Effect of Chitosan Nanoparticles Incorporation on Antibacterial Properties and Shear Bond Strength of Dental Composite Used in Orthodontics

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Abstract

Background: Plaque accumulation is a drawback of orthodontic treatment, which requires composite for bonding of brackets. As the antimicrobial properties of chitosan nanoparticles (NPs) have been proven, the aim of this study was to evaluate the antimicrobial and mechanical properties of composite resins modified by the addition of chitosan NPs.

Methods: Orthodontics composite containing 0%, 1%, 5% and 10% NPs were prepared. 180 composite disks were prepared for Eluted Component Test, Disk Agar Diffusion Test and Biofilm Inhibition Test to collect the counts of microorganisms on three days, measure the inhibition diameter and quantify the viable counts of colonies consequently. For shear bond strength (SBS) test 48 intact bovine incisors were divided into 4 groups. Composites containing 0%, 1%, 5% and 10% NPs were used for bonding of bracket. The bracket/tooth SBS was measured by Universal testing machine.

Results: All concentration of chitosan NPs had a significant effect on creation and extension of inhibition zone. For S. mutans and S. sanguinis all concentration of chitosan NPs caused reduction of the colony counts. Composite containing 10% chitosan NPs had significant effect on reduction of colony counts for S. mutans and S. sanguinis in all three days. The highest mean shear bond strength belonged to the 1% NPs composite while the lowest value was seen in control group.

Conclusions: Incorporating chitosan nanoparticles into composite resins confer antibacterial properties of adhesives, while the mean shear bond of composite containing NPs don’t change dramatically.

Keywords: Shear Bond Strength, Nanochitosan, Antimicrobial Effect, Stepotococcus, Mutans, Lactobacillus Acidophilus.

1. Background

Bonding technique with resin based composite as an adhesive agent has been primarily used in orthodontics for securing orthodontics brackets to the surface of the teeth. Unfortunately, in spite of the fact that bonding technique have many advantages such as high esthetic and simple procedure, still have some drawbacks such as plaque accumulation, development of white spot lesions and bond failure, which cause prolonging the treatment course, imposing high cost, consuming more chair time and a less than optimal esthetic result occurs after treatment due to demineralization of enamel adjacent to the brackets specially around the bracket margin, because of the remaining exposed composite surface (1-4).

Several methods have been used to inhibit biofilm growth that contributes to dental caries. For instance, one group of such efforts has been evaluation of effectiveness of incorporating different antimicrobial agents in the adhesives; two of the most common examples are fluoride and chlorhexidine (5-9).

Today, one of the most important advances in dental material field is the application of nanotechnology to resin composites. Many studies investigated the effect of antimicrobial nanoparticles incorporated into composite resins to prevent plaque accumulation and bacterial adhesion. Nanoparticles are believed to penetrate into the cell wall of bacteria efficiently due to their smaller size, exerting their antibacterial properties effectively (9, 10). A study evaluated the antibacterial effect of composite with different concentration of incorporated nanosilver, which has been used in medicine as an antimicrobial agent (11). The result of the study showed that nanosilver containing composite
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could confer surface antibacterial activity without significant difference on shear bond strength. One of the most important acquisition of nanotechnology is nanoparticles of chitosan, a linear polysaccharide gained by the deacetylation of chitin, that is a structural biopolymer existing in the exoskeletons of the crustaceans and mollusks (12). Chitosan is widely used in many fields including nutrition, pharmaceutical, cosmetics, and agriculture for its remarkable antiviral, antifungal, and antimicrobial activity. The antibacterial activity of chitosan is particularly interesting and has been observed against various bacteria (salmonella lyphimurium, pneumoniea, E.coli, S. aureus) (4, 13, 14).

The antibacterial mechanism of chitosan is due to the bonding of amino groups NH3 with positive charge and carbocyclic group with negative charge on the membrane of bacteria. The bonding changes the permeability of the cell membrane resulting in a weakness and tear in membrane that is followed by cellular leakage (15).

