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Comparative Evaluation of the Effect of Two Cavity Disinfectants on the Microtensile Bond Strength at Resin-Dentine Interface: In Vitro study

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Abstract

Aim: The study aimed to evaluate and compare the effect of two cavity disinfectants [Chlorhexidine Digluconate 2% and Octenidine Dihydrochloride 0.1%] on the micro-tensile bond strength at the resin-dentine interface and to observe modes of failures under Scanning Electron Microscope. (SEM)

Methods: 21 freshly extracted human permanent molars were sectioned with a double-sided diamond disc to expose mid-coronal dentine and were randomly divided into 3 groups: A, B and C. 7 flat dentine surface were then treated with 37% phosphoric acid gel for 15 seconds. All restored teeth then were thermocycled and longitudinally sectioned and were subjected to micro-tensile testing, and fractured specimens were observed under SEM.

Results: There was a statistically significant difference between Group A (Control) and Group C (OCT) in microtensile bond strength. ($p<0.05$) The difference was no statistically significant between Group A (Control) and Group B (CHX), Group B (CHX) and Group C (OCT).

Conclusion: The use of Octenidine Dihydrochloride 0.1% and Chlorhexidine Digluconate 2% solutions after etching the dentine enhanced micro-tensile bond strength of composite restorations.

Keywords: Resin-dentine bond strength, MMPs, Cavity disinfectant, Micro-tensile testing

Introduction

Although cavity preparation is an operative procedure that attempts to remove all infected dentine before placing a restorative material, bacterial remnants during and after the cavity preparation pose a significant problem in restorative dentistry.[1] Bacterial activity has proved to be the main causative factor for the placement and replacement of restorations.[2] It was confirmed histologically that fermentative organisms remained viable under non-antiseptic restorations for as long as 139 days. Only a portion of the tooth is sterile after the termination of routine cavity preparation.[1,2] This can lead to increased sensitivity and inflammation of the pulp and secondary caries, necessitating the restoration's replacement.[3,4] As infection beneath the restoration is considered a great threat to the pulp. The concept of toileting of the cavity is gaining wider acceptance with various commercially available dentine disinfectants launched into the market.[5]

Chlorhexidine Digluconate 2% is the most widely used oral antiseptic, with low toxicity and a broad spectrum of antibacterial activity. This was demonstrated from a recent in vivo study, in which the application of Chlorhexidine Gluconate 2%, known to have a broad-spectrum MMP-inhibitory effect, significantly improved the integrity of the hybrid layer in a six-month clinical trial.[1] Octenisept is an antiseptic for skin burns, wound disinfection and mouth rinses consisting of Octenidine Hydrochloride and Phenoxyethanol. Octenidine hydrochloride [N,N¢-(1,10 decanediyldi-1[4H]-pyridinyl-4-ylidene)bis(1-octanamine)dihydrochloride].[2] Octenidine Dihydrochloride 0.1% has been proved better as an endodontic irrigant based on its prolonged antimicrobial compared to Chlorhexidine Gluconate 2%. So it can also be used as cavity disinfectant.[2]

However, the use of a disinfectant could be a problem if it interferes with a hydrophilic resin's ability to wet and micro mechanically bond to dentine. In 1994, Sano et al. introduced the microtensile bond strength test.[6] This test exhibits, as one of its peculiarities, the tendency to result in higher bond strength values than do other tests, because it uses a smaller adhesion area than the others. According to Pashley et al., the microtensile test presents several advantages compared to the others, eg, permitting a greater number of adhesive failures, measuring regional bo nd strengths, and calculating values in one tooth, and the testing of very small areas.[7] Hence, the purpose of the present study was to evaluate and compare the effect of two cavity disinfectants [Chlorhexidine Digluconate 2% (AMMDENT) and Octenidine Dihydrochloride 0.1% (OCTENISEPT)] on the micro-tensile bond strength at the resin-dentine interface and to observe modes of failures under Scanning Electron Microscope. (SEM)

Materials and Methods

A total of 21 freshly extracted human permanent molars were selected for the study. After extraction, the teeth were kept in a hydrogen peroxide solution for 15 minutes and washed under running tap water for 15 minutes each. Later they were cleaned in pumice and stored in normal saline at room temperature until use. Then teeth were sectioned with a low-speed double-sided diamond disc underwater coolant to expose mid-coronal dentine. Each preparation was rinsed with distilled water for 20 seconds and dried for 20 seconds. Then the teeth were randomly divided into 3 groups A, B and C.

Group A (Control)

7 flat dentine surface were treated with 37% phosphoric acid gel for 15 seconds. Then gel was removed with water spray for 10 seconds. Then bonding agent was applied to dentine surface and was cured for 20 seconds. Then nano-hybrid restorative composite was applied on flat dentine surface up to 6 mm in increment and was light-cured.

Group B (Chlorhexidine Digluconate 2%)

7 flat dentine surface were then treated with 37% phosphoric acid gel for 15 seconds. Then gel was removed with water spray for 10 seconds. Then chlorhexidine digluconate 2% was applied on flat dentine surface with micro-applicators for 60 seconds and blot dry for 5 seconds. Then bonding agent was applied to the dentine surface and was cured for 20 seconds. Then nano-hybrid restorative composite was applied on flat dentine surface up to 6 mm in increment and was light-cured.

