Effect of Addition of Curcumin Nanoparticles on Antimicrobial Property and Shear Bond Strength of Orthodontic Composite to Bovine Enamel

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Abstract
Objectives: This study sought to assess the effect of curcumin nanoparticles (curcNPs) on antimicrobial property and shear bond strength (SBS) of orthodontic composite to bovine enamel.
Materials and Methods: In this *in vitro*, experimental study, 1%, 5% and 10% curcNPs were added to Transbond XT composite. Stainless steel brackets were bonded to 48 sound bovine incisors in four groups (n=12) using composite containing 0% (control), 1%, 5% and 10% curcNPs. The bracket-tooth SBS was measured by a universal testing machine. The adhesive remnant index (ARI) score was calculated after debonding using a stereomicroscope. Also, 180 discs were fabricated of the four composites; 108 were subjected to eluted component test, 36 were used for disc diffusion test and 36 were used for biofilm test to assess their antimicrobial activity against *Streptococcus mutans*, *Streptococcus sanguinis* and *Lactobacillus acidophilus*.
Results: The highest and lowest SBS belonged to control and 10% curcNP groups, respectively. The difference in SBS was significant among the four groups (P=0.008). The SBS of control group was significantly higher than that of 10% curcNPs (P=0.006). The four groups were not significantly different in terms of ARI score (P>0.05). Growth inhibition zones were not seen in any group. In biofilm test, the colony counts of all bacteria significantly decreased by an increase in percentage of curcNPs. Colony count significantly decreased only at 30 days.
Conclusions: At 1% concentration, curcNPs have significant antimicrobial activity against cariogenic bacteria with no adverse effect on SBS. However, insolubility of curcNPs remains a major drawback.
Keywords: Curcumin; Nanoparticles; Shear Strength; Composite Resins; Orthodontic Brackets; Anti-Bacterial Agents

INTRODUCTION

Bracket bonding has some drawbacks including low strength of bracket bond to tooth structure and subsequently high risk of debonding, high risk of plaque accumulation and consequent development of white spot lesions around brackets [1-4].

Prevention of enamel demineralization around orthodontic brackets is a major challenge in fixed orthodontic treatment [5]. Brackets and orthodontic appliances complicate oral hygiene and enhance the accumulation of microbial plaque [6]. Evidence shows that the population of cariogenic bacteria significantly increases during the course of orthodontic treatment [7-9]. Higher counts of mutans streptococci and lactobacilli have been reported in the oral cavity following placement of fixed orthodontic appliances [10]. At a pH of <5.5, these bacteria have anaerobic activity and produce organic acids, causing dental caries and white spot lesions [11]. These lesions are often irreversible and are a major concern for patients and orthodontists [12]. Thus, manufacturers have been in search of new
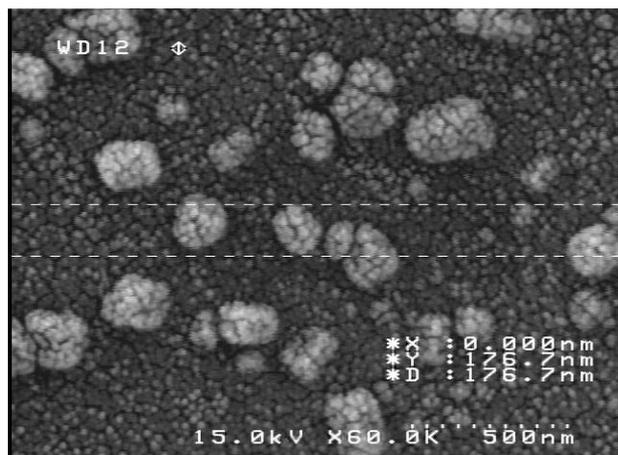
Fig. 1: Scanning electron microscopic micrograph of curcumin nanoparticles
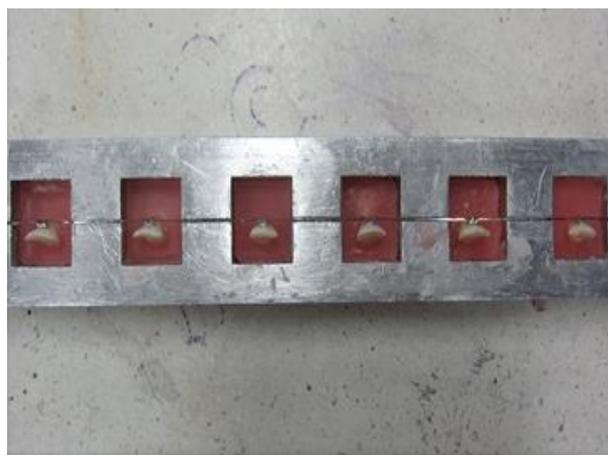
Fig. 2: Mounting of teeth in mold