Regarding the necessity of manufacturing of composites with antibacterial properties and the capacity of an appropriate banding strength, and also considering mentioned properties of nanochitosan the main goal of this study is to investigate the effect of nanochitosan NPs against bacteria and shear band strength of adhesive composite used in orthodontic treatment.

2. Methods

2.1. Preparation of Nano Chitosan

Chitosan bulk (Acros company USA) with a low molecular weight was dissolved in acetic acid (1%) then was adjusted to PH = 10 by adding NaOH (N = 10). 3 milliliter of this milky solution was added to 1 milliliter of Tripolyphosphates followed by rapid magnetic string mixing at the highest power. Then Nanoparticles were deposited using a centrifuge. The particles were washed using DI water. After freezing and crashing particles were prepared to use (Figure 1).

2.2. Nanocomposite Preparation

3600 mg of Transbond XT (3M Unitek, USA) composite was blended with 400 mg nano powder (containing 50/50 w/w NPs), using a mixing spatula on a glass slab in a semi-dark environment, until a uniform consistency was achieved. Then 1200 mg of the original composite was mixed with 1200 mg of 10% w/w blended composite to obtain a composite with 5% w/w NPs. Similarly, 240 mg of 10% w/w composite was blended with 2160 mg of original composite to obtain 1% w/w NP composite.

2.3. Shear Bond Strength Test

48 bovine central incisors with no visible cracks or caries were disinfected in 0.5% Chloramine-T solution (4°C) for one week. Specimens were randomly divided into four groups (N = 10) of composites with 0%, 1%, 5% and 10% NP content. Buccal surfaces were cleaned with a prophylaxis brush without powder, rinsed and dried; 35% phosphoric acid (Ultra etch, Ultradent, USA) was then applied to the buccal surfaces for 30 seconds, followed by 30 seconds of rinsing and gentle air drying. A uniform, thin layer of adhesive (3 M Unitek, USA) was applied to the buccal surfaces and light cured (Woodpecker, UK) for 10 seconds after placing the stainless steel orthodontic brackets (Standard edgewise, 0.18 slot, 12.62 mm² base area).

All specimens were thermocycled (Vafaei Industrial, Iran) for 1000 rounds in 24 hours to simulate oral environment. Each cycle consisted of 15 seconds of immersion in 5°C water bath, 10 seconds of dwell time and 15 seconds of immersion in 55°C water bath. The thermocycled teeth were then fixed to the corners of 2.5 cm diameter metal molds using rectangular wire and then the molds were filled with self-cured acrylic resin (Acropars, Iran) up to the level of the cementoenamel junction.

The SBS was measured using Roell-7060 universal testing machine (Zwick/Roell, Germany). Specimens were positioned in such way that the bracket base was parallel to the direction of the applied force. A 0.6 mm metal blade was used in an inciso-gingival direction at a crosshead speed of 0.5 mm/min to apply shear force to the composite interface. The obtained value (N) was divided by the bracket surface area (mm²) to calculate the SBS in Mega Pascal. Each tooth and bracket complex was then checked under a stereomicroscope (Nikon, SMZ800, Japan) at 10X magnification to score the amount of remaining adhesive.
using the ARI as follows: 0 = no adhesive on bracket, 1 = < 25% adhesive on bracket, 2 = 25 - 50 % adhesive on bracket, 3 = 50 - 75% adhesive on bracket and 4 = 75 - 100% adhesive on bracket.

2.4. Antimicrobial Test

Preparation of bacterial suspensions: Streptococcus Mutans ATCC25175, Streptococcus Sanguinis ATCC10556 and Lactobacillus Acidophilus ATCC4356 were supplied in lipophilised form and incubated in broth, in anaerobic and 37°C conditions for 48 hours. 108 CFU/mL microorganisms suspensions were prepared by spectrophotometer for determining the antimicrobial effect of TiO₂ NPs.

