Effect of Silver Nanoparticles Synthesized Using Betel Leaf Extract Added into Orthodontic Adhesive on the Bracket's Tensile Bond Strength

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Abstract

This study investigates the effect of silver nanoparticles synthesized from betel leaf extract (SNBL) added into the orthodontic adhesive on the bracket's tensile bond strength (TBS).

Forty extracted premolars were randomly allocated into four groups of 10 as follows: the negative control (NC) group, and the treatment groups (1% SNBL (BL1), 3% SNBL (BL3), and 5% SNBL (BL5), which was incorporated with the orthodontic adhesive primer to form a nano-adhesive). The SNBL was incorporated at various concentrations into the orthodontic adhesive and visually analyzed using a transmission electron microscope (TEM). The brackets were then bonded, and TBS testing was performed using a Universal Testing Machine. The adhesive remnant index (ARI) value was evaluated by a stereoscopic microscope. The TBS-generated value was analyzed with ANOVA, whereas the ARI score was analyzed using the Kruskal-Wallis test ($\alpha = 0.05$).

There was a significant TBS difference ($p < 0.05$) between the four groups. The group bonded with 5% SNBL showed the significantly lowest TBS than the others [5.92 (0.96) MPa]. In contrast, the NC group exhibited the significantly highest TBS value than the other groups [11.13 (1.57) MPa]. The ARI scores between the four groups showed no statistically significant difference ($p = 0.092$). Orthodontics brackets failure location of NC, BL1, and BL3 dominated by ARI scores of 2, representing the bond failure between the adhesive-bracket interfaces. However, the BL5 group dominated by a score of 0, which indicated that bond failure occurred between the adhesive-enamel surfaces.We concluded that the addition of SNBL above 3% concentration into orthodontic adhesive materials decreases the TBS of orthodontic brackets but was still in the recommended adequate bond strength range value of 5.9–7.8 MPa.

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Introduction

Orthodontic treatment is a procedure that moves the teeth to achieve good alignment, accomplishes appropriate occlusion, and corrects malocclusion.[1] The movement of the teeth with a fixed orthodontic appliance is achieved through the interaction between the bracket attached to the tooth surface and the archwire attached to the bracket.[2] The bracket can be attached directly to the tooth surface with an adhesive material that is better known as bonding.[3] Composite resin adhesive has become the first choice of most orthodontists as a bracket bonding material for decades. However, despite the popularity of this material, it has several shortcomings that cannot be completely resolved.[4] The remaining adhesive on the tooth surface around the bracket's edge can become an area of plaque accumulation. Then, a decalcification process occurs, especially in the area where the enamel connects with the adhesive material.[5] A previous study found that more bacteria were identified in the bonding adhesive than in the bracket material.[6] These findings indicate that the orthodontic adhesive is a risk factor for demineralization of enamel during orthodontic treatment. Therefore, an innovative technique is needed to produce an adhesive material with antibacterial properties that can reduce bacterial colonization.[7]

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The existence of nanotechnology enables the development of "experimental composite adhesives" using nanoparticles.[4] Silver nanoparticles (AgNP) are known to have antibacterial properties superior to gold or lead particles.[8] Silver nanoparticles have several advantages, including low toxicity, good biocompatibility with human cells, long-term antibacterial activity, and low bacterial resistance.[9] Silver nanoparticles directly affect the bacterial cell membrane and cause damage to cell structures and DNA that inhibit bacterial replication.[10] Silver nanoparticles of varying sizes and long-stable particle shapes can be produced by several methods. An environmentally friendly nanoparticle synthesis method using plant extracts is known as green synthesis.[11] Today, researchers are interested in biological reduction procedures using plant extracts for nanoparticle synthesis. The synthesis of silver nanoparticles can be performed using betel leaf extract (*Piper betle* L.) as a bioreductor agent. Phenol derivatives in betel leaf are expected to reduce silver ions (Ag^{+}) to silver nanoparticles because the –OH group of these compounds is easily oxidized. Betel leaf can be used to synthesize AgNPs for antibacterial product development and exhibit antibacterial drug activity against *Bacillus subtils* and Klebsiella planticola.[12]

Experimental adhesives produced to increase the protective properties against bacteria will be better if they do not negatively affect the mechanical properties of the material. An essential characteristic of orthodontic adhesives is their ability to withstand forces, which are generally evaluated through the bond strength test.[7] This study was conducted to investigate the effect of silver nanoparticles synthesized using betel leaf extract added into the orthodontic adhesive on the orthodontic bracket's tensile bond strength (TBS).