techniques and materials with anti-caries properties to minimize the occurrence of white spot lesions [13].

Use of nanotechnology for the fabrication of composite resins with unique properties is among the most important achievements in dental material science [14]. It has been documented that nanoparticles have superior physical, chemical, mechanical and optical properties compared to microparticles and they have been used to manufacture dental materials with high mechanical properties and antimicrobial effects [15]. Curcumin is a yellow, active ingredient of turmeric, which is derived from the underground stems of Curcuma longa. It is used not only as a spice, but also as a medicinal herb for treatment of many conditions [16]. Curcumin inhibits the growth and proliferation of many bacterial strains such as staphylococci, lactobacilli and streptococci [17].

The antibacterial activity of curcumin is attributed to destruction of peptidoglycan cell wall of bacteria [17]. Curcumin can be used in the form of micro- and nanoparticles. Nanoparticles have a high potential for use against pathogenic bacteria since they can well pass the bacterial cell membrane due to their small size [18].

Decreasing the risk of caries while maintaining

adequate bracket-tooth bond strength is important in success of orthodontic treatment. Considering the recent interest in antimicrobial activity of curcumin nanoparticles (curcNPs) and gap of information regarding their antimicrobial efficacy and effect on shear bond strength (SBS) when added to orthodontic composite, this study sought to assess the effect of addition of various concentrations of curcNPs to orthodontic composite on antimicrobial activity and SBS of bracket to bovine enamel.

MATERIALS AND METHODS

Fabrication of composite containing curcNPs: curcumin (100 mg, 0.27 mmol; Sigma Chemical Company, St. Louis, MO, USA) was obtained in dichloromethane (20 mL), and 1 mL of this solution was added to boiling water (50 mL) dropwise with a flow rate of 0.2 mL/minute within five minutes under ultrasonic conditions, with an ultrasonic power of 100W and a frequency of 30kHz. After sonication for 10 minutes, the contents were stirred at 200 rpm at room temperature for about 20 minutes until a clear orange-colored solution was obtained. The solution was concentrated under reduced pressure at 50°C and was then freeze-dried to obtain a pale orange powder and then subjected to scanning electron microscopy to ensure its
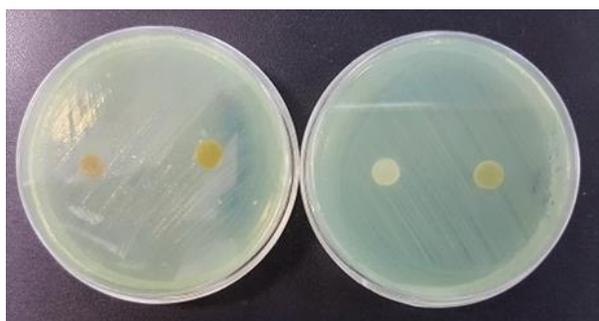
Fig. 3: Disc agar diffusion test

