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Effect of Chitosan Nanoparticles on Orthodontic Resin Composites: Antimicrobial Properties and Shear Bond Strength

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Abstract

Background and objectives: The aim of this *in-vitro* study was to evaluate the antibacterial effect of adding different concentrations of chitosan nanoparticles (CS-NPs) into composite resin and their effect on shear bond strength (SBS) of orthodontic brackets.

Materials and methods: 60 composite resins disks containing 0%, 1%, 5%, and 10% w/w CS-NPs were prepared for disk agar diffusion test on days 3, 15 and 30. SBS test was conducted on 60 intact maxillary first premolars which were bonded with Composite resins containing 0%, 1%, 5%, and 10% w/w CS-NPs. The bracket/tooth SBS was measured by Universal testing machine. ANOVA, Tukey HSD post hoc test, Kruskal-Wallis and Mann-Whitney statistical tests were used to analyze the data with significance set as 0.05.

Results: All concentrations of CS-NPs showed antibacterial activity, creation and extension of inhibition zone. Composite resin containing 10% w/w CS-NPs had significant effect on extension of inhibition zone for S.mutans on day 3 (P-value: 0.007). Inhibition zone was decreased in long term which was not significant in 5% and 10%w/w CS-NPs composite. The highest mean shear bond strength was observed in the 0% w/w CS-NPs composite, while the lowest value was recorded in the 10% w/w CS-NPs group. Tukey HSD analysis showed that the 10% w/w CS-NPs group had significantly lower SBS than the Transbond XT control and 1% w/w CS-NPs groups.

Conclusion: On the basis of this study, adding all concentrations of CS-NPs to orthodontic composite enhances its antibacterial activity with minimal adverse effect on shear bond strength.

Keywords: Composite resins; Chitosan; *Streptococcus mutans*.

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1. Introduction

Decalcification of the enamel, manifesting as white spot lesions, frequently occurs during fixed orthodontic treatment, especially in areas surrounding the brackets (1) . Using composite resins for bonding orthodontic brackets is simple and esthetically acceptable however the exposed bonding material may facilitate bacterial adhesion and plaque accumulation, potentially leading to enamel demineralization (2,3). Thus, ensuring that oral hygiene is maintained at a satisfactory level is imperative to prevent these side effects. Young orthodontic patients, particularly those who are under 13 years of age, frequently show a lack of commitment to a regular tooth brushing schedule. Establishing an antimicrobial bonding system has the potential to address the challenges posed by inadequate cooperation. Numerous research efforts have been directed towards improving the antibacterial properties of composite resins, frequently involving the addition of various nanoparticles such as Cu_2O, ZnO, Ag, and Hydroxyapatite (4-7). The primary objective of the research was to enhance the ability of nanoparticles to penetrate the cell wall of bacteria, specifically *Streptococcus mutans*, which is the predominant microorganism present in cariogenic plaques (8,9).

Chitosan is a biocompatible biodegradable and bioactive linear aminopolysaccharide known for its antiviral, antifungal, and antimicrobial properties (10). The antibacterial mechanism of chitosan relies on the presence of amino groups (NH3) within its molecular structure. These groups can interact with the bacterial membrane, compromising its structural integrity and leading to the leakage of intracellular components from the bacteria (11).

Aiming to investigate the effects of Chitosan nanoparticles on *Streptococcus mutans* and the shear bond strength of adhesive composites used in orthodontic applications, this study considered the benefits associated with the manufacture of composite resins that exhibit antibacterial properties and appropriate bonding strength.

