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Comparative Evaluation Of Microtensile Bond Strength Of 8th Generation Bonding Agent Modified With Chitosan And Copper Nanoparticles On Sound And Simulated Demineralized Dentin – An In Vitro Study

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Abstract
Background: *Secondary caries arising from microleakage in resin restorations remains a leading cause of restoration failure. Modification of adhesive systems with antibacterial agents is a promising strategy. Copper nanoparticles (CuNPs) possess potent broad-spectrum antimicrobial activity, while chitosan offers biocompatibility and collagen cross-linking ability. This study evaluated and compared the microtensile bond strength (μTBS) of an 8th generation bonding agent modified with 0.12% chitosan and 0.2% copper nanoparticles on sound and simulated demineralized dentin.*
Methods: *Forty-two extracted premolars were randomly assigned to three groups (n=14) and six subgroups (n=7). Dentin was exposed by horizontal sectioning at the dentinoenamel junction (DEJ). Demineralized dentin was simulated by placing 21 extracted teeth in 10% citric acid solution for 4 hours. Group I: 8th generation bonding agent (G-Premio BOND, GC Corporation, Japan) [control]; Group II: modified with 0.12% chitosan; Group III: modified with 0.2% CuNPs. Composite resin build-up was completed and specimens sectioned into 1 mm² beams. After 24-hour water storage μTBS was tested using a universal testing machine.*
Results: *μTBS was significantly greater in sound dentin than demineralized dentin in all three groups ($p<0.001$). For both substrates, μTBS ranking was: Group III (CuNPs) > Group II (chitosan) > Group I (control). Post-hoc Tukey analysis revealed significant differences between all group pairs ($p<0.05$).*
Conclusions: *Both modified bonding agents demonstrated significantly higher μTBS than the unmodified control on sound and demineralized dentin. The 0.2% CuNP-modified bonding agent showed the highest μTBS, followed by 0.12% chitosan-modified. Antibacterial modification of 8th generation bonding agents represents a promising avenue for improving restoration longevity.*
Keywords: *Copper nanoparticles; simulated demineralized dentin; chitosan; 8th generation bonding agent; microtensile bond strength (μTBS)*

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I. Introduction

Establishing durable adhesion between dentin, enamel and resin-based restorative materials remains a fundamental challenge in restorative dentistry.[1] The introduction of bonding agents alongside composite resin has transformed modern restorative practice. Since their inception, bonding systems have been the subject of intensive research focusing on material chemistry, clinical simplification and enhancement of bond strength.[2] Contemporary adhesive materials rely primarily on micromechanical retention of the adhesive to both the restorative material and tooth structure, with chemical bonding playing a secondary role.[3]

Fusayama's research described two distinct layers of carious dentin: the outer caries-infected layer, which is highly demineralized and harbors microorganisms with irreversibly denatured collagen unsuitable for remineralization; and the inner caries-affected (firm) dentin, which is partially demineralized but physiologically remineralizable with intact collagen, and therefore should be preserved during cavity preparation.[4] Clinically, minimally invasive dentistry mandates preserving this inner layer while removing only the infected outer layer, necessitating reliable adhesive bonding to demineralized substrates.[5]

The 8th generation bonding agent, (Voco Futurabond Germany, 2010) incorporates nano-sized filler particles that enhance hybrid layer thickness and resin monomer penetration, with dual-cure capability providing improved mechanical properties and versatility across a wide range of clinical applications.[6] There is growing interest in modifying 8th generation bonding agents with antibacterial nanoparticles, to enhance both the biological and mechanical performance of adhesive systems. Limited research is available on the bonding performance of modified adhesives on simulated demineralized dentin. Thus, the aim of the study was to evaluate and compare

the μTBS of an 8th generation bonding agent modified with 0.12% chitosan and 0.2% copper nanoparticles on sound and simulated demineralized dentin in an in vitro study.