Materials and methods

Biosynthesis of silver nanoparticles

This experimental study passed the Eligible Ethics from the Research Ethics Committee. A total of 5 kg finely cut betel leaves were collected and washed using deionized water. Then, it was distilled using a steam distillation reactor with a pressure of 40 Psi. Next, 100 mL of the distillate was extracted with 25 mL of chloroform. Then, the solvent was evaporated by an evaporator at 60°C. Next, 1 g of chitosan was dissolved in 100 mL of 1% acetic acid solution and stirred using a magnetic stirrer at room temperature for 18 hours. Then, 100 mL of a total of 100 ppm AgNO3 solution was added dropwise to 100 mL of chitosan solution at 50°C. The solution was kept stirring for one hour, then 100 mL was added to the solution containing 0.1 g of green betel leaf extract. The solution was heated at 100°C for two hours. The color change from clear to yellow indicates that colloidal silver nanoparticles have formed. The result of the synthesis in the form of colloidal silver nanoparticles at a concentration of 50 ppm was added to the adhesive primer (Transbond XT, 3M, USA) with a micropipette and transferred to an Eppendorf tube.

The colloidal silver nanoparticle and Transbond XT primer mixture were vortexed for about two minutes until homogeneous, then stored in a heatproof and lightproof place (Reddy et al., 2016). Then, the mixture was viewed using a transmission electron microscope (TEM) to visualize the dispersion and size of the silver nanoparticles in the adhesive primer. Silver nanoparticles synthesized from betel leaf extract (SNBL) were incorporated into the orthodontic adhesive primer to form a nano-adhesive. The nano-adhesive was prepared in three formulations as follows: 1% SNBL (BL1), which was 0.99 mL Transbond XT primer plus 0.01 colloidal silver nanoparticles equal to 0.5 µg silver nanoparticles; 3% SNBL (BL3), which was 0.97 Transbond XT primer plus 0.03 mL colloidal silver nanoparticles equal to 1.5 µg silver nanoparticles; 5% SNBL (BL5), which was 0.95 mL Transbond XT primer plus 0.05 colloidal silver nanoparticles equal to 2.5 µg silver nanoparticles.

Bonding brackets

Forty sound maxillary first bicuspids, recently extracted for orthodontic reasons, with no defects, intact enamel surfaces, caries-free, and devoid of any restorations, were chosen. The teeth were cleaned of soft tissue and remaining blood with running water and then sterilized using 0.5% chlorine for one week at room temperature, which was replaced once for two days for decontamination. Self-cured acrylic was stirred and cut into pieces of aluminum blocks measuring 20 × 10 × 10 mm. Each tooth was implanted into acrylic before hardening to the cervical level vertically for the tensile strength test. All teeth were divided into four groups randomly (n = 10) as follows: the negative control (NC) and the treatment groups (1% SNBL (BL1); 3% SNBL (BL3); 5% SNBL (BL5).

Before bracket adhesion, the prophylaxis procedure was performed on the tooth's buccal area, followed by etching. The bracket was held by a bracket holder. Afterward, a layer of primer with silver nanoparticle concentration (as much as 0.1 mL) was smeared once using a microbrush on the buccal area in each group. Transbond XT composite resin was applied on the bracket base and then placed right in the middle of the tooth buccal surface under 300 g of compressive strength for five seconds, measured by the tension gauge. Any excess adhesive was cleaned using a dental probe, followed by light curing two mm away from the bracket on each occlusal, cervical, mesial, and distal side for 10 seconds. All specimens were stored in distilled water at 37°C for 24 hours following bonding before TBS analysis.

Tensile bond strength analysis

Bracket TBS was analyzed using the Universal Testing Machine (Pearson Panke Equipment, London) with a 50 kg load cell. A mounting jig aligned the bracket base parallel to the acrylic mold and perpendicular to the force during the TBS test (Figure 1).

Debonding was done by moving the machine at a speed of 0.5 mm/s from the bracket ligature to the machine's head until the bracket came off. The tensile strength measured was the maximum strength used when the bracket detached from the tooth surface.

