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Original Article

The effect of silver and calcium fluoride nanoparticles on antibacterial activity of composite resin against *Streptococcus mutans*: An *in vitro* study

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ABSTRACT

Background: Recurrent caries were attributed to the lack of antibacterial properties of the dental materials. Silver nanoparticles (AgNPs) and calcium fluoride nanoparticles (CaF2NPs) are broad-spectrum antibacterial agents. The object of the study was to investigate the antibacterial properties of composite-incorporated AgNPs and CaF2NPs on *Streptococcus mutans*.

Materials and Methods: This experimental study forty-eight disks containing 0.5, 1, and 1.5% wt AgNPs s (n = 24) and 5, 10, and 15% wt CaF2NPs were prepared from flowable composite resin (n = 24). The third group consisted of 9 types of the combination of AgNPs and CaF2NPs (n = 72). A field emission scanning electron microscope with an energy-dispersive X-ray spectroscopy analysis system was used to test for the presence of nanoparticles in composite resins. The antibacterial efficacy of dental composite was evaluated by disk diffusion agar test. The minimum inhibitory concentration (MIC) and minimal bactericidal concentration were conducted. Data were analyzed using one-way ANOVA and multiple Tukey HSD (Honestly Significant Difference) tests. Significance level was set at 0.05.

Results: Nanoparticles added to composite produce bacterial inhibition zone. The greatest inhibition of bacterial growth was recorded in the third group which contained both nanoparticles ($P < 0.05$). MIC values decreased after adding CaF2 NPs to the AgNPs-containing composite. The results of the FE-SEM test indicate the presence of AgNPs and CaF2NPs in the dental composite resin sample. On the other hand, the formation of AgNPs and their elemental nature were proved using energy dispersive X-ray microanalysis EDX analysis. According to the results, composite resins containing 0.5% of AgNPs s and 15% of CaF2NPs exhibited a significantly lower antibacterial activity compared to the 1.5% and 1% of AgNPs s with 15% of CaF2NPs ($P < 0.05$).

Conclusion: Dental composite resins-containing CaF2NPs and AgNPs showed anti-bacterial activity against *S. mutans*.

Key Words: Anti-bacterial agents, calcium fluoride, composite resins, nanoparticles, silver

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INTRODUCTION

Dental caries is one of the most frequent bacterial diseases, that causes tooth structure loss.[1] Currently, composite resins are used in dentistry as tooth-colored restorative materials in a wide range of applications, due to good maneuverability and excellent esthetic.[2] The bacterial accumulation on the surfaces of composite resin restorations compared to other dental materials can lead to the creation of a bacterial source and the development of secondary caries.[3] Nanotechnology could make available novel techniques in the prevention of demineralization and treatment of dental caries. The antibacterial activity of nanoparticles could control dental plaque biofilms and prolong restoration service life.[4,5]

Silver nanoparticles (AgNPs) have a strong antimicrobial activity and effectively oppose *Streptococcus mutans* of the human oral cavity. The antimicrobial activity of AgNPs is typically determined by the size and shape of the particles.[6,7] Studies have proven that AgNPs can avoid acid production through dental plaque by growing inhibiting *Lactobacillus* and *S. mutans*.[8,9] This nanoparticle had an excessive surface area-to-mass ratio; therefore, a small quantity of it was sufficient for strong antibacterial protection of the composite. According to some earlier reports, AgNPs have been incorporated into the composite and did not compromise the physical and mechanical properties of the composite as well as color and esthetics.[10,11] Barot *et al*.[11] investigated the effect of AgNPs immobilized halloysite nanotubes (HNT/Ag) fillers on the mechanical properties of resin composite and observed that 10% HNT/Ag exhibited a weakening effect on the mechanical properties. However, they provided the best antimicrobial effects.

Recently, a study combined calcium fluoride nanoparticles (CaF_2NPs) with restorative material to help remineralization capabilities and reduce secondary caries.[12] Studies evaluated that the release of F and Ca ions could accelerate the remineralization of the tooth structure.[13,14] Some studies confirmed that the mechanical features of the CaF_2NPs were several times better than that of resin-modified glass ionomer and matched those of composites with little F release.[12,15]

Therefore, it might be notably proper to mix AgNPs with CaF_2NPs to gain the double benefits of antibacterial effects and remineralization capabilities. There has been no report on the synergic effect of AgNPs and CaF_2NPs on antibacterial activity. The objectives of this *in vitro* study were to examine the antibacterial activity of composite resin with nanoparticles in opposition to *S. mutans*.