II. Materials And Method

Forty-two healthy single rooted premolars extracted due to orthodontic or periodontal indications were included. The sample size was determined using G-Power 3.1.9.2 software (alpha = 0.05, power = 80%, effect size = 0.50). Teeth were randomly divided into three equal groups (n=14) and 2 subgroups (n=7) each.

Group I – 8th generation bonding agent (G-Premio BOND, GC Corporation, Tokyo, Japan) [control group]:
Group IA – μTBS on sound dentin;
Group IB – μTBS on simulated demineralized dentin.

Group II – 8th generation bonding agent modified with 0.12% chitosan:
Group IIA – μTBS on sound dentin;
Group IIB – μTBS on simulated demineralized dentin.

Group III – 8th generation bonding agent modified with 0.2% copper nanoparticles (CuNPs):
Group IIIA – μTBS on sound dentin;
Group IIIB – μTBS on simulated demineralized dentin.

Preparation of Modified Adhesives

The chitosan-modified adhesive was prepared by dissolving chitosan powder in 1% (v/v) acetic acid to yield a 0.12% (w/w) chitosan solution. An equal volume (one drop each) of chitosan solution and the 8th generation bonding agent was mixed under controlled temperature and humidity in a dark room immediately prior to application.

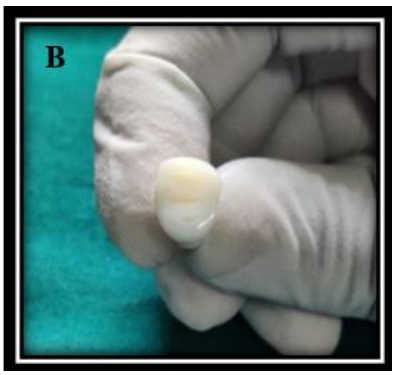
The CuNP-modified adhesive was prepared by suspending copper nanoparticles in the 8th generation bonding agent to achieve a final concentration of 0.2% (w/v). Uniform dispersion was achieved by 30-second sonication (ultrasonic bath, 40 kHz) at room temperature immediately before use, under controlled light conditions to minimize photo-activation.

Simulation of Demineralized Dentin

Simulation of demineralized dentin was done by placing 21 extracted teeth in 10% citric acid solution for 4 hours.

Study Procedures

All 42 teeth were sectioned horizontally in the mesiodistal direction at approximately the level of the dentinoenamel junction (DEJ) to expose the dentin surface. Two coats of the respective adhesive (control or experimental) were applied with a disposable applicator tip to the dentinal surface of each sectioned tooth (sound or demineralized). The adhesive was agitated for 10s, air-dried for 5 seconds and light-cured for 10 seconds using a dental curing unit (Woodpecker, Henan, China; wavelength 470 nm, light source standardized at 5 mm from the dentin surface).