The data obtained were converted to mega-pascals (MPa) by dividing the debonding force (in Newtons) by the surface area of bracket mesh (10.64 mm2).

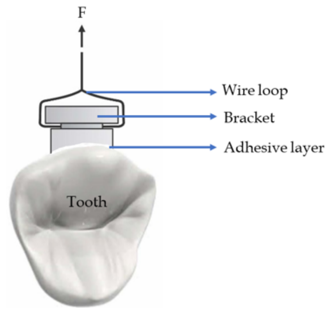

Figure 1. An illustration of the test construction for determining tensile bond strength.

Adhesive remnant index observation

The adhesive remnant index (ARI) test was conducted to determine the amount of residual adhesive. This test was done by examining the tooth surface under a stereoscopic microscope (Nikon, SMZ-2T, Japan) with 10x magnification connected to a digital camera. The ARI scores were categorized according to the classification of Artun and Bergland as follows: a score of 0 if no adhesive attached to the tooth surface; a score of 1 if less than half of the adhesive attached to the tooth surface; a score of 2 if more than half of the adhesive attached to the tooth; and a score of 3 if all the adhesive attached to the tooth surface.[13] Measurements were performed by two qualified examiners who were blinded to the samples. The examiners' analysis showed a high intra- and inter-examiner agreement and reliability (Kappa index value 0.83).

Statistical analysis

All data were analyzed utilizing the statistical package for the social sciences software (version 24.0, Chicago, IL). First, all data were subjected to a test of normality and homogeneity. A one-way ANOVA was used to determine the significance of the TBS value between the groups. If there was a significant difference, then the Tukey post hoc test was used. Data tabulation of the ARI test was analyzed using the Kruskal-Wallis test. $P < 0.05$ was considered statistically significant.

Results

Visualization of silver nanoparticle dispersion and size in adhesive primer

The morphology, dispersion, and size of AgNPs were determined using high-resolution TEM. A representative TEM image showed that nanoparticles had a spherical shape and the average diameter of biosynthesized AgNPs was less than 65 nm (Figure 2). The observed nanoparticles in the TEM image were in the range of 20.88 nm to 40.62 nm in diameter for the BL1 group, 20.92 nm to 40.81 nm in diameter for the BL3 group, and 25.31 nm to 64.76 nm in diameter for the BL5 group. Increased concentrations of silver nanoparticles showed a higher agglomeration tendency because silver nanoparticles accumulate and form clusters, as seen in the BL3 and BL5 groups.

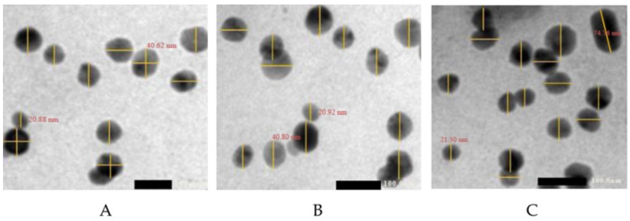

Figure 2. A TEM image showing the formation of nanosized particles in each group after incorporating colloidal silver nanoparticles into the orthodontic adhesive primer, A indicates the BL1 group, B indicates the BL3 group, and C indicates the BL5 group.

TBS and ARI values

The TBS values of the four groups are shown in Table 1 [Mean (standard deviation)]. These descriptive statistics indicate the variation in TBS between the four groups with the maximum TBS value in the NC group [11.13 (1.57) MPa]. However, groups treated with SNBL 5% exhibited the lowest TBS value compared with other groups [5.92 (0.96) MPa]. The normality test of the four groups showed that the values were normally distributed (*P* > 0.05). Data homogeneity was then tested using Levene's test, which showed that the tested variants were homogeneous (*P* > 0.05), suggesting that further analysis can be continued using the one-way ANOVA. The results shown in Table 1 reveal that there was a significant difference in the TBS between the four groups (*P* < 0.05). The significance of various silver nanoparticle concentrations' effect between groups was determined by a post hoc Tukey honestly significant difference LSD test. The Tukey test showed that the TBS of the NC group was significantly higher than the other groups. Furthermore, there was a significant difference in the TBS value between the BL5 and the other groups (*P* < 0.05). In contrast, no significant difference in the TBS value was found between the NC group and the BL1 and BL3 groups (*P* > 0.05) (Table 1).