2. Materials and methods

2.1. Preparation of chitosan NPs

The Chitosan powder, (Sigma Aldrich, USA) possessing a low molecular weight with 75-85% deacetylation, was lightly agitated in a 1% acetic acid solution for 24 hours until it dissolved completely. The pH was then adjusted to 7 by adding 2 molars of NaOH. A filter with a pore size of 1 micrometer (Millipore) was employed to isolate the undissolved substances. Following this, Sodium Triphosphate was gradually incorporated into the chitosan solution, maintaining a stirring speed of 800 rpm for 45 minutes. The resultant particles were then separated using a centrifuge set at 2500 rpm for 30 minutes. The particles were thoroughly washed with deionized water several times before being freeze-dried by Lyophilize freeze-dryer. Once frozen, the particles were crushed to yield nanoparticles ready for application.

2.2. Preparation of modified composites

To create 10%, 5%, and 1% concentrations, CS-NPs were integrated into the conventional composite (Transbond XT, 3M Unitek, USA) following a specific procedure. The mixing process commenced with a spatula on a glass slab in a semi-dark environment. This was followed by the use of a Vortex mixing machine (Heidolph, Germany) for a duration of 5 minutes to achieve a consistent mixture.

2.3. Preparation of bacterial suspensions

Streptococcus mutans (Strain ATCC 25175) was obtained from the Iranian Research Organization for Science and Technology (IROST). The organism was then seeded onto Mitis Salivarius agar (Merck, Germany) with the addition of 1% potassium tellurite, in accordance with standard protocols, to yield fresh colonies for the preparation of a microbial suspension. The density of the resulting suspension was modified using sterile phosphate buffered saline (PBS) to conform to the 0.5 McFarland standard concentration, which was utilized in the experimental procedures.

2.4. Composite disc preparation

Standard ring-shaped molds with a diameter of 5mm were positioned between glass slides to achieve a smooth surface after being filled with composite materials, comprising 15 samples of conventional composite and 45 samples of CS-NPs composite at concentrations of 1%, 5%, and 10% (15 each). A visible light cure at a wavelength of 450 nm was administered for a duration of 40 seconds using the Smart Lite Max LED Curing Light from Dentsply-Milford, USA. All specimens, totaling 60, underwent sterilization through UV radiation.

2.5. Disc agar diffusion test (DAD)

Sixty composite discs, comprising conventional and CS-NPs composites, were randomly assigned into four groups of 15 discs. These discs were placed on BHI agar plates, which contained 200 µL of bacterial solution, maintaining a separation of 2 cm between them. After an incubation period of 48 hours at 37°C, the bacterial inhibition zones were measured. This evaluation took place on the third, fifteenth-, and thirtieth-days following incubation in artificial saliva.

2.6. Shear bond strength test (SBS)

A total of sixty intact maxillary premolars, exhibiting no visible cracks or caries, were stored in a 0.1% Tymol solution at a temperature of 4°C for one week. These specimens were randomly divided into four groups of 15, each subjected to a bracket bonding procedure with one of the following options: conventional composite (control), composite with 1% CS-NPs, composite with 5% CS-NPs, or composite with 10% CS-NPs. The buccal surfaces were cleaned using a prophylaxis brush without powder, followed by rinsing and drying. Afterward, 37% phosphoric acid (Fineetch, Korea) was applied to the buccal surfaces for a duration of 30 seconds, followed by a 15-second rinse and gentle air dryingA uniform, thin layer of adhesive (3M Unitek, USA) was uniformly applied to the buccal surfaces and light cured with a SmartLite Max LED Curing Light (Dentsply-Milford, USA) for 15 seconds. Subsequently, stainless steel orthodontic brackets (Dentaurum, Discovery, Germany, Standard edgewise, 0.22 slot, 12.62 mm² base area) were affixed at a distance of 4 mm from the tip of the buccal cusp and light cured for 10 seconds on each side. The specimens were subjected to thermocycling for 1500 cycles within a 24-hour timeframe to mimic the oral environment. Each cycle comprised 15 seconds of immersion in a 5°C water bath, a 10-second dwell period, and 15 seconds of immersion in a 55°C water bath. Then, the thermocycled teeth were embedded in plastic molds with a diameter of 2.5 cm, which were subsequently filled with self-cured acrylic resin (Acropars, Iran) to the level of the cement-enamel junction. The shear bond strength was assessed utilizing a universal testing machine (Walter + baiag, Switzerland). The specimens were arranged so that the base of the bracket was aligned parallel to the direction of the applied force. A metal blade was employed in an inciso-gingival orientation at a crosshead speed of 0.5 mm/min to exert shear force on the composite interface. The resulting force value (N) was divided by the bracket surface area (12.62 mm^2) to determine the SBS in MegaPascal.