Optical density for L. acidophilus in 600 nm is 1, (OD = 1); which is 10⁸ cells per ml. this density was then diluted ten times and inoculated on BHI (brain heart infusion) agar. OD = 0.2 for the other two organisms was equivalent to 10⁷ cells per ml.

Composite disc preparation: 5 mm diameter standard ring-shaped molds were placed between glass slides, for attaining a smooth surface, after being filled with composite. Visible light cure (470 nm) was applied for 30 seconds (Bluphasee® 16i, Ivoclar Vivadent AG, Australia). Discs were cured another 10 seconds after application of a thin layer of bonding.

All specimens (n = 180) were sterilized in Iran’s Nuclear Science and Tech gamma radiation center with 25 KGy dosages.

2.5. Biofilm Inhibition

Three-day biofilms were generated on composite discs (n = 36) using 24-well plates. Each well was inoculated with adjusted bacterial inoculum. Biofilms were grown at 37°C. At the end of the third day, each disc was rinsed with sterile saline to remove loosely absorbed proteins and biofilm matrix residues. To count the colony forming units (CFUs) responsible for biofilm formation, specimens were sonicated in sterile saline and then vortexed. CFU/mL of the microorganism present in the suspension was counted with drop-plate method using rapid dilution in microtiter plates.

2.6. Disc Agar Diffusion Test (DAD)

Antibacterial activity of discs via solubility and diffusion of chitosan NPs was examined by this test. Composite discs (n = 36) were placed, 2 cm apart, on BHI agar plates, which were inoculated with a 200 μL bacterial solution (~ 10⁸ CFU/mL) by a sterile swap. After 48-hour incubation, the bacterial growth inhibition diameter was optically measured.

2.7. Antibacterial Properties of Eluted Components

Antibacterial activity of possible Eluted components from nanoparticle comprising composite discs was also evaluated. Composite discs (n = 108) were placed in tubes containing 5 mL BHI media. BHI media was then aspirated from the tubes on days 3, 15 and 30 and placed in other 15 mL plastic tubes; tubes were inoculated with 50 μL of bacterial culture (final ~ 25 × 10⁸ CFU in 5 mL media) and shaken at 300 rpm rate in 37°C for 24 hours.

2.8. Statistical Analysis

Shear bond strength test results were analyzed using one-way ANOVA followed by Post Hoc Tukey’s HSD test. The Kruskal-Wallis test was also applied to analyze the ARI results. Antimicrobial test results were analyzed with multiple statistical tests. One-way ANOVA was first used for biofilm inhibition test followed by Tukey HSD test. Kruskal Wallis Test was used to analyze data attained from DAD test. Two-way ANOVA was first used to analyze day × concentration relation in Eluted components test. For those groups with significant difference one-way ANOVA was used.

3. Results

3.1. Biofilm Inhibition Test

Mature biofilm on four different composite groups were recorded after 3 days. Descriptive results are shown in Table 1. All tests were carried out three times for each group. Kruskal-Wallis Test showed that S. mutans and S. sanguis colonies were meaningfully lowered in all three groups of composites containing NPs (P < 0.05). However, for L. acidophilus colonies counts reduced as CS-NPs nanoparticles concentration was increased but this difference was significant only in 10% NPs (Figure 2-4).

3.2. DAD Test

In all three repeated tests, in the all three groups of composites containing NPs had a significant diameter of bacterial growth inhibition for all three microorganisms. Kruskal-Wallis Test did not show any significant difference between 1%, 5% and 10% NPs groups. The results are illustrated in Table 2.