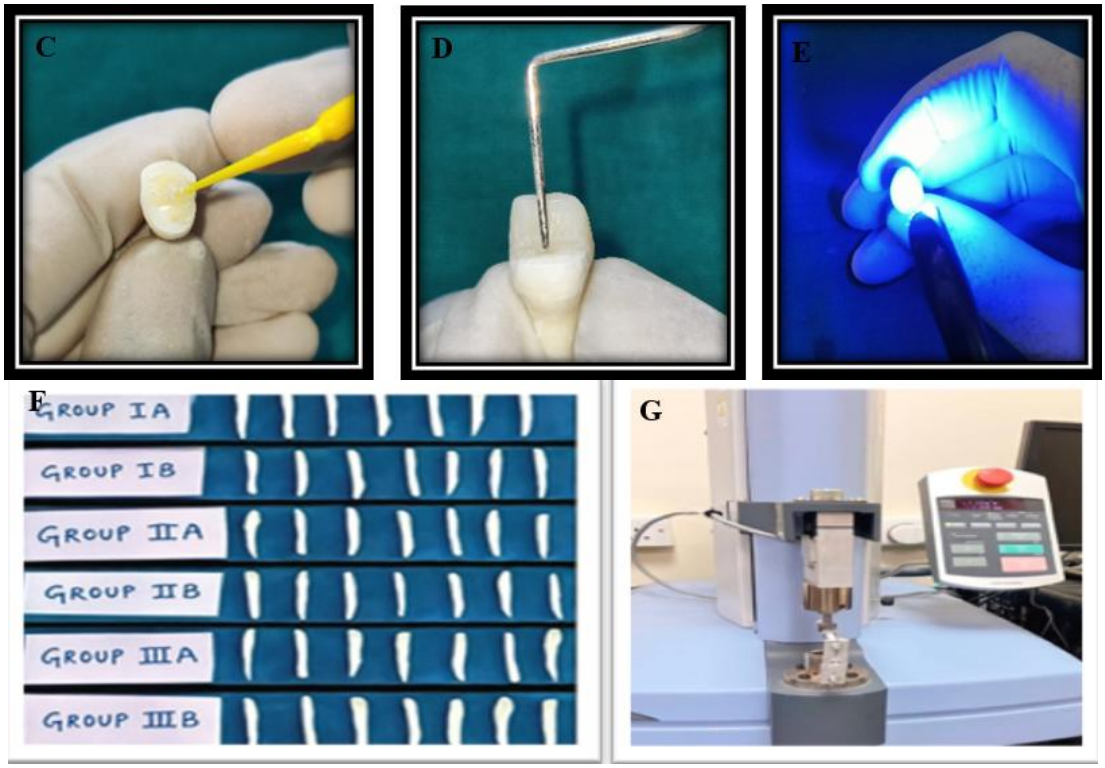


Figure 1: Evaluation of microtensile bond strength: (A) Forty-two extracted teeth; (B) simulated demineralized dentin; (C) application of bonding agent; (D) 4-mm composite build up; (E) curing of composite; (F) vertical sectioning of the teeth; (G) testing microtensile bond strength using universal testing machine

Following adhesive application, A 4-mm composite resin (3M™ Filtek™ Z350 XT, St. Paul, MN, USA) build-up was performed using incremental layering technique and each layer was cured for 20 seconds (470 nm). Bonded specimens were stored in distilled water at 37°C with 100% humidity for 24 hours prior to sectioning.

The bonded teeth were then sectioned vertically into serial slabs and further into beams with a 1 mm² cross-sectional area using a low-speed diamond saw under water coolant (17). µTBS was measured using a universal testing machine. Specimens were attached to a jig using cyanoacrylate adhesive, the crosshead speed of 1mm/min at room temperature was selected and teeth were subjected to failure. Load required to debond the adhesive interphase of tooth-composite was recorded. The µTBS was then calculated by dividing the load at failure (F) by the cross-sectional area (A). The µTBS was calculated in MPa by using the formula: µTBS = F/A

Statistical Analysis

Data was analysed by using SPSS version 26.0 software. Independent t-test was used for intragroup comparison between sound and demineralized dentin, while one-way ANOVA followed by post-hoc Tukey test was used for intergroup comparison. Statistical significance was set at $p < 0.05$.

III. Results

The mean micro-tensile bond strength (µTBS) values for sound dentin were 30.44 ± 1.27 MPa for the control group (G1: 8th-generation bonding agent), 38.44 ± 0.60 MPa for the copper-modified bonding agent (G2), and 35.36 ± 0.64 MPa for the chitosan-modified bonding agent (G3). For demineralized dentin, the µTBS values were 20.63 ± 0.91 MPa (G1), 28.33 ± 0.43 MPa (G2), and 25.09 ± 0.31 MPa (G3). One-way ANOVA revealed statistically significant differences among the three groups for both sound and demineralized dentin ($p < 0.0001$).

Pairwise comparisons also confirmed that copper-modified bonding agents (G2) had significantly higher µTBS than chitosan-modified bonding agents (G3) ($p < 0.0001$).

Table 1. Descriptive statistics for microtensile bond strength (MPa).