optimal particle size (Fig. 1). For the fabrication of composite containing different concentrations of curcNPs, 200mg of curcNP powder was manually mixed with 1800mg of Transbond XT composite (3M Unitek, Monrovia, CA, USA) to obtain 2000mg of composite containing curcNPs with 10% concentration. To fabricate composite containing 5% curcNPs, 600mg of composite containing 10% curcNPs was mixed with 600mg of plain composite. To fabricate composite containing 1% curcNPs, 120mg of 10% curcNPs was mixed with 1080mg of plain composite. Thus, 1280mg of composite containing 10% curcNPs, 1200mg of composite containing 5% curcNPs and 1200mg of composite containing 1% curcNPs were obtained as such.
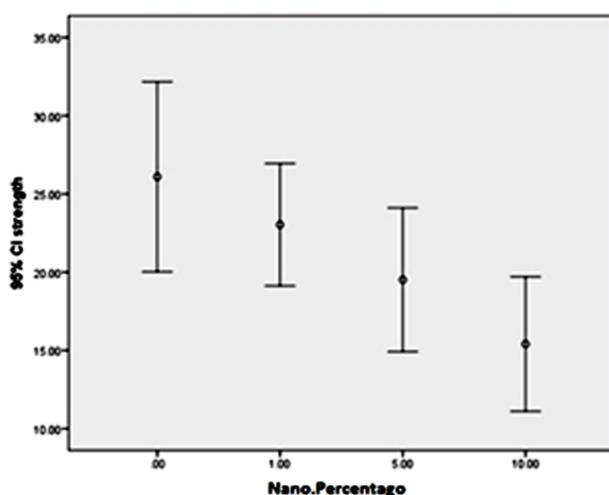
Fig. 4: The mean shear bond strength of bracket to enamel in the four groups with 95% confidence interval

For the process of mixing, curcNPs and conventional composite were weighed using a digital scale (U.S. Solid, ND, USA) with 0.0001g accuracy and mixed on a glass slab using a spatula in a dimly lit room. Mixing was continued until homogenous color and consistency were obtained. The fabricated composites were stored in a dark environment at room temperature.

Bracket bonding: A total of 48 bovine incisors without enamel cracks, caries, wear or fracture were randomly selected and immersed in 0.5% chloramine T solution for one week at 4°C. The samples were randomly divided into four groups of 12 for 0%, 1%, 5% and 10% concentrations of curcNPs. Teeth surfaces were first cleaned with a prophy brush without pumice paste, rinsed and dried. The buccal surface of teeth was etched with 35% phosphoric acid gel (Ultra etch; Ultradent Products Inc., South Jordan, UT, USA) for 30 seconds, rinsed with water spray for 30 seconds and air-dried with oil- and moisture-free air. A thin layer of adhesive (3M Unitek, Monrovia, CA, USA) was gently applied on the surface and light cured with a LED light-curing unit (Demetron, Kerr, Orange, CA, USA) for 10 seconds. Equal amounts of composite were placed on the bracket base of standard edgewise 0.18-inch slot stainless steel brackets (American Orthodontics, Sheboygan, WI, USA) with 12.62mm2 base area. Transbond XT composite without curcNPs (0%) was used in the control group while composite resins containing 1%, 5% and 10% curcNPs were used in the remaining three groups. Brackets were then bonded at the center of buccal surface of teeth mesiodistally and occlusogingivally. After aligning the longitudinal axis of brackets with the longitudinal axis of the tooth, excess adhesive was removed by a scaler and each tooth was light-cured for a total of 40 seconds from the mesial, distal, occlusal and gingival directions.

The teeth were then thermocycled (Vafaei Industria, Tehran, Iran) to simulate stresses and

Table 1: The mean shear bond strength of bracket to enamel in the four groups (MPa)

Curcumin nanoparticle percentage (n=12)	Minimum	Maximum	Mean	Standard deviation
0%	13.35	42.50	26.09	9.55
1%	13.54	33.09	23.03	6.15
5%	5.56	32.95	19.51	7.23
10%	7.47	30.60	15.40	6.76

physical conditions of oral environment. The teeth were subjected to 1,500 thermal cycles between 5-55°C within 24 hours with a dwell time of 15 seconds and transfer time of 10 seconds.

After completion of thermocycling, the samples were mounted in metal molds measuring 2.5×2.5×3cm containing auto-polymerizing acrylic resin. The teeth were fixed to a rectangular stainless steel piece (16×22 inches) with ligature wire. Self-cure acrylic resin paste was placed in the mold up to the level of cementoenamel junction of teeth (Fig. 2). After its polymerization, the teeth were removed from the metal mold.