Group	n	TBS (MPa)	Sig*	*p*-value		
				BL1	BL3	BL5
NC	10	**11.13 ± 1.57**	*p* = 0.041*	0.136	0.095	0.001*
BL1	10	**10.86 ± 1.49**			0.083	0.001*
BL3	10	**10.77 ± 1.67**				0.001*
BL5	10	**5.92 ± 0.96**				

Data from four independent determinations are expressed as mean ± SD (n=10).
*by one-way ANOVA followed by Tukey's test, significant differences between groups (p < 0.05).
NC: negative control group; BL1: 1% nanoparticles synthesized from betel leaf extract; BL3: 3% nanoparticles synthesized from betel leaf extract; BL5: 5% nanoparticles synthesized from betel leaf extract.
ANOVA, Analysis of variance; TBS, tensile bond strength.

Table 1. Results of the one-way ANOVA comparing the TBS between the four groups tested.

The ARI scores for all groups are listed in Table 2. The results of the Kruskal-Wallis test showed no significant differences between the groups (P = 0.055). Orthodontics bracket failure located in the NC, BL1, and BL3 groups was dominated by an ARI score of 2, representing bond failure between adhesive-bracket interfaces. In contrast, those in the BL5 group were dominated by a score of 0, indicating bond failure between the adhesive-enamel surface.

Group	ARI values								Kruskal-Wallis
	0		1		2		3		
	n	%	n	%	n	%	N	%	*P*-value
NC	0	(0)	2	(20)	5	(50	3	(30)	0.055
BL1	0	(0)	2	(20)	6	(60	2	(20)	
BL3	1	(10)	2	(20)	5	(50)	2	(20)	
BL5	5	(50)	3	(30)	1	(10)	1	(10)	

Data from four independent determinations are presented as numbers (%).
*by Kruskal-Wallis, significant differences between groups (*P* < 0.05).

ARI scores: 0, no adhesive on the tooth; 1 = less than half of the adhesive left on the tooth surface; 2 = half of the adhesive or more left on the tooth surface; 3 = all adhesive left on the tooth surface, failure between the adhesive and bracket base.
ARI, Adhesive remnant index; TBS, tensile bond strength; NC: negative control group; BL1: 1% nanoparticles synthesized from betel leaf extract; BL3: 3% nanoparticles synthesized from betel leaf extract; BL5: 5% nanoparticles synthesized from betel leaf extract.

Table 2. The adhesive remnant index (ARI) values on enamel tooth surfaces in the four groups tested.

Discussion

The incorporation of AgNPs green synthesis from betel leaf extract into the orthodontic material has revealed its promising potential regarding antimicrobial properties. Silver (Ag) has antiviral and antibacterial properties.[14] Resins containing NPs of silver were synthesized that have antibacterial characteristics. These properties are mainly due to the release of Ag^+ ions, which are more numerous when fine AgNPs are used (<10 nm particle size) for antibacterial action compared with larger Ag particles.[15] The results indicated that the average TBS value of the groups with added SNBL decreased with the increase in silver nanoparticle concentration. The highest average TBS value was seen in the NC group, whereas the lowest TBS score was observed in the 5% nanoparticle concentration group. Study findings indicated that the number of silver nanoparticles in the adhesive primer could reduce the bond strength of the bracket. The effect of silver nanoparticles on the adhesive primer, which decreased the TBS of the bracket, was suspected as being due to a defect in the resin matrix. This defect might be a result of silver nanoparticle agglomeration that produces larger particle formation. In accordance with the Salem et al.[16] study, the addition of silver nanoparticles to the orthodontic adhesive primer significantly reduces the strength of the material as the agglomeration of the silver nanoparticles in the primer affects flow-ability and creates a weak point that can inhibit the curing process of the adhesive. ANOVA results showed that variations in the concentration of SNBL in the orthodontic adhesive affected the TBS of the bracket. The addition of silver nanoparticle concentrations of 5% and 10% to the adhesive primer reduces the bond strength of the orthodontic bracket.[4] Incorporating more than 5% nanoparticles can inhibit the formation of organized polymer networks, leading to modified physical properties or reduced adhesive strength.[8]