Group	Condition	Mean (MPa)	SD
G1 (Control G-Premio BOND)	Sound Dentin	30.44	1.27
	Demineralized Dentin	20.63	0.91
G2(Copper Nanoparticles)	Sound Dentin	38.44	0.60
	Demineralized Dentin	28.33	0.43
G3 (Chitosan)	Sound Dentin	35.36	0.64

	Demineralized Dentin	25.09	0.31

ANOVA p-values show a statistically significant difference ($p < 0.0001$) among the three groups for both sound and demineralized dentin.

Table 2. Post-hoc Tukey test for intergroup comparison among the three groups.

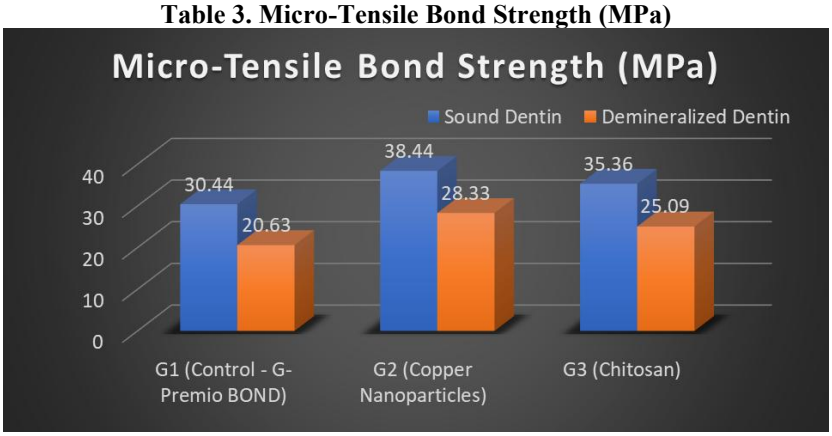
Sound Dentin

Comparison	Mean Difference	*p*-value	95% CI (Lower)	95% CI (Upper)
G1 (Control) vs G2 (Copper)	**8.0000**	**<0.0001**	6.79	9.21
G1 (Control) vs G3 (Chitosan)	**4.9143**	**<0.0001**	3.70	6.13
G2 (Copper) vs G3 (Chitosan)	**-3.0857**	**<0.0001**	-4.30	-1.87

Demineralized Dentin

Comparison	Mean Difference	*p*-value	95% CI (Lower)	95% CI (Upper)
G1 (Control) vs G2 (Copper)	**7.7000**	**<0.0001**	6.87	8.53
G1 (Control) vs G3 (Chitosan)	**4.4571**	**<0.0001**	3.63	5.29
G2 (Copper) vs G3 (Chitosan)	**-3.2429**	**<0.0001**	-4.07	-2.41

Table 3. Micro-Tensile Bond Strength (MPa)



IV. Discussion

The use of 8th generation bonding agent is crucial due to its advantages of improved adhesion, enhanced penetration and hybrid layer formation, optimized mechanical properties, versatility of the material, and dual cure polymerization utilization.[7] The 8th generation bonding agent contains nano-sized fillers that increase the penetration contributing to the formation of a thicker and more effective hybrid layer and enhancing the bond strength between the tooth and the restorative material. This improved hybrid layer thickness and resin penetration lead to enhanced mechanical properties of the adhesive such as better resistance to wear, stress and forces experienced during mastication. Moreover, it can bond with all the substrates allowing for a wide range of applications in restorative and cosmetic dentistry, thereby indicating its versatility.[8]

The present study evaluated and compared the μTBS of an 8th generation bonding agent, unmodified and modified with either 0.12% chitosan or 0.2% copper nanoparticles, on sound and simulated demineralized dentin substrates. The results demonstrated that both experimental modifications significantly improved bond strength compared to the unmodified adhesive. Among all groups, the CuNP-modified adhesive showed the highest μTBS, followed by the chitosan-modified adhesive, whereas the control group exhibited the lowest values. Furthermore, sound dentin consistently demonstrated significantly greater bond strength than simulated demineralized dentin.