Bond strength testing: After mounting the teeth in acrylic blocks, SBS of bracket to enamel was measured by a universal testing machine (Zwick Roell, Ulm, Germany). The teeth were placed on the jig in such a way that bracket base was parallel to the load application vector. Metal blade of the machine with a tip thickness of 0.6mm was adjusted to apply load incisogingivally to the composite-tooth interface at a crosshead speed of 0.5mm/minute until bracket debonding. The load causing bracket debonding was recorded in Newtons and then divided by the bracket base area in mm2 to obtain the SBS value in Megapascals (MPs).

Calculation of the ARI score: After debonding, the teeth and brackets were inspected under a stereomicroscope (SMZ800, Nikon, Tokyo, Japan) at ×10 magnification. The ARI score was determined based on the following scoring system:

Score 0: All adhesive remnant on the bracket surface

Score 1: More than 50% of adhesive remaining

on the bracket surface

Score 2: Less than 50% of adhesive remaining on the bracket surface

Score 3: No adhesive remnant on the bracket surface.

Microbial tests: Metal washers were placed on a glass slab and inside the washers, composite was applied with 5mm diameter and 0.64mm thickness. Next, a thin layer of bonding agent was applied on the samples. The reason behind selection of this particular diameter of metal washers was their equal size to bracket base. A smooth thin glass slab was placed on top of the samples and mildly compressed to obtain a smooth composite surface with no porosity, and also to obtain equal thickness in all samples. Each sample was light-cured by a light-curing unit for 40 seconds. After completion of curing, composite samples were separated from the washers by finger pressure. The samples were then sterilized by gamma radiation with a minimum dose of 25kGray.

standard strains of *Streptococcus mutans (S. mutans,* ATCC35668), *Streptococcus sanguinis* (*S. sanguinis*, ATCC10556) and *Lactobacillus acidophilus* (*L. acidophilus*, ATCC314) were obtained from the Iranian Biological Resource and incubated at 37°C for 48 hours.

Biofilm inhibition test: Microbial biofilm was created by three-day culture of bacteria on composite discs in a 96-well plate. For this purpose, composite discs were placed in the wells and microbial suspension with a concentration of 1.5×10^8 colony forming units (CFUs)/mL was added to each well; the plate was incubated at 37°C in order for the biofilm to form. After 72 hours, composite discs were rinsed with saline to eliminate planktonic

Table 2: Pairwise comparison of the four groups in terms of shear bond strength

Groups	P value
Control-1% curcNPs	0.753
Control-5% curcNPs	0.157
Control-10% curcNPs	0.006
1% curcNPs-5% curcNPs	0.664
1% curcNPs-10% curcNPs	0.077
5% curcNPs-10% curcNPs	0.546

CurcNPs: Curcumin nanoparticles

bacteria. To isolate biofilm-forming bacteria, composite discs were subjected to sonication and vortexed. The obtained suspension was serially diluted in microtiter plates and spread-cultured in trypticase soy agar (TSA); the bacterial colonies were then counted using drop-plate method and reported in CFUs/mm2 of discs.

Disc agar diffusion test: This test was used to assess the antimicrobial property of composite discs containing curcNPs by assessing the release of nanoparticles from the discs. For this purpose, bacterial suspension (1.5×10^8 CFUs/mL) was spread on the surface of plate containing TSA by a sterile swab; composite discs were then placed on the surface of plates with 2cm distance from each other. After incubation of plates for 48 hours, the diameter of growth inhibition zones was measured by a ruler (Fig. 3).

Eluted component test: This test was used to assess the antimicrobial activity of curcNPs released from composite discs. Composite discs were placed in tubes containing 0.5 mL of brain heart infusion broth. After three, 15 and 30 days, the brain heart infusion broth was transferred to 15 mL plastic tubes and 50 μL of the bacterial culture was added to each tube containing 5 mL of TSA (final concentration of 10^5 CFUs/mL in 1 mL of medium). The tubes were shaken at 300 rpm at 37°C for 24 hours. The obtained suspension was serially diluted in microtiter plates and spread cultured in TSA. The bacterial colonies (CFUs/mL) were counted using drop-plate method.

