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A Comparative Evaluation of Chitosan Preconditioning Versus Chitosan Incorporated Adhesive on Dentin Bond Strength: An *In Vitro* Study

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KEYWORDS

Biomodification, Chitosan, Dentin bond strength, Microtensile test, Universal adhesive.

ABSTRACT:

Introduction: The breakdown of the resin-dentin bond over time is still a major challenge in adhesive dentistry. Chitosan, a natural biopolymer with collagen-stabilizing and antimicrobial properties, has been explored to improve bond durability. However, limited data exist comparing chitosan preconditioning and chitosan-modified adhesives.

Objectives: To compare the effect of chitosan preconditioning and chitosan incorporated adhesive on the dentin bond durability of a universal adhesive system.

Methods: A total of 36 extracted human molars were collected and flattened to expose mid-coronal dentin and randomly divided into three groups (n = 12): Group 1 (Control): Selective enamel etching followed by Clearfil S3 Bond Universal and composite restoration. Group 2 (Chitosan incorporated Adhesive): Clearfil S3 Bond Universal modified with 0.5% (w/w) chitosan nanoparticles. Group 3 (Chitosan Preconditioning): Dentin preconditioned with 1% (w/v) chitosan nanoparticles in 1% acetic acid for 60 seconds followed by Clearfil S3 Bond Universal. The samples were filled with resin nanohybrid composite and analyzed for microtensile bond strength (μTBS) on a universal testing equipment. The data was examined with one-way ANOVA and Tukey's post hoc test ($p < 0.05$).

Results: The mean μTBS values (MPa) were: Control = 39.82 ± 5.29, Chitosan incorporated Adhesive = 41.33 ± 6.39, and Chitosan Preconditioning = 46.46 ± 7.12. Although the chitosan-treated groups exhibited higher mean bond strength than the control, there was no statistically significant difference ($p = 0.0658$).

Conclusions: In comparison to the control, dentin bond strength was enhanced by both chitosan preconditioning and chitosan incorporated into adhesive. Nevertheless, there was no statistically significant change. The highest mean bond strength was achieved using chitosan preconditioning, which may improve long-term bond durability.

1. Introduction

The longevity of adhesive dental fillings depends largely on stability of dentin-resin interface. Although adhesive materials have evolved considerably, the hybrid layer continues to be vulnerable to both hydrolytic and enzymatic breakdown, which gradually weakens the bond and can result in restoration failure over time [1, 2]. The deterioration of collagen within this layer is mainly attributed to the intrinsic enzymes action, particularly matrix metalloproteinases (MMPs) along cysteine cathepsins [3, 4].

Various approaches were suggested to enhance the durability of hybrid layer, such as promoting collagen crosslinking, inhibiting enzymatic activity, and modifying the dentin substrate through biomodification techniques [5, 6]. Among these, chitosan, a naturally derived polysaccharide obtained from chitin, has shown promise because of its biocompatibility, antibacterial properties, ability to cross-link with collagen fibrils [7]. Chitosan can interact with collagen through hydrogen and ionic bonding, thereby increasing its mechanical stability and resistance to enzymatic degradation [8,9].

Chitosan has been applied both as a dentin preconditioning agent and as a nanoparticle additive incorporated into adhesive systems [10-12]. While both approaches aim to enhance bonding performance,

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comparative evidence regarding their relative effectiveness remains scarce.

Therefore, this *in vitro* investigation sought to evaluate how chitosan application as a preconditioning agent and its incorporation into the adhesive influence the dentin bond strength of a universal adhesive system.

2. Objectives

- To evaluate whether chitosan preconditioning improves the durability of the resin–dentin bond compared to the control group.
- To assess if incorporating chitosan nanoparticles (0.5% w/w) into a universal adhesive enhances bond strength compared to the unmodified adhesive.
- To determine which chitosan application method i.e: preconditioning vs incorporation, provides superior bond performance.

3. Materials and Methods

Specimen preparation

Thirty-six undamaged, caries-free human molars were extracted after informed consent and ethical approval processes. Until the experiment, the samples were kept in distilled water at 4°C. A water-cooled, low-speed diamond saw was used to cut the occlusal enamel in order to reveal a flat mid-coronal dentin surface. After that, a consistent smear layer was created by polishing the exposed dentin for about 30 seconds using 600-grit silicon carbide abrasive paper.

Grouping of Specimens:

The specimens were randomly allocated into three experimental groups (n = 12):

• **Group 1 (Control):** Selective enamel etching was performed using 37% phosphoric acid for a duration of 15 seconds, followed by Clearfil S3 Bond Universal application and nanohybrid composite restoration.

• **Group 2 (Chitosan Incorporated Adhesive):** Clearfil S3 Bond Universal modified with 0.5% (w/w) chitosan nanoparticles, applied as per manufacturer's instructions.

• **Group 3 (Chitosan Preconditioning):** The dentin surface was conditioned with a 1% (w/v) chitosan nanoparticle solution prepared in 1% acetic acid for 60 seconds. After gentle air-drying, Clearfil S3 Bond Universal was applied, followed by placement of the composite resin restoration.

Microtensile bond strength testing

After the restoration technique, the samples were submerged in distilled water and stored at 37°C for 24 hours. The teeth were sectioned perpendicular to the bonded interface, resulting in resin-dentin beams having a cross-sectional area of approximately 1mm^2. The sticks were tested for tensile strength using a universal testing equipment with a crosshead speed of 1 mm/min. The microtensile bond strength (μTBS, in MPa) was calculated by dividing the measured failure load (N) by the cross-sectional area (mm^2).

Statistical Analysis

The data was analyzed using one-way ANOVA and Tukey's post hoc comparison test (SPSS software, version 25.0; IBM Corp., USA). A p-value less than 0.05 was used to signify statistical significance.