The results of the present study are in accordance with the reported literature. The study by Nakajima et al. demonstrated significantly lower tensile bond strength on caries-affected firm dentin compared to sound dentin, attributed to alterations in dentin composition and structure.[9] Similarly, Yoshiyama et al. focused on the effect of total-etch adhesive and an experimental self-etching adhesive in the bond strength when applied over sound, firm, and soft dentin. The study demonstrated that bond strength was significantly higher for the sound dentin followed by affected firm dentin and the least with the infected soft dentin.[10]

The chitosan-modified adhesive also demonstrated significantly greater μTBS than the control group in both substrates, although the values were lower than those obtained with CuNP modification. Chitosan is a biocompatible cationic polysaccharide capable of interacting chemically with dentinal collagen through hydrogen bonding and electrostatic interactions.[11] It acts as a collagen cross-linking agent and matrix metalloproteinase (MMP) inhibitor, thereby stabilizing the collagen fibrils and reducing enzymatic degradation within the hybrid layer.[12]

The improved bond strength observed with chitosan modification may also be related to enhanced preservation of the collagen scaffold and improved hybrid layer integrity. de Carvalho Nunes et al. reported that addition of 0.12% chitosan to adhesive systems improved dentin bond strength without adversely affecting polymerization or adhesive performance.[13] Kishen et al. demonstrated that chitosan nanoparticles stabilize collagen against enzymatic degradation and preserve hybrid layer integrity.[14]

In the present study, incorporation of copper nanoparticles (CuNPs) into the 8th generation bonding agent resulted in the highest μTBS values in both sound and simulated demineralized dentin. The superior bonding performance of the CuNP-modified adhesive may be attributed to their extremely small particle size and large surface area, CuNPs can penetrate deeply into dentinal tubules and the exposed collagen network, thereby enhancing micromechanical interlocking and increasing the effective bonding surface area. In addition, copper nanoparticles may function as reinforcing nanofillers within the adhesive resin matrix, improving mechanical properties such as stiffness, elastic modulus, and resistance to cohesive failure.[15]

Another possible explanation for the improved bond strength observed in the present study is the potent antibacterial activity of CuNPs. Copper nanoparticles are capable of generating reactive oxygen species and disrupting bacterial cell membranes, proteins, and enzymatic pathways, thereby reducing bacterial colonization at the adhesive interface. Reduction in bacterial activity may minimize degradation of the hybrid layer and contribute to improved long-term adhesive stability.[16] Xu et al. reported that incorporation of copper nanoparticles into restorative dental materials improves both biological and physicochemical properties because of their nanoscale interaction with the resin matrix and their ability to enhance material performance.[17] Copper nanoparticles enhance material performance by uniformly dispersing within the adhesive resin matrix, where they act as nanofillers that occupy microscopic voids and strengthen the polymer network, thereby improving mechanical properties such as flexural strength, elastic modulus and wear resistance.

However, despite these beneficial effects, the chitosan-modified adhesive demonstrated lower μTBS than the CuNP-modified adhesive in the present study. This difference may be related to the stronger nanofiller reinforcement effect produced by copper nanoparticles within the adhesive resin matrix. CuNPs may improve mechanical reinforcement more effectively than chitosan because of their inorganic nanoparticulate structure and greater ability to enhance stress distribution within the polymerized adhesive layer.[15]

V. Conclusion

Within the limitations of this in-vitro study: μTBS was significantly greater in sound dentin than in simulated demineralized dentin across all groups. Both the 0.12% chitosan-modified and 0.2% CuNP-modified 8th generation bonding agents demonstrated significantly higher μTBS than the unmodified control on both dentin substrates. The 0.2% CuNP-modified bonding agent provided the highest μTBS overall, followed by the 0.12% chitosan-modified bonding agent. Modified antibacterial bonding agents represent a promising future direction in adhesive dentistry for reducing secondary caries and improving restoration longevity.

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