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Original Article

Antibacterial activity and shear bond strength of fiber-reinforced composites and bonding agents containing 0.5%, 1%, 2.5%, and 5% silver nanoparticles

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ABSTRACT

Background: Bonded composites may increase bacterial accumulation and caries formation risk. Therefore, assessment of methods to decrease bacterial activity around them would be valuable. The literature on the efficacy of adding silver nanoparticles to fiber-reinforced composite (FRC) or adding them to bonding agents in terms of their antibacterial activity and/or shear bond strength (SBS) is scarce. Thus, we aimed to assess the antibacterial activity of flowable composites and bonding agents containing various percentages of experimental silver nanoparticles (nanosilver) against *S. mutans* and to evaluate the SBS of FRC and bonding agents containing different amounts of nanosilver to enamel.

Materials and Methods: In this preliminary study, 0% (control), 0.5%, 1%, 2.5%, and 5% nanosilver were added to flowable composite and bonding agent. Syntheses of nanosilver and nanosilver-incorporated composite specimens were approved using X-ray diffraction spectroscopy and scanning electron microscopy. Antibacterial effects of the produced materials on *S. mutans* were evaluated by colony count with serial dilution method (n = 7 groups × 10 [n = 70] specimens) and agar disc diffusion test (n = 6 groups × 5 [n = 30] composite specimens + n = 6 groups × 5 [n = 30] light-cured bonding + n = 6 groups × 5 [n = 30] uncured bonding) against negative control and cefotaxime antibiotic. Moreover, SBS values of various FRC blocks bonded to enamel using various bonding agents were measured (n = 9 groups × 6 [n = 54] human premolars). Data were analyzed using Kruskal–Wallis, Dunn, two-way analysis of variance, and Tukey's tests (α = 0.05).

Results: Composite discs containing all concentrations of nanosilver reduced *S. mutans* colony counts (P < 0.05); bacterial growth was ceased at samples containing 2.5% and 5% of nanosilver. The reduction in the SBS of FRCs was significant only for 5% nanosilver (P < 0.05).

Conclusion: Adding 0.5%, 1%, and 2.5% nanosilver to composite and 0.5% or 1% nanosilver to bonding agent led to a significant antibacterial behavior against *S. mutans* while not significantly affecting the SBS of FRC.

Key Words: Antibacterial efficacy, bonding agents, dental materials, fiber-reinforced composite, flowable composite, nanoparticles, orthodontics, *Streptococcus mutans*

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INTRODUCTION

Fiber-reinforced composites (FRCs) can be used for numerous applications such as orthodontic fixed retainers, splinting teeth without the need for leveling and aligning for certain orthodontic movements in the active phase of the treatment, or splinting the teeth after trauma, or maintaining the space of missing teeth until proper prosthodontic restoration.[1-5] Flowable resin composite (FC) is another important adhesive that has numerous uses such as attaching fixed retainers to the lingual enamel of the teeth.

Composites bonded to the teeth may cause difficulty in oral hygiene control.[6-8] Therefore, better materials and/or methods are warranted to minimize microbial growth around composites and bonding agents.

Various antimicrobial agents have been added to different dental materials to improve plaque control.[9-18] Metal nanoparticles are known as antibacterial agents that can be more effective than metal particles, due to their higher effective surface areas.[14] Titanium, zinc, silver, and gold nanoparticles are among the antibacterial particles that have been recently introduced to dentistry.[9-14,18-22] Among these, silver has shown the highest antimicrobial effect with relatively lower doses.[23,24] Silver nanoparticles have been added to dental materials and showed to be effective in reducing *Streptococcus mutans*, the main oral cariogenic bacteria.[23-26]

Adding sliver nanoparticles ("Ag nanoparticles" or AgNPs) to clinically used materials will be useful only if it does not compromise their mechanical properties. There are only a few controversial studies assessing the antibacterial effects of adhesive containing silver nanoparticles and their shear bond strength (SBS),[26,27] and they are about SBS of brackets and not FRC. Degrazia *et al*.[26] showed that adding 11%, 18%, and 33% of weight (wt%) AgNP to orthodontic adhesive results in a significant reduction of *S. mutans in vitro*; the SBS of their brackets decreased significantly with adding AgNP in all three concentrations, yet the remaining extent of SBS seemed sufficient to bond brackets successfully compared to the clinically proper values suggested by Reynolds.[26,28] However, the other study did not show a significant difference between SBS of control versus brackets bonded with nanosilver-incorporated resin.[27]

For a bonded composite, bond strength and low caries formation risk are both important. Therefore, assessment of ways to improve their anticaries properties when maintaining their proper SBS would be of clinical importance. Therefore, the purpose of this study was to investigate the antibacterial activity and SBS of AgNP-incorporated FRCs and bonding agents for the first time.