2.7. Statistical analysis

The data were analyzed using one-way ANOVA, Tukey HSD post hoc test, Kruskal-Wallis test, and Mann-Whitney U test, with the significance level set at 0.05. The data were first evaluated for normality, revealing that the shear bond strength data followed a normal distribution, while the anti-microbial results did not. Accordingly, the shear bond strength data were analyzed using one-way ANOVA, followed by Tukey HSD post hoc test for pairwise comparisons. In contrast, the antimicrobial data were assessed using the Kruskal-Wallis test, followed by Mann-Whitney U tests for pairwise comparisons.

1. Results

3.1. DAD test

The average values of the bacterial growth inhibition zones are displayed in Table 1. In all repeated tests conducted on days 3, 15, and 30, the resin composite containing 10% w/w CS-NPs exhibited the largest diameters of inhibition zones (8.54, 7.58, and 6.86, respectively), whereas the conventional composite with 0% particles demonstrated no inhibition zone. Notably, on days 3, 15, and 30, the 10% w/w CS-NPs showed a significantly larger inhibition zone compared to the other groups in the study. Significant differences were noted in the 1% w/w CS-NPs relative to the control group only on day 3. On days 15 and 30, there were no significant differences among the groups (1%, 5%, and 10% w/w CS-NPs), as presented in Table 2. Furthermore, the findings indicated a decline in the inhibition diameter over the course of days 3, 15, and 30 in composite resins that incorporated nanoparticles, as detailed in Table 3.

3.2. SBS test

Table 4 presents the descriptive statistics of the SBS test. The mean ± SD SBS values were 13.94 ± 1.43 MPa for Transbond XT, 13.75 ± 1.52 MPa for 1% w/w CS-NPs, 12.13 ± 2.61 MPa for 5% w/w CS-NPs, and 10.96 ± 2.43 MPa for 10% w/w CS-NPs. The highest mean SBS was observed in the Transbond XT control group, whereas the lowest value was recorded in the 10% w/w CS-NPs group.

One-way ANOVA showed a statistically significant difference in SBS among the study groups, $F(3,56) = 6.996$, $P < 0.001$ (Table 5). Tukey HSD post hoc analysis showed that the 10% w/w CS-NPs group had significantly lower SBS values than both the Transbond XT control group and the 1% w/w CS-NPs group. No statistically significant differences were observed among the remaining pairwise comparisons (Table 6).

Table 1. *S. mutans* inhibition diameter (mm) in 1%, 5%, and 10% w/w CS-NPs containing composite and control groups in DAD test.

Composites	Day	N	Mean ± SD	Minimum	Maximum
Transbond XT (0%)	3	5	0	0	0
	15	5	0	0	0
	30	5	0	0	0
1% w/w CS-NPs	3	5	6.96 ± 0.50	6.40	7.50
	15	5	6.72 ± 0.40	6.30	7.20
	30	5	6.34 ± 0.35	6.00	6.70
5% w/w CS-NPs	3	5	7.52 ± 0.37	7.20	8.10
	15	5	6.96 ± 0.28	6.70	7.40
	30	5	6.32 ± 0.24	6.00	6.50
10% w/w CS-NPs	3	5	8.54 ± 1.17	7.50	10.00
	15	5	7.58 ± 0.83	6.50	8.50
	30	5	6.86 ± 0.62	6.00	7.60

*Statistically significant at $P < 0.05$.