The post hoc test revealed a significant difference in the TBS between the BL5 and the other groups. This difference indicated that the addition of silver nanoparticles at a concentration of 5% initiated a significant decrease in shear strength. In general, silver nanoparticles can agglomerate and progressively form large clusters, experience precipitation, and nano-scale deviation. This can interfere with the effectiveness and application of nanoparticles.[17] Efforts to prevent agglomeration of silver nanoparticles have been performed in this study by adding chitosan as a stabilizer during the nanoparticle synthesis process. According to Hettiarachchi and Wickramarachchi[18], silver nanoparticles in medical applications require additional steps to eliminate synthetic polymers, such as polyvinyl pyrrolidone, polyvinyl alcohol, and polyvinyl chloride, which lead to a complex and costly synthesis process. When the stabilizer is removed, there is a tendency for silver nanoparticles to become unstable and change particle size. Therefore, researchers use a chitosan stabilizer, such as a biopolymer, because it is a natural polysaccharide, environmentally friendly, biodegradable, easy to obtain, and does not require removal like synthetic polymers.

The silver nanoparticles used in this study are colloidal nanoparticles. Colloids are solid materials that are dispersed in the solution phase or water. Greater concentrations of silver nanoparticles in the adhesive primer in this study parallel with the water concentration from colloidal nanoparticles. Water plays a vital role in the degradation or erosion of composite materials due to its ability to diffuse into the material and trigger oxidation and hydrolysis degradation stages.[19] Water absorption in orthodontic composites occurs in the resin matrix. The dispersion of water can cause erosion of the unreacted monomer and filler particles in the composite material. This degradation process can reduce the mechanical strength of the material. The minimum recommended average bond strength value was 5.9–7.8 MPa, which is an adequate bond strength for most orthodontic needs during clinical use.[20] Although the results showed a trend toward a decrease in TBS, the bond strength value of the nano-adhesive used in the present study was to fulfill the minimum requirement. However, it was still expected to resist the stresses from orthodontic forces.

In contrast to restorative composite materials in conservative dentistry that are intended to be applied for an extended period, orthodontic adhesives must be easily removed at the end of treatment without damaging the tooth structure. The main goal of the debonding process is to remove the bracket and adhesive resin and restore the enamel surface to its pre-orthodontic treatment state.[21] During removal of the bracket, bond failure is likely to occur at the adhesive-enamel interface (adhesive failure), within the adhesive material (cohesive failure), or a combination of adhesive and cohesive failure (mixed failure).[22] The ARI observation results indicated variations of failure location in each group. Even so, this variation does not have a significant difference. Orthodontic bracket failure located in the NC, BL1, and BL3 groups was dominated by an ARI score of 2, representing bond failure between adhesive-bracket interfaces. In contrast, those in the BL5 group were dominated by a score of 0, indicating bond failure between the adhesive-enamel surface.

Currently, there are debates in the literature regarding the ideal location of attachment failure. The less residual adhesive material on the teeth after the debonding process decreases the cleaning time required for the orthodontist. A low ARI score is beneficial in this situation.[23] However, when a bond failure occurs between the adhesive and the enamel surface, there is always partial enamel loss due to the micromechanical bond between the adhesive and the enamel surface.[24] The adhesion failure between the bracket adhesive (mixed) or within the adhesive (cohesive failure) indicates less chance of enamel damage during debonding. The higher the frequency of remaining adhesive on the tooth surface may indicate that the enamel is protected during the debonding process.[25] This finding is supported by the findings of Scribante et al.[26], who reported that a score of “0” on the ARI test was associated with the low shear force and was often connected with contaminants in enamel that can reduce the bond strength. However, a score of “3” on the ARI test represented a slight risk of enamel fracture during the debonding procedure but prolonged the polishing process. It is expected that adhesive orthodontic materials meet the characteristics of mixed adhesion (ARI scores of “1” and “2”).

Conclusions

The addition of silver nanoparticles from the green betel leaf biosynthesis at adhesive orthodontic materials above 3% concentration decreases the TBS of orthodontic bracket attachment but was still in the recommended adequate bond strength range value of 5.9–7.8 MPa. It is necessary to perform further evaluations investigating the addition of silver nanoparticles from green betel leaf biosynthesis on orthodontic adhesive using a concentration between 0% and 3% for a longer period to determine the stability of silver nanoparticles in the orthodontic adhesive.

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Declaration of Interest

All authors have none to declare

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