MATERIALS AND METHODS

This *in vitro* explorative experimental study had three independent phases: nanosilver synthesis, antimicrobial assessment, and SBS measuring. The study and its ethics were approved by the research committee of the university.

Phase I: Synthesis of nanosilver

Synthesis of AgNP was performed according to the method used by Zhou and Wang.[29] In order to synthesize silver nanoparticles, aqueous solution containing silver nitrate (Merk, Frankfurt, Germany) was first prepared and heated to the boiling point. Then, as soon as the aqueous solution containing silver nitrate boiled, the aqueous solution containing sodium citrate (Merk) was added gradually. As the sodium citrate solution was being added, it slowly turned to a yellowish gray. This color conversion represents the reduction of silver nitrate and the production of silver. The solution was then cooled to room temperature and then placed at 100°C to evaporate its water. Scanning electron microscopy (SEM) (Philips, Eindhoven, the Netherlands) and X-ray diffraction (XRD) spectroscopy (Philips, Eindhoven, the Netherlands) were used to characterize the synthesized nanoparticles. The size of silver nanoparticles in SEM image was measured using ImageJ software (National Institutes of Health, Bethesda, MD, United States). The XRD spectra showed that the particles produced were silver of high purity; in the XRD pattern, all the peaks belong to silver and no impurity peaks were observed [Figure 1]. The SEM image shows that the average particle size of the silver was 17.145 nm in the agglomerate state [Figure 2].

Preparation of flowable composite and Ag nanoparticles-incorporated bonding agent

A digital scale was used to weigh materials at 0.00001 g (Adam Equipment Co., Milton Keynes, UK). The synthetized AgNP was added to flowable composite (Master-Dent, Dentonics Inc., Monroe, NC, USA) and bonding agent (Universal Bonding, Dentonics Inc., Monroe, NC, USA) with 0.5, 1, 2.5, and 5 weight percentage (wt%) and mixed with

magnetic stirrer (Heidolph Instruments GmbH& Co., Schwabach, Germany) with 200 rpm for 20 min. The experimental composite and bonding agent were stored in sterile and light-protective containers.

Preparation of composite discs

To minimize the microbial contamination, the instruments were sterilized and the surfaces were disinfected. A custom-made cylindrical polytetrafluoroethylene mold [Figure 3] with dimensions close to antibiotic discs was used to form the composite discs. Prepared flowable composites with different concentrations of AgNP were injected into the mold. The composite was then poured with different percentages of nanoparticles into Teflon mold made of polyether tetra fluorine with inner diameter of 6 mm and height of 1.5 mm [Figure 3]. Using a glass slab, material excesses were removed and a uniform surface was prepared. The experimental composite in the mold was light-cured (LED.D, Woodpecker, Beijing, China) for 60 s at an intensity of 1700 mW/cm^2 from each side and then ejected from the mold. Twenty discs were made of each concentration of AgNP in the composite. A total of 100 discs were prepared and sterilized by autoclaving at 121°C and 15 psi for 30 min. To assess the distribution of AgNP in the cured composite, a SEM image was taken from a randomly selected disc of each concentration. Provided images confirmed relatively uniform distribution of nanoparticles [Figure 4].

Preparation of bonding agent containing nanosilver

Using a digital scale, silver nanoparticles were added to the bonding agent at 0.5, 1, 2.5, and 5 wt% and mixed using a magnetic stirrer for 20 min. It was then transferred to small sterile containers. The outer surfaces of the containers were carefully covered to prevent light exposure.

Phase II: Antibacterial tests

The antimicrobial property assessment methods used in this study are based on the standard methods published by the Clinical and Laboratory Standards Institutes (CLSI) published in 2017.[30]

Colony count with serial dilution method

A bacterial suspension equivalent to 0.5 McFarland standard (contained 1.5 × 10^8 CFU/ml bacteria) was obtained from a 24-h pure culture of *S. mutans* (Persian Type Culture Collection [PTCC] 1683). The bacterial suspension was then diluted 1:100 using normal saline to contain 1.5 × 10^6 CFU/ml bacteria. Seventy