3.3. Eluted Components

For S. mutans no significant difference was found for any of the groups in any day (P = 0.144). Groups of composites containing more than 5% NPs on all days significantly reduced colony counts of S. mutans (P < 0.001). Statistically differences in all three days were found for S. sanguis (P = 0.004) and L. acidophilus (P = 0.020) so each of the categories were analyzed by One-way ANOVA, which showed in Figures 5 and 6.
Table 1. Colony Counts (CFU/mm²) in 1%, 5% and 10% NPs Containing Composite and Control Group in Biofilm Inhibition Test

<table>
<thead>
<tr>
<th>Percent</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
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<tr>
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Table 2. Microorganism Inhibition Diameter (Millimeter) by Nanoparticles Diffusion

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<tr>
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<tr>
<td>mutans 5%</td>
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<td>7</td>
<td>8</td>
<td>7.67</td>
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<tr>
<td>mutans 10%</td>
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<td>10</td>
<td>13</td>
<td>11.33</td>
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</tr>
<tr>
<td>Lactobacillus.ac</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1%</td>
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<td>6</td>
<td>8</td>
<td>7.33</td>
<td>1.155</td>
</tr>
<tr>
<td>5%</td>
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<td>6</td>
<td>9</td>
<td>8</td>
<td>1.732</td>
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<tr>
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<td>13</td>
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<td>0.577</td>
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<tr>
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<td>6</td>
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<tr>
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<td>3</td>
<td>9</td>
<td>11</td>
<td>10.33</td>
<td>1.155</td>
</tr>
</tbody>
</table>

3.4. Shear Bond Strength

The result of shear bond strength can be seen in Table 3 shows that maximum mean of the shear band strength belongs to the group with Nanochitosan concentration of 1% and the least belongs to the control group. One-sample kolmogrove-simirnove tests showed a normal distribution among all groups. According to the result of one way ANOVA difference was not significant between groups (P = 0.730).

The Kruskal-Wallis test did not show any significant difference among groups in terms of the ARI (P = 0.823).

4. Discussion

The application of nanotechnology in dental composite resins has been introduced to enhance the long-term antimicrobial properties and provide superior mechanical strength simultaneously (9, 16).

On the basis of studies that have shown antibacterial effect of chitosan particles and according to the fact that nanoparticles have greater effect on membrane of bacteria, it seems like the chitosan NPs has induced an antibacterial activity in resin composite (17).

The antibacterial effect of chitosan NPs against Strepto-
Table 3. Descriptive Data of Shear Bond Strength in Groups

<table>
<thead>
<tr>
<th>Nano</th>
<th>percentage</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
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coccus mutans ATCC 25175, Streptococcus sanguis ATCC10556 and Lactobacillus acidophilus ATCC4356 was evaluated in this study. Criteria for selection of these types of microorganisms were their presence as a normal flora of the mouth, their large population and their important role in causing oral disease (18, 19). 1%, 5% and 10% concentrations of NPs were used for purpose of comparing this NP with the previously studied documents (4).

The result demonstrated that bacterial biofilm inhibition in composite containing NPs is significantly more than conventional composite resins. This effect increased as the percentage of NPs in the composites increased in such a way that the composite containing 1% NPs significantly reduced S. mutans and S. sanguis. However this had no effect on biofilm inhibition of L. acidophilus unless 10% NPs. L. acidophilus help the development of caries and are found in more advanced lesions. Therefore, the biofilm

Figure 2. Viable Counts of Streptococcus Mutans Biofilms on the Following Composites: 0%, 1%, 5% and 10% Nanoparticles-Containing Composite Discs

Figure 3. Viable Counts of Streptococcus sanguis Biofilms on the Following Composites: 0%, 1%, 5% and 10% Nanoparticles-Containing Composite Discs

Figure 4. Viable Counts of Lactobacillus acidophilus Biofilms on the Following Composites: 0%, 1%, 5% and 10% Nanoparticles-Containing Composite Discs
that containing these microorganisms are very resistant and is not effected by adding NPs to the composite resins in low concentration. This finding is in agreement with Poosti who investigated the inhibition of bacterial biofilm only on S. mutans and showed that the inhibition is more prominent with composite containing 1% TiO2NPs (20).

The results of Eluted Component Test showing continuity of antibacterial effect indicated significant reduction of the colony count of S. mutans and S. sanguis, only for 10%NPs group. Similar results were showed in other NPs such as ZnO, and TiO2 compared to chitosan (4, 21). They showed NPs maintain its properties for a long time up to three weeks. Because just a slight increase in the number of colonies was observed unlike L. acidophilus that by increasing time the number of colonies remains constant.