For statistical analysis, the data were statistically analyzed using SPSS version 20 (SPSS Inc., IL, USA). One-way ANOVA was applied to compare the four groups. Since one-way ANOVA found a statistically significant difference in SBS of the four groups, pairwise comparisons were made using Tukey's HSD test. The adhesive remnant index (ARI) score was analyzed using the Kruskal-Wallis test. For the biofilm test, one-way ANOVA was used. Since the results were significant, post-hoc Tukey's test was applied for pairwise comparisons. Analysis of the results of eluted component test for assessment of bacterial proliferation at three different time points was done by two-way ANOVA. P<0.05 was considered statistically significant.

RESULTS

The SBS test results: The highest and the lowest mean SBS belonged to the control (26.09±9.55MPa) and 10% curcNP (15.40±6.76MPa) groups, respectively. Table 1 presents the mean SBS in the four groups. According to one-way ANOVA, a significant difference existed in SBS among the four groups (P=0.008). Considering the equality of variances, Tukey's HSD test was applied for pairwise comparison of the groups (Table 2), which revealed that SBS in the control group was significantly higher than that in 10% curcNP group (P=0.006). Despite lower bond strength in 1% (P=0.753) and 5% (P=0.157) curcNP groups than the control group, these differences did not reach statistical significance. Figure 4 shows the mean SBS of bracket to enamel in the four groups with 95% confidence interval. Table 3 shows the frequency of ARI scores in the four groups. As seen in Table 3, the four groups were not significantly different in terms of ARI scores (P>0.05).

Biofilm test results: The mean colony count for each bacterial strain in the four groups is shown in Table 4. The results of post hoc test for *S. mutans* count revealed that *S. mutans* colony

Table 3: The frequency of adhesive remnant index (ARI) scores number & percentage in the four groups

Groups	ARI score			
	0	1	2	3
Control	1 (8)	3 (25)	4 (33)	4 (33)
1% curcNPs	2 (16)	2 (16)	4 (33)	4 (33)
5% curcNPs	1 (8)	3 (25)	5 (41)	3 (25)
10% curcNPs	2 (16)	2 (16)	4 (33)	4 (33)

CurcNPs: Curcumin nanoparticles

count in all concentrations of curcNPs was significantly lower than that compared to the control group (plain composite) and reached zero (all P<0.001). However, *S. mutans* colony count was not significantly different among the three groups with 1%, 5% and 10% concentrations of curcNPs (all P>0.999). The colony count of *S. sanguinis* significantly decreased in all three concentrations of curcNPs compared to the control group (all P<0.001). Comparing the colony count in three groups with 1%, 5% and 10% concentrations of curcNPs revealed that colony count decreased with an increase in concentration of curcNPs; however, the differences were not statistically significant (all P>0.999). The colony count of *L. acidophilus* significantly decreased in all three concentrations of curcNPs compared to the control group (all P<0.001). However, *L. acidophilus* colony count was not significantly different among the three groups with 1%, 5% and 10% concentrations of curcNPs (P=0.968 for the comparison of 1% and 5%, P=0.884 for the comparison of 1% and 10% and P=0.992 for the comparison of 5% and 10%). Disc agar diffusion test results: In the agar diffusion test, no growth inhibition zone was noted around composite discs in any group. Eluted component test: Analysis of the results of eluted component test for assessment of bacterial proliferation at three different time points by two-way ANOVA revealed that the interaction effect of time and concentration was not significant for *S. mutans* (P=0.985), *S. sanguinis*

(P=0.980) or *L. acidophilus* (P=0.955). The effect of concentration was not significant either for *S. mutans* (P=0.486), *S. sanguinis* (P=0.794) or *L. acidophilus* (P=0.954). Assessment of the effect of time revealed that a significant reduction in colony count only occurred at 30 days for all microorganisms (P<0.001).