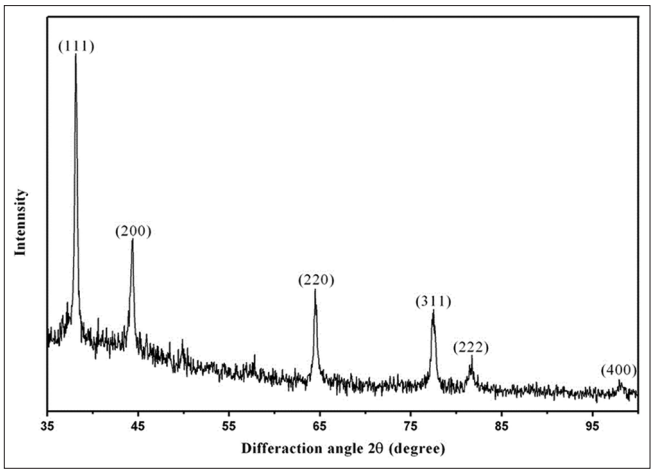

Figure 1: The XRD pattern showing silver particles of high purity. XRD: X-ray diffraction.

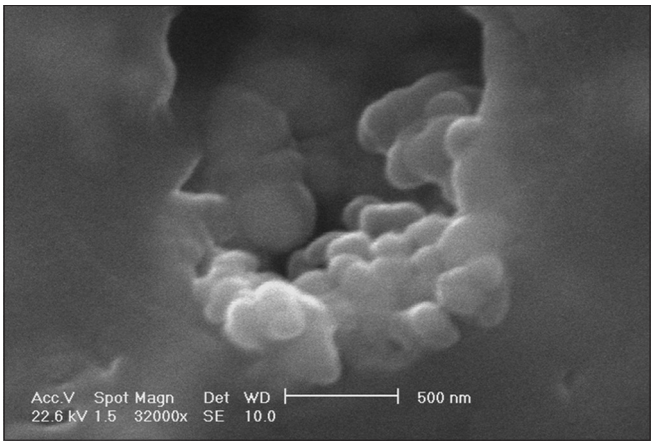

Figure 2: The SEM image showing an average particle size of 17.145 nm in the agglomerate state. SEM: Scanning electron microscopy.

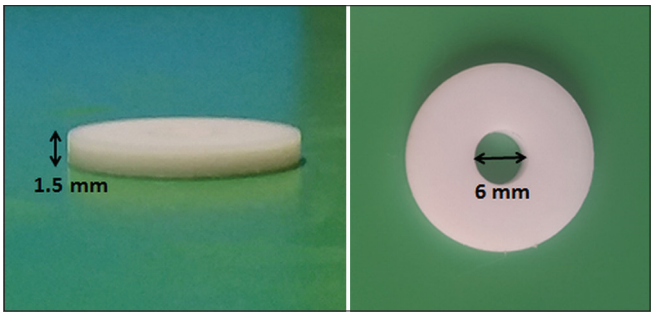

Figure 3: The custom-made cylindrical polytetrafluoroethylene mold.

sterile test tubes were divided into 7 groups of ten. Then, 950 µL brain–heart infusion broth (BHI, Pronadisa, Madrid, Spain) and 50 µL of the diluted bacterial suspension were added to all the tubes using a sampler (Cleaver Scientific, Warwickshire, United Kingdom) and disposable sterile heads (Pol

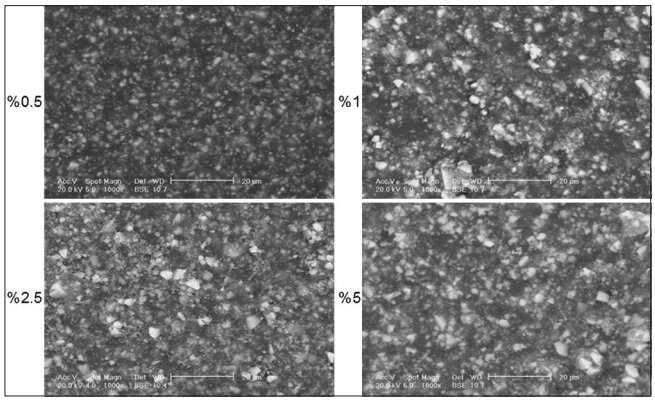

Figure 4: Composite discs at × 1000 confirming relatively uniform distribution of nanosilver.

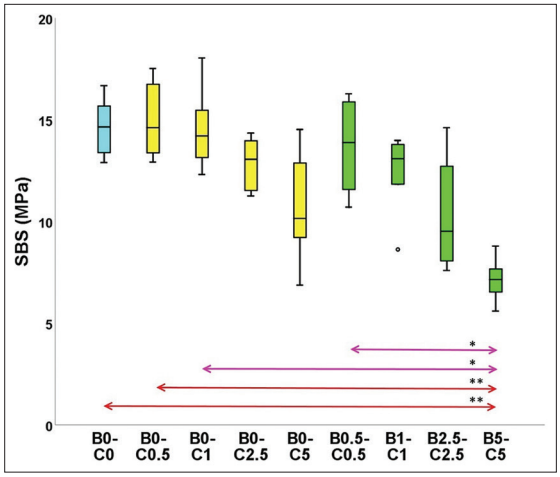

Figure 5: Box plots showing descriptive SBS statistics (MPa). Significant Dunn pairwise comparisons are marked with double-arrows: *$P < 0.05$; **$P < 0.01$. SBS: Shear bond strength; MPa: Mega Pascal.