Table 2. Pairwise comparison of inhibition zone diameter by adding 1%, 5%, and 10% w/w CS-NPs to resin composite in day 3.

Study Groups	1% w/w CS-NPs	1% w/w CS-NPs	1% w/w CS-NPs
1% w/w CS-NPs	-	0.451	0.016*
5% w/w CS-NPs	0.451	-	0.57
10% w/w CS-NPs	0.016*	0.79	-

Table 3. Pairwise comparison of inhibition zone diameter by adding 1% w/w CS-NPs to resin composite by time (P-value).

Day	Day 3	Day 15	Day 30
Day 3	-	0.052	*0.012
Day 15	0.52	-	*0.012
Day 30	*0.012	*0.012	-

*Statistically significant at $P < 0.05$.

Table 4. Descriptive data of SBS test in groups (Mpa).

Study groups	Mean ± SD	Minimum	Maximum
Transbond XT	13.94±1.43	11.19	17.09
1% w/w CS-NPs	13.75±1.52	11.23	16.38
5% w/w CS-NPs	12.13±2.61	9.53	18.21
10% w/wCS-NPs	10.96±2.43	6.96	15.01

Table 5. ANOVA test results.

SBS	Sum of squares	df	Mean square	F	P-value
Between Groups	90.054	3	30.018	6.996	<0.001*
Within Groups	240.284	56	4.291		
Total	330.339	59			

*Statistically significant at $P < 0.05$.

Table 6. Tukey HSD post hoc pairwise comparisons of shear bond strength among study groups.

Comparison	Mean difference (MPa)	CI 95%	Adjusted P-value
Transbond XT vs 1% w/w CS-NPs	0.188	-1.815 to 2.191	0.995
Transbond XT vs 5% w/w CS-NPs	1.812	-0.191 to 3.815	0.090
Transbond XT vs 10% w/w CS-NPs	2.981	0.979 to 4.984	0.001*
1% w/w CS-NPs vs 5% w/w CS-NPs	1.624	-0.379 to 3.627	0.151
1% w/w CS-NPs vs 10% w/w CS-NPs	2.793	0.791 to 4.796	0.003*
5% w/w CS-NPs vs 10% w/w CS-NPs	1.169	-0.834 to 3.172	0.418

*Statistically significant at $P < 0.05$.

4. Discussion

This *in-vitro* study aimed to analyze the antibacterial efficacy of different concentrations of Chitosan nanoparticles (CS-NPs) when added to composite resin, along with their impact on the shear bond strength of orthodontic brackets. According to the finding of the study, enhancing the antibacterial activity of orthodontic composite resins can be achieved by integrating all concentrations of CS-NPs but the adverse effects on shear bond strength of brackets varies due to various concentrations.

The Effective oral hygiene plays a vital role in the success of orthodontic treatment, heavily relying on the compliance of the patient. Exploring approaches that lessen the dependence on patient cooperation, such as the integration of antimicrobial agents within composite resins, could be advantageous (12). Previous research has demonstrated that various nanoparticles, including chitosan, exhibit antimicrobial properties (7,13,14). The current research investigated the antibacterial efficacy of CS-NPs on *S. mutans*, the principal cariogenic microorganism found in the oral cavity, which significantly contributes to the demineralization of enamel (15). The assessment of the antimicrobial properties of chitosan nanoparticles (CS-NPs) over an extended period was crucial due to the prolonged nature of orthodontic treatments. The findings of this study indicated that, in the short term (specifically over a duration of three days), all groups containing nanoparticles exhibited markedly superior antimicrobial effects in comparison to Transbond XT. Furthermore, the 10% w/w CS-NPs demonstrated a significantly larger inhibition zone than the 1% w/w CS-NPs. However, as time progressed (on days 15 and 30), the antimicrobial efficacy diminished across all three groups, resulting in no significant differences among them. The study conducted by Mirhashemi et al. (16), highlighted the antimicrobial effects achieved by the incorporation of chitosan nanoparticles (CS-NPs) and zinc oxide nanoparticles (ZnO-NPs) into composite resin. The results demonstrated that this combination possessed significant antibacterial properties, particularly at elevated concentrations of the nanoparticles. The antimicrobial effects of the 1% w/w CS-NPs group were found to be similar to those of the control group, in contrast to the 5% and 10% w/w CS-NPs groups, which significantly decreased the growth of *S. mutans* organisms.