DISCUSSION
Researchers have long been in search of methods to prevent enamel demineralization during orthodontic treatment without adversely affecting the bond strength [19,20]. Addition of nanoparticles to composite resin has been documented as an effective strategy to prevent enamel demineralization [21,22]. Advancements in nanotechnology have enabled the fabrication of nanoparticles with improved properties. However, aside from their antimicrobial efficacy, their effects on physical and mechanical properties of orthodontic composites must be evaluated [23]. Optimal antimicrobial activity of curcumin has been documented against *Enterococcus faecalis* [16] and *S. mutans* [24]. The cariostatic effect of curcumin is mediated by prevention of bacterial adhesion to enamel and destruction of bacterial cell wall via disrupting the peptidoglycan layer [24].

Moreover, despite its strong antimicrobial activity, curcumin is non-toxic and safe; Fernandes et al, [25] reported that cell viability of human gingival fibroblasts was not affected by exposure to curcumin in short or long-term. Yen et al, [26] showed improved physicochemical properties of curcNPs compared to curcumin. They added that curcNPs have higher solubility and bioavailability than curcumin extract emulsion and can better pass through the cell membrane; as the result, curcNPs have greater efficacy in lower dose. Thus, in the current study, curcNPs were added to orthodontic composite to assess their antimicrobial efficacy and effect on SBS of brackets to enamel. Shear bond strength testing is commonly used for assessment of bond

Table 4: Descriptive values of colony count for each bacterial strain in the four groups (CFUs/mm2)

curcNPs%	Bacterial strain	Minimum	Maximum	Mean	Standard deviation
0	*S. mutans*	20000	130000	66666	56862
	S. sanguinis	440000	680000	570000	121243
	L. acidophilus	140000	190000	160000	26457
1	*S. mutans*	0	0	0.0	0.0
	S. sanguinis	1100	2900	1800	964
	L. acidophilus	7100	9900	8633	1418
5	*S. mutans*	0	0	0.0	0.0
	S. sanguinis	197	450	325	126
	L. acidophilus	3100	4300	3766	611
10	*S. mutans*	0	0	0.0	0.0
	S. sanguinis	11	40	26	14
	L. acidophilus	350	1130	783	397

strength of brackets to enamel [21]. To simulate the thermal and physical stresses applied to teeth in the oral environment, we performed thermocycling according to a previous study [19]. Also, we used bovine teeth due to their easy availability. Selection of central incisors was due to the fact that they have a smoother surface than other teeth and allow optimal adaptation of bracket to tooth structure; this increases the accuracy of measurement of SBS [21].

The results of the current study revealed that by addition of curcNPs up to 5%, the SBS of Transbond XT composite to enamel did not change significantly and was within the clinically acceptable range of 6-8MPa. However, the SBS in 10% curcNP group was significantly lower than that in the control group. Thus, this concentration is not optimal for use in the clinical setting. Since no previous study was found on the effect of curcNPs on bond strength and other properties of composites, we compared our findings with the results of other studies on some other nanoparticles.

Poosti et al, [21] assessed the effect of incorporation of TiO2 nanoparticles on SBS of composite and reported that 1% concentration of TiO2 yielded a bond strength value similar to the control group; this finding was similar to our results; however, they used TiO2 instead of curcNPs. Moreover, they mixed the nanoparticles with composite using a mixer while we mixed them manually. Also, they stored the samples at 37°C for 24 hours while we performed thermocycling; the latter further decreases the bond strength. Akhavan et al, [19] reported an increase in bond strength following addition of 1% silver nanoparticles/ hydroxyapatite while bond strength decreased following the addition of 5% and 10% silver nanoparticles/hydroxyapatite. In terms of dose-dependent results, their findings were in line with ours. However, their results regarding 1% concentration were different from our findings, which is attributed to the main differences between the two studies including the type of nanoparticles used and type of teeth. They reported that increased bond strength in 1% group was due to the ability of silver nanoparticles/hydroxyapatite to enhance the adhesion at the interface of restorative material-enamel via increasing the mechanical strength of the adhesive layer and reinforcing the supporting structures. However, it should be noted that increased bond strength is not always optimal

and if it exceeds a certain amount, it can cause enamel damage at the time of debonding. Moreover, it should be noted that addition of silver nanoparticles even in concentrations as low as 1% can cause significant discoloration of composite, which compromises esthetics.