Ideal Pars, Tehran, Iran). Hence, each tube contained approximately 5 × 10^5 CFU/ml bacteria.

Composite discs with 0, 0.5, 1, 2.5, and 5 wt% were inserted, respectively, into tubes pertaining to groups 1–5; in group 6 (positive control), instead of composite discs, cefotaxime was added to tubes to attain 4 µg/ml of antibiotic in each tube. In group 7 (negative control), neither composite disc nor antibiotic was added. Hence, the tubes in this group were contained only bacteria and BHI [Table 1]. The role of group 7 as the negative control was to evaluate the viability and growth of the bacteria and to confirm the intended concentration of the bacteria in the test tubes.

All tubes except for the tubes in group 7 (negative control) were incubated at 35°C and 5% CO_2 atmosphere for 24 h). In the case of group 7, 10 µL

Table 1: Contents of each group in serial dilution test

Group	*n*	Contents
1	10	5×10^5 CFU/ml bacteria + composite disc with 0 wt% AgNP
2	10	5×10^5 CFU/ml bacteria + composite disc with 0.5 wt% AgNP
3	10	5×10^5 CFU/ml bacteria + composite disc with 1 wt% AgNP
4	10	5×10^5 CFU/ml bacteria + composite disc with 2.5 wt% AgNP
5	10	5×10^5 CFU/ml bacteria + composite disc with 5 wt% AgNP
6 (positive control)	10	5×10^5 CFU/ml bacteria + 4 µg/ml of cefotaxime
7 (negative control)	10	5×10^5 CFU/ml bacteria

AgNP: Ag nanoparticles; wt%: Weight percentage

of the content of tubes was immediately (without incubation) cultured on blood agar and incubated simultaneously with tubes in group 1–6 under the same conditions of incubation.

After incubation, 10 µL of contents of each tube in group 1–6 was cultured on blood agar plates (Becton, Dickinson and Company [BD], Franklin Lakes, USA). All blood agar plates were incubated for 24 h at 35°C and 5% CO_2 atmosphere. Afterward, colonies formed on each plate were counted and multiplied by 100 to estimate the bacterial concentration in each tube.

Agar disc diffusion test

This test was performed to check whether in the absence of any fluid solvents, nanosilver can leak from the composite or bonding agent into the solid medium. First, a 24-h culture of *S. mutans* PTCC 1683 (PTCC, Tehran, Iran) was prepared using a half-McFarland suspension. Afterward, a sterile cotton swab was immersed in the prepared suspension. The swab was then removed from the suspension and the excess swab moisture was collected by pressing the swab into the inner wall of the tube.

The microbial suspension was cultured (lawn method) on the surface of Müeller–Hinton Agar (Becton, Dickinson and Company [BD], Franklin Lakes, USA) to which 5% sheep blood (Darwash, Tehran, Iran) was added. The plate was then rotated 60°, and this was repeated again. The swab was then rotated around the plate. The culture remained for 15 min to dry on the plate.

Finally, composite discs with different concentrations of nanoparticles (one disc per the 0, 0.5, 1, 2.5, and 5 wt% concentrations on each plate) were placed on the

plate with a forceps to ensure the contact of the entire surface of the disc on agar. In addition to composite discs, a number of antibiotic cefotaxime discs were also placed on each plate as positive control. The minimum distance from the center of one disc to the center of the other disc was 24 mm according to the CLSI-2017 standard.

Five plates were prepared as described above and incubated at 35°C for 48 h. Then, the diameter of the bacterial growth inhibition zone around the discs was evaluated under reflective light.

In accordance with the method described above, the bacterial growth inhibition halo measurement was repeated with 6 mm diameter sterile paper discs coated with bonding agents containing different percentages of silver nanoparticles (0, 0.5, 1, 2.5, and 5 wt%). Coating was done using a sampler; different sizes of sampler heads were tested to make sure that paper discs become wetted completely but not over- or underwetted. Two different groups of bonding agents were tested: in the first group, the bonding agent was light-cured to test if the light-cured agent allows the propagation of nanosilver into the agar medium or not. In the second group, to facilitate propagation of the bonding agent to the culture medium, paper discs were not light-cured. A paper disc coated with the antibiotic cefotaxime was placed on each plate as the positive control. The minimum distance from the center of one paper to the center of the other paper was 24 mm. Five plates were prepared according to the above method and incubated at 35°C for 48 h. The diameter of the bacterial growth inhibition halo around the paper discs was evaluated under the reflected light.