Sodagar et al. (17), demonstrated that adding TiO_2 nanoparticles into orthodontic composite resins increases antimicrobial effect against composite while the mean shear bond strength of composite containing 1% and 5% NPs has sufficient bond strength. Khan et al. (18), has also observed that ZnO and Cu2O nanoparticles can inhibit bacterial growth and colonization. However, it is important to highlight that the combination of silver and Cu2O nanoparticles with composites resulted in color alterations, which undermines the aesthetic objectives and the polymerization process of the composites.

The augmentation of antibacterial efficacy in resin composites is advantageous; however, it may compromise the shear bond strength associated with bracket bonding. This study's results revealed a decline in shear bond strength for the control and the 1%, 5%, and 10% w/w CS-NP groups, with measurements

of 13.94, 13.75, 12.13, and 10.96 MPa, respectively. Reynolds showed 6 to 8 MPa shear bond strength as the minimum acceptable shear bond strength for routine clinical application (19) and Kim et al. demonstrated that a shear bond strength of up to 14 MP is deemed acceptable for orthodontic procedures, aiming to reduce potential damage to enamel during the debonding of brackets (20). The findings of this study indicated that the results for shear bond strength fell within an acceptable range. It is important to highlight that the shear bond strength observed *in-vivo* is lower than that measured *in-vitro*. The disparity may reach as high as 40%-60% for Transbond Plus and Transbond XT, respectively. This variation can be attributed to factors such as tensile, shear, and torsional forces, as well as the presence of humidity and microbial plaque in the oral cavity, all of which can negatively affect shear bond strength (21-23). Poosti et al. reported that incorporation of 1% w/w TiO_2 nanoparticles significantly reduced shear bond strength compared with the control group. In the present study, however, the 1% w/w CS-NPs group showed no statistically significant reduction in SBS compared with Transbond XT, whereas a significant reduction was observed only in the 10% w/w CS-NPs group (14).
Akhavan et al. also indicated that the incorporation of 1% and 5% silver and hydroxyapatite nanoparticles did not negatively impact the shear bond strength (SBS); however, the addition of 10% of these nanoparticles resulted in a significant decrease in SBS (17).

5. Limitations

Consistent with the nature of in-vitro studies, the current research faces limitations in accurately simulating the oral cavity environment, which underscores the necessity for randomized controlled trials (RCTs) to substantiate the findings. Furthermore, to support the application of nanoparticles, it is imperative to explore their influence on all the mechanical and chemical properties of bonding resin composites.

6. Conclusion

On the basis of this study, adding CS-NPs at all tested concentrations to orthodontic composite resins enhanced their antibacterial activity. The 1% w/w CS-NPs composite resin showed the most favorable balance, with minimal adverse effect on shear bond strength and antibacterial activity comparable to the 5% and 10% w/w CS-NPs groups on day 30.

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None.

Ethics

This study is conducted with the approval by the ethics committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.REC.1395.175).

Using artificial intelligence (AI)

No artificial intelligence-based tools were employed in any stage of this research (i.e., study design, analysis, manuscript preparation, etc.).

Author contributions

Mohammad Hossein Toodehzaeim: Supervision
Zahra Moradi: Investigation, Formal analysis, Writing – Original draft
Elaheh Rafiei: Supervision
Mostafa Mohebbi: Writing – Review & editing
Pedram Toodehzaim: Writing – Review & editing

Conflict of interest

The authors declare no conflicts of interest.

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