Materials used in this study.

| Material | Manufacturer | Main components | Lot n. |
|---------------------|--------------|---|--------|
| Adper Single Bond | 3M Espe | Ethyl alcohol 30–40%; bis-GMA 15–25%; HEMA 10–20%; glycerol 1, 3-dimethacrylate 5–15%; acrylic acid copolymer and itaconic acid 5–15%; diurethane dimethacrylate 2–8%; water 2–8% | 7KJ |
| Clearfil Tri S Bond | Kuraray | HEMA; ethanol; Bis-GMA; MDP; colloidal silica; di-camphorquinone; water; initiators; accelerators | 040219 |
| Z250 | 3M Espe | | 7WW |

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