Phase III: Shear bond strength of fiber-reinforced composites containing silver nanoparticles

Preparation of fiber-reinforced specimens and human premolars

The ethics of this study were approved by the ethics committee of the university. The teeth collected for the study had been extracted solely for the purpose of treatments. Of intact premolars without decay extracted due to orthodontic treatment plan, kept at room temperature in distilled water (0.1 wt/vol) at room temperature, 54 teeth without enamel hypoplasia, enamel crack, or any enamel defects on the buccal surface were selected. The teeth were carefully cleaned using an ultrasonic scaler (Woodpecker, China) from soft tissue debris and then randomly divided into 9 groups of 6 each.

The buccal surfaces of all teeth were etched with 37% phosphoric acid (Dentonics, Inc., Monroe, NC, USA) for 20 s and then rinsed with water for 20 s and finally dried with oil-free air until appearance of the dry, matt enamel. The bonding agents with different concentrations of silver nanoparticles [Table 2] were applied to the dried enamel surface using a microbrush and were gently dispersed by an oil-free air pressure and then light-cured for 20 s. Polyethylene fiber (BioDental Technologies Pty Ltd, Macksville, Australia) 3 mm thick was first immersed into the bonding agent and formed. The fibers used in this study were continuous unidirectional type with which FRC was stronger than other fiber types.[31]

Five millimeters of resin-soaked fiber was placed on the buccal surface of the prepared premolar teeth and light-cured for 20 s, so that the surface of the fiber-tooth contact was 5 mm long and 3 mm wide. The fibers were then coated with a flowable composite in each group in a way that they did not exceed the fiber surface. They were then light-cured again for 40 s.

To obtain a larger volume of composite-reinforced fibers to calculate the SBS, two additional layers of fibers were placed on the previous layer by the said process. Bonding agent and flowable composite with different percentages of nanoparticles in accordance with Table 1 were used for preparing samples in each group. At each stage, the distance between the light-curing device and the specimen was not more than 2 mm. The specimens were mounted in a self-setting polymethyl methacrylate (TDV dental, Santa Catarina, Brazil) inside a cylindrical mold with a diameter of 1.7 cm and a height of 6 cm. After complete setting of acrylic resin, the samples were taken out and stored in distilled water for 24 h.

Shear bond strength assessment

After 24 h, the samples were removed from distilled water and dried. They were then mounted on the

Table 2: Concentrations (%) of nanosilver in the bonding agent and composite

Group	*n*	Bonding agent	Composite
1	6	No nanosilver	No nanoparticles
2	6	No nanosilver	0.5% nanosilver
3	6	No nanosilver	1% nanosilver
4	6	No nanosilver	2.5% nanosilver
5	6	No nanosilver	5% nanosilver
6	6	0.5% nanosilver	0.5% nanosilver
7	6	1% nanosilver	1% nanosilver
8	6	2.5% nanosilver	2.5% nanosilver
9	6	5% nanosilver	5% nanosilver

jig of the Universal Electromechanical Testing Machine (Walter + Bai, Löhningen, Switzerland) in a way that the blade exerted the force into FRC-tooth junction. The shear force was divided by the surface area to calculate the SBS in mega Pascal (MPa).

Statistical analysis

The raw data and descriptive statistics were presented. The KolmogorovSmirnov test showed that data pertaining to antimicrobial assessments were nonnormal. Therefore, nonparametric KruskalWallis and Dunn *post hoc* tests were used for their analysis. The data pertaining to the SBS measurements were normally distributed and further investigations showed that SBS residuals as well follow a normal distribution; therefore, two-way analysis of variance (ANOVA) and Tukey's *post hoc* tests were used to assess the effect of incorporation of nanosilver particles in bonding agent and flowable composite, separately. Since SBS subgroups were small (n of each of the 9 subgroups = 6), nonparametric KruskalWallis and Dunn *post hoc* tests were used to compare the 9 SBS subgroups with each other. The level of significance was set at 0.05.

RESULTS

Agar disc diffusion test

By assessing composite discs coated with silver nanoparticles, no bacterial growth inhibition halo was observed around any of the discs except for the antibiotic at the center. Examination of paper discs wetted by the bonding agent with different percentages of silver nanoparticles showed bacterial growth inhibition halos around all paper discs.