Group C (Octenidine Dihydrochloride 0.1%)

7 flat dentine surface were then treated with 37% phosphoric acid gel for 15 seconds. Then gel was removed with water spray for 10 seconds. Then octenidine dihydrochloride 0.1% was applied on flat dentine surface with micro-applicators for 60 seconds and blot dry for 5 seconds. Then bonding agent was applied to the dentine surface and was cured for 20 seconds. Then nano-hybrid restorative composite was applied on flat dentine surface up to 6 mm in increment and was light-cured. All the restored teeth then were stored in distilled water for 24 hours, at 37°C and then subjected for thermal cycling with 500 cycles between water baths of 5°C-55°C with a dwell time of 30 seconds. Teeth were then longitudinally sectioned across the bonded interface in sections perpendicular to the pulpal floor. A diamond saw to produce a series of 1 mm x 1 mm x 20 mm beams. 2 to 3 beams were obtained from each preparation. Total of 45 samples, 15 samples per group. Each specimen was then individually fixed to a Universal Testing Machine and subjected to tensile load at a crosshead speed of 3 mm/min until failure. All fractured specimens were then observed under a scanning electron microscope with 85x magnification at 15 kV.

The fracture modes were classified as :

(1) Failure in the resin composite
(2) Failure in the bonding resin
(3) Failure in the hybrid layer
(4) Failure in dentine.

In cases of uncertainty, we used higher magnifications (500-4000x) to confirm the fracture's nature. The percentage of each fracture mode was then estimated for each specimen.

Statistical Analysis

Data analysis was done using Statistical Package for Social Sciences (SPSS) for Windows 26.0 (SPSS, Inc. Chicago, Illinois). Confidence intervals were set at 95%, and a p-value $\leq$ of 0.05 was considered statistically significant. One way ANOVA was used to compare the effects of the treatment modes on the microtensile bond strength at resin-dentine interface and distribution of failure modes. Tukey's Post Hoc test was computed to analyze in between-group differences for microtensile bond strength.

Results

There was a statistically significant difference between Group A (Control) and Group C (OCT) in microtensile bond strength. ($p<0.05$) The difference was no statistically significant between Group A (Control) and Group B (CHX), Group B (CHX) and Group C (OCT). ($p>0.05$) Most of the specimens showed mixed failures, with failures at the bottom of the hybrid layer for the control specimens. In contrast, group B (Chlorhexidine Digluconate 2%) and group C (Octenidine Dihydrochloride 0.1%) were a mostly cohesive failure within the adhesive layers and composite resin.

Discussion

The effectiveness of CHX as a cavity disinfectant is very well documented in literature.[8] CHX is amongst the well-known antimicrobial agents and matrix metalloproteinase inhibitors.[9-12] Soares *et al.*,[12] and Brackett *et al.*[13] have reported that using CHX with etch-and-rinse adhesives either enhance or not affect the bonding mechanism. In the present study, the application of 2% chlorhexidine gluconate after acid etching enhanced the bond strength compared to the control group. No cavity disinfectant was used, which was in accordance with previous studies.

Octenisept belongs to the bipyridines group carrying two cationic active centres per molecule and demonstrates broad-spectrum antimicrobial effects covering both the Gram-positive and the Gram-negative bacteria, fungi and several viral species.[15] Octenidine dihydrochloride is a cationic antimicrobial substance, which as a result of two positive charges in relation to the molecular weight of 437 daltons, is strongly adsorbed onto negative cell surfaces. It reacts with the polysaccharides in the cell wall of microorganisms, attacks the enzymatic systems there, destroys cell function and leads to leakage of the cytoplasmic membrane. As a result, the mitochondrial function is also disturbed. Some findings indicate strong adherence to lipid components in cell membranes (e.g. cardiolipin), which might explain the high antimicrobial activity together with good tolerability for human epithelium and wound tissue.[16-19] Octenidine dihydrochloride shows broad antimicrobial activity against Gram-positive and Gram-negative bacteria, chlamydiae and fungi. Microbiostatic and microbicidal efficacy ranges about 10 times higher than that of chlorhexidine. A particular feature is the marked residual effect. Octenidine dihydrochloride's minimal microbicidal concentration (MMC 5min contact time, _5 log reduction) for Staphylococcus aureus, Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa ranges at 250 mg/ml, and for Candida albicans 100 mg/ml. On average, octenidine dihydrochloride is 3 to 4 times more effective than chlorhexidine digluconate. Phenoxyethanol, an ethanol derivate, serves as a preservative component in Octenisept, which is also supposed to improve the antibacterial activity of octenidine synergistically.