4. Results

Table 1 shows the mean μTBS values for the three groups. The control group exhibited a mean bond strength of 39.82 ± 5.29 MPa, the chitosan incorporated adhesive group 41.33 ± 6.39 MPa, and the chitosan preconditioning group 46.46 ± 7.12 MPa.

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Table 1: Pair-wise differentiation of mean microtensile bond strength values (MPa) using Tukey's post hoc procedure:

Groups	Control group	Chitosan incorporated adhesive group	Chitosan Preconditioning group
Mean ± SD	39.82 ± 5.29	41.33 ± 6.39	46.46 ± 7.12
Control	—	P = 0.7576	P = 0.0600
Chitosan incorporated adhesive	P = 0.7576	—	P = 0.2367
Chitosan Preconditioning	P = 0.0600	P = 0.2367	—

P>0.05 indicate no significant difference. SD- Standard deviation

One-way ANOVA revealed no significant difference between the groups (F = 2.958, p = 0.0658). Both chitosan-treated groups showed a numerical increase in their bond strength compared to the control group, however the difference was not significant (Table 2). Post-hoc Tukey's analysis revealed no significant pairwise differences (p > 0.05).

Table 2: Microtensile bond strength values (Mpa) comparison by one-way ANOVA for all the groups:

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between groups	2	224.717	112.358	2.958	0.0658
Within groups	33	1253.688	37.991		
Total	35	1478.405			

P>0.05 indicate no significant difference.

Among the groups, the chitosan preconditioning group demonstrated the highest mean μTBS, followed by the chitosan incorporated adhesive group, while the control group recorded the lowest values.

5. Discussion

The purpose of this *in vitro* study was to evaluate and compare the effect of chitosan preconditioning and integration into the adhesive on dentin bond strength achieved using a universal adhesive system. Although there were no significant differences between the three experimental groups, specimens treated with chitosan had greater mean μTBS values than the control group, with preconditioning having the highest mean strength.

The improvement in bond strength observed in the chitosan preconditioning group can be attributed to chitosan's multiple beneficial properties. Chemically, chitosan is a cationic biopolymer containing amino and hydroxyl functional groups that allow it to form hydrogen and ionic bonds with the negatively charged carboxyl groups of collagen fibrils. This interaction contributes to strengthening the collagen framework, making it more mechanically stable and less prone to enzymatic breakdown by endogenous enzymes such as MMPs and cysteine cathepsins [8, 10]. In addition, chitosan functions as a gentle cross-linking agent that enhances the rigidity and resilience of demineralized dentin matrices against both mechanical stress and chemical degradation [11].

From a surface chemistry perspective, chitosan preconditioning increases dentin surface energy and wettability, thereby promoting improved resin monomer penetration and the development of a more uniform hybrid layer [12, 13]. The increased diffusion of resin into the collagen framework helps create a stable, well-integrated interface, which may enhance both the initial and long-term durability of the adhesive bond.

The chitosan incorporated adhesive group also showed a slight increase in μTBS compared to the control, which may be related to the biofunctional properties of chitosan nanoparticles. Their addition to adhesive systems can enhance bioactivity, mechanical strength, and antibacterial performance of the adhesive layer [14]. Nanoparticles can also act as reinforcing fillers, improving the polymer network density and stress distribution within the adhesive layer [8]. However, the lack of statistical significance may be due to limited nanoparticle loading (0.5% w/w) and potential issues

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such as nanoparticle agglomeration or altered viscosity, which could reduce effective wetting and polymerization efficiency [15, 16].

The non-significant difference between groups might also be attributed to the short-term nature of this study. Previous reports have shown that the beneficial effects of chitosan are more prominent after long-term storage or thermocycling, where enzymatic degradation of the hybrid layer becomes more evident [16]. Chitosan's potential to inhibit MMP activity and preserve collagen integrity becomes increasingly valuable under conditions simulating intraoral aging, suggesting that further studies incorporating artificial aging, thermocycling, and nanoleakage assessment would provide a more comprehensive understanding of its long-term effects.

Additionally, differences in experimental variables, such as chitosan concentration, particle size, and solvent system, can influence its performance. For example, Epasinghe et al. reported significant improvement in bond strength when chitosan was applied at concentrations above 1%, while Fawzy *et al*. [8] demonstrated enhanced mechanical resilience of collagen matrices with chitosan-riboflavin cross-linking. This indicates that optimizing the formulation and application parameters of chitosan could yield more consistent and clinically relevant improvements.

Clinically, the incorporation of chitosan as a biomodifier offers a biocompatible and cost-effective strategy to enhance the durability of adhesive restorations. Its dual function stabilizing collagen and imparting antibacterial properties, may contribute to reduced secondary caries and longer restoration longevity. Furthermore, chitosan's compatibility with universal adhesives and its potential to integrate into simplified bonding protocols align with modern minimally invasive restorative approaches.

While this study did not yield statistically significant differences, the observed positive trend with chitosan preconditioning indicates its potential as an adjunctive strategy for dentin biomodification. Subsequent research should incorporate aging protocols to evaluate long-term bond durability, employ scanning electron microscopy (SEM) to examine alterations in the hybrid layer, and assess nanoleakage patterns to verify sealing effectiveness. These methodologies will facilitate validation of clinical performance and guide formulation optimization in adhesive dentistry.

6. Conclusion

Considering the constraints of this in vitro experiment, both the chitosan preconditioning and chitosan-incorporated adhesive group's demonstrated greater dentin bond strength than the control, though the differences did not reach statistical significance. Among the tested conditions, chitosan preconditioning achieved the highest average bond strength, suggesting its potential as an effective approach to improve the longevity of the resin–dentin bond.

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