Colony count with serial dilution method

In the experimental group 1 (0 wt% AgNP), after overnight incubation, heavy turbidity was seen which was representative of progressive bacterial growth. Hence, linear culture of this group was not performed on blood agar. The mean colony counts were 55,000 and 12,500, respectively, in the groups 2 and 3 [0.5% and 1% AgNP, respectively, Table 3]. In the positive control group (group 6 containing antibiotic) as well as in the experimental groups 4 and 5 (2.5% and 5% nanosilver, respectively), no colonies were observed on the plates after overnight incubation [Table 3]. In the negative control group (group 7), all plates showed approximately 500,000 colonies, which confirmed the health of used bacteria and proper dilution method. The negative control was excluded from statistical analyses because its role was not quantitative.

Table 3: The raw data pertaining to colony counts (unitless) as well as descriptive statistics

Plate	Negative control	Experimental: Silver nanoparticles					Antibiotic
		0%	0.5%	1%	2.5%	5%	
1	500,000	500,000	50,000	10,000	0	0	0
2	500,000	500,000	65,000	25,000	0	0	0
3	500,000	500,000	75,000	10,000	0	0	0
4	500,000	500,000	50,000	15,000	0	0	0
5	500,000	500,000	55,000	15,000	0	0	0
6	500,000	500,000	35,000	10,000	0	0	0
7	500,000	500,000	80,000	5000	0	0	0
8	500,000	500,000	55,000	10,000	0	0	0
9	500,000	500,000	65,000	10,000	0	0	0
10	500,000	500,000	20,000	15,000	0	0	0
Mean	500,000	500,000	55,000	12,500	0	0	0
SD	0	0	17,950.6	5400.6	0	0	0
Median	500,000	500,000	55,000	10,000	0	0	0

SD: Standard deviation

The KruskalWallis test was used to compare the experimental groups and the positive control. It showed a significant difference among the groups ($P < 0.0001$). The Dunn *post hoc* test indicated significant differences between negative control or 0.5% nanosilver with each of the groups '2.5% nanosilver, 5% nanosilver, and antibiotic' [Table 4]. The difference between the group 1% nanosilver and the groups 2.5%, 5%, and antibiotic was not significant [Table 4].

Shear bond strength results

The results indicated that by the addition of silver nanoparticles, the median bond strength could reduce [Table 5 and Figure 5]. The KruskalWallis test indicated that there was a significant overall difference among the 9 subgroups ($P = 0.0003$). The Dunn *post hoc* test showed that there was no significant difference between the control group (no nanosilver) with any of the subgroups (all the 8 $P > 0.1$) except with the 9th subgroup which had 5% nanosilver in both the bonding agent and compote. According to the Dunn test, most other pairwise comparisons were insignificant as well ($P > 0.05$); the only significant pairwise comparisons were between the following subgroups: between control (bonding agent without nanosilver + composite without nanosilver) versus the 9th group (bonding with 5% nanosilver + composite with 5% nanosilver [$P < 0.01$]), between Bonding agent without nanosilver + Composite with 0.5% nanosilver vs Bonding agent with 5% nanosilver + Composite

with 5% nanosilver ($P < 0.01$), between Bonding agent without nanosilver + Composite with 1% nanosilver versus Bonding agent with 5% nanosilver + Composite with 5% nanosilver ($P < 0.05$), and between Bonding agent with 0.5% nanosilver + Composite with 0.5% nanosilver versus Bonding agent with 5% nanosilver + Composite with 5% nanosilver [$P < 0.05$, Figure 5].

Assessing the effects of the incorporation of nanosilver into bonding agent and composite, the two-way ANOVA showed significant effects for the incorporation of nanosilver into the bonding agent ($P = 0.004$) and composite ($P = 0.002$). Out of the 10 pairwise comparisons between different percentages of nanosilver within the bonding agent, 5 were significant [Table 6]. Of the pairwise comparisons between composites with different amounts of nanosilver, 6 were significant [Table 7].