Anuradha et al. also evaluated the antimicrobial efficacy of OCT and NaOCl against E. faecalis by irrigating infected dentine blocks with 3ml of irrigant for 1min and found OCT to be more effective than 5% NaOCl.[20] Makkar et al. compared various concentrations of OCT and CHX against E. faecalis with different contact time and found the number of CFU's dropped to zero after 3min and remained zero after 5min and 10 minutes contact time, with 0.1% OCT and 2% CHX.[21]

In the present study, the application of octenidine dihydrochloride 0.1% after acid etching enhanced the bond strength compared to the control group, where no cavity disinfectant was used. In the group, chlorhexidine digluconate 2% was used. There was a significant difference between control and group C. Even-though there was no statistically significant difference between group B and group C, the bond strength obtained by octenidine dihydrochloride 0.1% was better compared to the chlorhexidine digluconate 2% group.

The probable reason for the enhanced antimicrobial effect of octenidine can be attributed to the cation-active structure that tends to bind readily to negatively charged bacterial cell envelope, automatically disrupting the cell membrane's vital functions killing the cell. Preliminary results point to a strong adherence, particularly to the lipid components (e.g., cardiolipin), which is prominent in bacterial cell membranes explaining high antimicrobial efficacy without adversely affecting human epithelia or wound tissue. Octenidine has been demonstrated to be more effective than chlorhexidine as a means for prolonged bacterial anti-adhesive activity.[22] It also proved to resists organic challenges by maintaining its antimicrobial property in the presence of organic material when compared to chlorhexidine and iodine.

Other factors that could have a role in better penetration and better bond strength of OCT than CHX can be correlated to two important parameters: (i) surface tension and (ii) viscosity. The surface tension of the solution governs the capability of its penetration into the dentinal tubules. Dynamic viscosity is an essential parameter related to fluid flow; the resistance exhibited by a fluid while tensile or shear stresses are deforming it. The lesser the viscosity, the easier is the fluid movement. Other reason could be its good MMP inhibition activity. It can be a good MMP inhibitor. But no study is existing at present for this evidence.[17]

The ability of μTBS methods to produce fractures in the adhesive joint has been described as a distinct advantage over the traditional strength-based testing methods by previous investigators.[16-19] According to Pashley et al., the micro-tensile test presents several advantages compared to the others, eg, permitting a greater number of adhesive failures, measuring regional bond strengths, and calculating values in one single tooth and testing of very small areas.[7]

Conclusion

Within this study's limitations, it can be concluded that the use of Octenidine Dihydrochloride 0.1% and Chlorhexidine Digluconate 2% solutions after etching the dentine enhanced micro-tensile bond strength of composite restorations. So the null hypothesis in this study, that Octenidine Dihydrochloride 0.1% can also be equally effective as Chlorhexidine Digluconate 2% as cavity disinfectant, was accepted. However, further long term studies are still required to check for their efficacy and long term stability to be used in dentistry.

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Conflicts of interest : Nil

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Figure 1: Distribution of mean values of micro-tensile bond strength in Group A, Group B, and Group C.

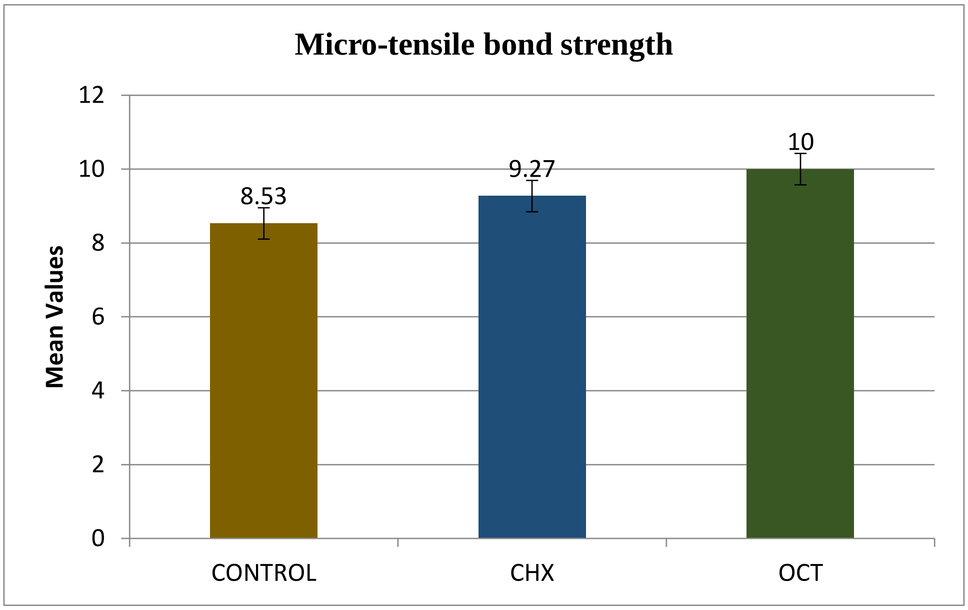

Table 1: Pairwise comparison of Micro-Tensile Bond Strength

Micro-Tensile Bond Strength	Control	Chlorhexidine Digluconate	Octenidine Dihydrochloride
Control	-	0.05*	0.05*
Chlorhexidine Digluconate	0.05*	-	0.21
Octenidine Dihydrochloride	0.051*	0.21	-

*Significant