Presence of S. sanguis in the mouth, reducing the population of S. mutans, these bacteria are in balance (22). If the number of colonies remain constant or not significantly decreased in the presence of chitosan NPs the desired result is achieved. However, due to the reduced number of colonies of S. sanguis in this study can be concluded that the chitosan NPs reduce both S. sanguis and S. mutans unslected. But since S. mutans have reduced more than S. sanguis finally, create a favorable effect.

Mirhashemi et al. also reported the antimicrobial effect of chitosan NPs in combination with ZnO-NPs, results showed that ZnO-NPs and Chitosan NPs cause inhibition of bacterial biofilm, which is more prominent with higher NP concentrations. The 1% group was almost similar to the control group while the 5% group significantly reduced S. mutans and the 10% group inhibited S. sanguis and all three organisms significantly (4). This result supposedly caused by the addition of Chitosan NPs because the studies on ZnO-NPs solely decreased significantly only the L. acidophilus on day 30 for the Eluted components test which shows an enhanced antibacterial activity in the combination of NPs compared to ZnO-NPs used alone, since the latter showed no significant difference on any day for any concentrations (21). Pinto et al. in which examined the antimicrobial effect of chitosan NPs in combination with Silver NPs against microorganisms commonly found in food, showed that the effect of chitosan matrix is depend on silver containing; and chitosan NPs alone have a bacteriostatic effect and inhibits growths of microorganisms, although the choice of microorganisms was different (23). Ahn et al. also demonstrated silver NPs have antimicrobial effect against refractory bacteria. But Silver NPs in combination with composite caused color modification which defies the esthetic purposes of composites (24).

For other NPs such as TiO2 the biofilm inhibition was similar in the 1% group with present study, Poosti et al. showed that the Light cure orthodontic composite con-

Figure 5. Colony Counts of Streptococcus Sanguinis on the Following Composites: 0%, 1%, 5% and 10% NPs Containing Composite Discs in Eluted Component Test

Figure 6. Colony Counts of Lactobacillus Acidophilus on the Following Composites: 0%, 1%, 5% and 10% NPs Containing Composite Discs in Eluted Component Test
taining 1% (w/w) TiO$_2$ nanoparticles was quite effective in inhibiting bacterial growth in long term (20).

Properties of composites change by adding NPs to it. So that is important by adding chitosan NPs by means of decrease plaque accumulation, another properties of composite such as mechanical and physical properties remain approximately without changes (25, 26). The aim here is to evaluate the SBS modification caused by the addition of CS-NPs to resin composite used in orthodontics.

Results of this study show that addition of chitosan NPs increase SBS of composite. So that the Transbond XT composite as a gold standard has the lowest strength and the 1% NPs containing composite has the highest strength and it is within the acceptable range 6 - 8 MPa. The bond strength is decreased as the percentage of NPs increase in composite, So that with increasing concentration of 1% to 5% and from 5% to 10% shear bond strength are reduced. However, there is no significant difference between any of the groups. According to the bond strength and scattering of data to use of 1% weight of NP might be advisable with mechanical modifications only in the acceptable range.

Compared with other studies examining the effects of other nanoparticles on shear bond strength of resin composite, in one study, Poosti et al. showed that there is no significant difference between TiO$_2$ NPs containing composite and Transbond XT, Which is similar to the present study (20). Results revealed that adding TiO$_2$ nanoparticle to composite in long term enhance its antibacterial effects without compromising the physical properties.

In another study Akhavan and sadogar concluded that the 1% Silver/hydroxyapatite NPs containing composite has higher SBS than the control group, while in 5% and 10% groups it was decreased. The similar results with this study were obtained that the bond strength is decreased as the percentage of NPs increase in composite (25).

4.1. Conclusion

Finally, the findings of the present study indicate that, dental composite containing chitosan NPs had a quite antimicrobial effect by means of decreasing the number of the colonies, in long term without compromising the shear bond strength.

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