Table 4: The results of the Dunn *post hoc* test

Compared groups	Difference in rank sum (unitless)	*P*
Negative control versus 0.5%	10.10	>0.05
Negative control versus 1%	19.90	>0.05
Negative control versus 2.5%	40.00	<0.0005
Negative control versus 5%	40.00	<0.0005
Negative control versus antibiotic	40.00	<0.0005
0.5% versus 1%	9.800	>0.05
0.5% versus 2.5%	29.90	<0.0005
0.5% versus 5%	29.90	<0.0005
0.5% versus antibiotic	29.90	<0.0005
1% versus 2.5%	20.10	>0.05
1% versus 5%	20.10	>0.05
1% versus antibiotic	20.10	>0.05
2.5% versus 5%	0.0000	>0.05
2.5% versus antibiotic	0.0000	>0.05
5% versus antibiotic	0.0000	>0.05

DISCUSSION

Our findings regarding the efficacy of nanosilver were similar to those of Degrazia *et al.*,[26] who showed that 0.11, 0.18, and 0.33 wt% nanosilver added to orthodontic composite could significantly reduce *S. mutans*. However, in their study, there was no significant difference between bactericidal efficacies of those three concentrations, and none of them was able to reduce bacteria count to zero.[26] In their study, each of nanosilver concentrations was able to reduce bacteria count about 2 logs. However, in our study, the 0.5% group was able to reduce bacterial count for only about 1 log, despite being more concentrated than nanosilver amounts used by Degrazia *et al*.[26] On the other hand, Sodagar *et al*.[32] who had used higher percentages of nanosilver in their study (1, 5, and 10 wt%) observed less reductions in colony count. This can be due in part to the size of nanoparticles. Degrazia *et al*.[26] used nanoparticles 25–100 nm in size, Sodagar *et al*.[32] used particles of 55–65 nm, and we used agglomerated particles of 117.45 nm size in average. Apart from the differences in the size of the silver nanoparticles, other factors differed between studies including the shape of the nanoparticles, the method of incorporating the silver nanoparticles into the composite, the dimensions of the composite discs, as well as the methods for evaluating the efficacy of nanoparticles. Despite differences in efficacy, all three studies unanimously showed that all percentages of nanosilver tested in these three studies can significantly decrease the number of *S. mutans* colonies. Even the addition of 0.025 and 0.05 wt% nanosilver to orthodontic composite has been shown to decrease *S. mutans* growth speed despite the smoother surface of control composite

Table 5: The raw data pertaining to all shear bond strength values (mega Pascal) as well as relevant descriptive statistics for each subgroup

Sample	B0 + C0	B0 + C0.5	B0 + C1	B0 + C2.5	B0 + C5	B0.5 + C0.5	B1 + C1	B2.5 + C2.5	B5 + C5
1	12.89	17.52	15.05	11.25	10.18	10.7	13.78	14.6	8.79
2	15.26	14.68	12.31	11.52	14.52	14.34	11.83	8.97	5.59
3	16.67	13.37	18.03	14.34	6.87	16.27	13.98	8.06	7.49
4	13.38	16.74	15.46	13.96	9.21	11.57	8.62	12.71	6.79
5	15.67	14.54	13.14	12.75	12.87	13.4	12.62	10.07	7.66
6	14.02	12.92	13.35	13.35	10.12	15.87	13.55	7.59	6.53
Mean	14.65	14.96	14.56	12.86	10.63	13.69	12.40	10.33	7.14
SD	1.46	1.83	2.08	1.27	2.71	2.25	2.02	2.77	1.10
Median	14.64	14.61	14.20	13.05	10.15	13.87	13.09	9.52	7.14

B0: Bonding agent without any nanosilver; B0.5: Bonding agent with 0.5 wt% nanosilver; B1: Bonding agent with 1 wt% nanosilver; B2.5: Bonding agent with 2.5 wt% nanosilver; C0: Composite without any nanosilver; C0.5: Composite with 0.5 wt% nanosilver; C1: Composite with 1 wt% nanosilver; C2.5: Composite with 2.5 wt% nanosilver; C5: Composite with 5 wt% nanosilver; SD: Standard deviation; wt%: Weight percentage

Table 6: Results of the Tukey's *post hoc* test, comparing the shear bond strength values (mega Pascal) of different percentages of nanosilver added to the bonding agent

Nanosilver	Diff (MPa)	*P*	95% CI
0%			
0.5%	−0.16	0.9998	−2.73-2.41
1%	1.13	0.7195	−1.43-3.70
2.5%	3.20	0.0080	0.63-5.77
5%	6.39	<0.00005	3.82-8.96
0.5%			
1%	1.30	0.8007	−2.02-4.61
2.5%	3.36	0.0459	0.04-6.67
5%	6.55	<0.00005	3.23-9.87
1%			
2.5%	2.06	0.4046	−1.25-5.38
5%	5.26	0.0004	1.94-8.57
2.5%			
5%	3.19	0.0644	−0.12-6.51

Diff: Difference between the SBS values (MPa) of nanosilver percentages; CI for the mean difference. CI: Confidence interval; SBS: Shear bond strength; MPa: Mega Pascal