The ARI score is an important parameter in selection of orthodontic adhesive by clinicians. In the current study, no significant difference was noted among the four groups in terms of ARI scores, which was in agreement with the results of a previous study [19].

The current study also assessed the antimicrobial activity of Transbond XT composite containing 1%, 5% and 10% concentrations of curcNPs against *S. mutans, S. sanguinis* and *L. acidophilus*. The three selected bacterial strains are the main constituents of dental plaque. Initiation of caries mainly depends on the activity of *S. mutans* while lactobacilli (mainly *L. acidophilus*) are responsible for progression of caries. Presence of *S. sanguinis* in the oral cavity decreases the population of *S. mutans* and these two are in equilibrium [27]. Biofilm inhibition test was carried out to assess the antimicrobial activity of composites since it has been shown that bacteria in the form of biofilm are four times more resistant to antibacterial agents compared to planktonic form [28]. The current results showed that addition of curcNPs to composite significantly decreased the bacterial count of all three strains compared to the control group in all three concentrations. The results for *S. mutans* were highly favorable since *S. mutans* colony count in presence of all concentrations of curcNPs decreased to zero. This indicates low minimum inhibitory concentration and minimum bactericidal concentration of curcNPs against *S. mutans* [29]. This finding is clinically significant since *S. mutans* is the main cariogenic microorganism in the oral cavity. On the other hand, *L. acidophilus* showed higher resistance, which may be due to its role in progression of caries and formation of a very strong biofilm. In

contrast to our findings, Mirhashemi et al, [30] showed that only 10% concentration of nano-zinc oxide/nano-chitosan significantly decreased all three microorganisms; in their study, 5% concentration of nanoparticles was ineffective on *L. acidophilus* and 1% concentration had no effect on any microorganism. Difference between the results of the two studies is due to difference in type of nanoparticles used. These findings indicate the superior antimicrobial activity of curcNPs compared to other nanoparticles tested in the above-mentioned studies.

In the current study, the antimicrobial effects due to release of nanoparticles in composite samples were assessed by disc agar diffusion test. This test is important because white spot lesions are often formed around brackets (and not beneath them); thus, an ideal antimicrobial agent for addition to orthodontic composite must be able to diffuse into the environment. The current results showed no growth inhibition zone around discs in any group. This indicates insolubility and poor diffusion of curcNPs in the medium around composite discs. Thus, curcNPs do not have non-contact (long-distance) antimicrobial activity. Zinc nanoparticles also have low solubility similar to curcNPs and Aydin Sevinic and Hanley [20] showed that despite optimal antimicrobial activity, zinc nanoparticles did not form growth inhibition zone in disc diffusion test. Growth inhibition zone was noted around 10% concentration of chitosan/zinc oxide nanoparticles in the study by Mirhashemi et al [30]. Eluted component test shows the antimicrobial activity of a solution containing nanoparticles released from composite discs over time and indicates the substantivity of antimicrobial activity.

The results of eluted component test in the current study revealed a significant reduction in all three bacterial colony counts only at 30 days, irrespective of the concentration of curcNPs, which indicates low solubility and low diffusion

of curcNPs in an aqueous environment. Similarly, Mirhashemi et al, [30] showed that *L. acidophilus* colony count only decreased at 30 days following exposure to chitosan/zinc oxide nanoparticles. Thus, considering the low solubility and poor diffusion of curcNPs, further studies must focus on its use along with a nano-carrier to enhance its diffusion in the environment and improve its release profile.

CONCLUSION

Considering the significant effect of 1% concentration of curcNPs on antimicrobial property of orthodontic composite and no significant difference between 1% concentration and control group in terms of SBS, as well as the insolubility and low diffusion of curcumin, we suggest adding another soluble nanoparticle with high diffusion to 1% concentration of curcNPs.

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