Table 7: Results of the Tukey's *post hoc* test, comparing the shear bond strength values (mega Pascal) of different percentages of nanosilver added to flowable composite

Nanosilver	Diff (MPa)	*P*	95% CI
0%			
0.5%	0.32	0.9977	−2.55-3.19
1%	1.17	0.7742	−1.70-4.04
2.5%	3.05	0.0324	0.18-5.92
5%	5.76	<0.00005	2.89-8.64
0.5%			
1%	0.85	0.8402	−1.50-3.20
2.5%	2.73	0.0152	0.38-5.07
5%	5.44	<0.00005	3.10-7.79
1%			
2.5%	1.88	0.1714	−0.47-4.22
5%	4.59	<0.00005	2.25-6.94
2.5%			
5%	2.71	0.0161	0.37-5.06

Diff: Difference between the SBS values (MPa) of nanosilver percentages; CI for the mean difference. CI: Confidence interval; SBS: Shear bond strength; MPa: Mega Pascal

without nanosilver.[27] In their study, particle sizes were about 5 nm[27] and smaller particles can be more bactericidal due to their higher surface-to-volume ratios.[33] Furthermore, smaller particles have a higher chance to penetrate prokaryote cells; however, in addition to enhancing antibacterial activity due to the ease of penetration of particles into prokaryotic cells, nanoparticles penetrate the eukaryotic cells more easily and thus increase the toxicity of the particles and become unfavorable for clinical use.[33-35] Despite these findings, we could not observe any inhibition halos around composite discs in agar diffusion test, which was in line with findings of Degrazia *et al*.[26] and Ahn *et al*.,[27] who similarly observed no inhibition halos around composite discs despite observing proper antimicrobial activity in fluid culture. The only study that reported inhibition zones around discs was done by Sodagar *et al*.,[32] who noted such inhibition halos only around composite discs incorporated with 5 and 10 wt% nanosilver and not 1% nanosilver. The diameter of inhibition zone depends on the amount of released material in the disc and its diffusion potential in a dry, solid environment.[36] Therefore, the results of this test in our study and earlier studies cannot be generalized to the oral environment with significant saliva flux that can be considered a fluid medium. To check the reason for failure of this test in inhibiting bacterial growth, we also used uncured bonding agent incorporated with nanosilver placed using paper discs on agar culture. It turned out that when nanosilver particles were not trapped within polymerized networks of cured composite, they were capable of inhibiting bacterial growth. Although this can be an advantage regarding antimicrobial activity, at the same time, this shows that if nanosilver particles are supposed to disrupt bacteria, they need to be released which this can make them potentially cytotoxic agents, probably with systemic harms. Therefore, studies are needed to be done on biocompatibility of this material.

Nanosilver release might negatively affect SBS. It was shown that 1 wt% nanosilver in either the bonding agent or composite retained the SBS more than other groups. Still, addition of nanosilver up to 2.5 wt% to composite and up to 1 wt% or even 2.5 wt% to the bonding agent provided SBS rates that were not significantly different compared to SBS of control (0% nanosilver) and also were above or at the acceptable range of 6–8 MPa.[26,28] This means that considering the results of antimicrobial tests, we can count on these concentrations as providing enough SBS rates as well as proper antimicrobial capacity. Since most caries happen at the enamel-adhesive interface,[37,38] adding nanosilver to the bonding agent can be of clinical use. No other studies had evaluated SBS of FRCs. However, SBS of orthodontic brackets has been examined when bonded using nanosilver-incorporated composite. A study showed a decrease in SBS compared to control, although all SBS rates with or without nanosilver (15.3–17.6

MPa) were above clinically acceptable range, i.e., 6–8 MPa.[26,28] In another study, the SBS was not decreased significantly by nanosilver incorporation, although in that study, the concentration of nanosilver was very low (0.025–0.05 wt%) and nanoparticles were very small (5 nm).[27] Most of the few available studies in this regard are about titanium oxide nanoparticles. Poosti *et al*.[39] observed proper antimicrobial activity after the addition of titanium oxide nanoparticles to the composite without serious reduction in the SBS of brackets.[39] Behnaz *et al*.[40] showed that the incorporation of titanium oxide can reduce bracket SBS, though their compromised SBS results were still quite high compared to the clinically acceptable values suggested by Reynolds.[28]

CONCLUSION

Within the limitations of this preliminary explorative study, it can be concluded that the incorporation of 0.5%, 1%, and 2.5% nanosilver to composite and 0.5% or 1% nanosilver to the bonding agent can considerably reduce bacterial growth when maintaining a proper SBS. Future biocompatibility and antibacterial studies are needed to optimize these concentrations.

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Conflicts of interest
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