MATERIALS AND METHODS

This experimental study was approved by the Ethics Committee of Ardabil University of Medical Sciences (IR.ARUMS.REC.1399.240). In this *in vitro* study, flowable composite resin (DenFil Flow-Vericom, Korea) was used. The AgNPs (Sigma-Aldrich, Germany) had a concentration of 0.5, 1, and 1.5% wt, with an average particle size of ≤50 nm, and CaF2NPs, (Sigma-Aldrich, Germany) powder had a concentration of 5, 10, and 15% wt, were incorporated in the structure of the composite resin. The third group consisted of a combination of AgNPs and CaF2NPs[16] [Table 1].

Preparation and combination of silver nanoparticles and calcium fluoride nanoparticles with dental composite resin

The nanoparticles were mechanically blended with the composite resin with the aid of using a plastic spatula constantly for 30 min in a dark room. Round mold was fabricated from polyvinyl chloride with a diameter of 10 mm and a thickness of 2 mm. The nanocomposite resin (Ag, CaF_2) was inserted into the mold and two glass slides were immediately placed on the bottom and the top of the mold. Then, the

Table 1: Average diameters of bacterial growth inhibition zones (mm) in each group

Groups	Percentage of nanoparticle		Mean±SD	*P**
	Ag	CaF_2		
Group 1	0	5	2.57±0.41	0.778
	0	10	2.42±0.61	
	0	15	2.32±43	
Group 2	0.5	0	2.27±0.50	0.137
	1	0	2.70±0.88	
	1.5	0	3.30±0.48	
Group 3	0.5	5	2.20±0.35	0.008
	1	5	2.75±0.50	
	1.5	5	3.00±0.41	
	0.5	10	2.77±0.75	
	1	10	3.30±0.38	
	1.5	10	3.40±0.29	
	0.5	15	3.45±1.10	
	1	15	3.80±0.355	
	1.5	15	3.72±0.33	

SD: Standrad deviation. *The level of significance was set to 0.05

specimens were cured using an LED light-curing unit from both sides. The final composite was stirred, then molded and cured for 60 s by LED (Demi LED Light Curing System, Kerr Corp, Orange, CA, USA) light-curing unit with a light intensity of 800 mW/cm^2. To gain highly polished samples with the same surface roughness values, all the samples were polished with 600, 800, and 1200 grit silicon carbide papers (Stark Sandpaper, German) for about 2 min.

Before the relevant tests are carried out, the final composite is kept at room temperature and away from any direct light for 24 h.

Structural characterizations

To determine morphology, size of nanoparticle, and structural characterization of obtained dental composite resin with AgNPs and CaF_2NPs, the samples were broken into two pieces, samples were appended on an aluminum stub, sputter included with gold, and photograph captured with the aid of using a field emission scanning electron microscopy (FE-SEM; MIRA3TESCAN-XMU, Razi Metallurgical Research Center, IRAN) at × 5.00. The nanoparticle diameters were directly read from FE-SEM. To gather an average value, five points were determined by a single FE-SEM image obtained from at least five different spots on dental composite samples. Furthermore, the same device was used to perform energy dispersive X-ray microanalysis (EDAX) analysis, to detect the elements in the composite.

In vitro antibacterial activity evaluations

Disc agar diffusion test was used three times for each group to evaluate the antibacterial activity of composites containing nanoparticles. Flowable composite resin (DenFil Flow-Vericom, Korea) and gentamicin were used as negative and positive controls, respectively. *S. mutans* bacterial suspension (Persian type culture collection (PTCC® 1683.) in brain–heart infusion (BHI) broth with a concentration of 0.5 McFarland was prepared. 0.5 McFarland suspension was diluted 1,000 times to obtain a concentration of 1.5 × 105 bacteria in 1 mL. Bacterial suspensions were spread on the Hinton agar culture medium. Sterilization of composite discs was done in an autoclave. Composite discs have been positioned on the floor of plates at a 2-cm distance from each other. The culture media in an anaerobic jar containing gasp (A) were used to empty the culture medium for *S. mutans* and incubated at 37°C for 48 h. Subsequently, incubated for 48 h, the increased inhibition growth zones were recorded.

To determine morphology, size of the nanoparticle, and structural characterization of obtained dental composite resin with AgNPs and CaF2NPs, the samples were broken into two pieces, samples were appended on an aluminum stub, sputter included with gold, and photographs captured with the aid of using a field emission scanning electron microscopy (FE-SEM; MIRA3TESCAN-XMU, Razi Metallurgical Research Center, Iran) at × 5.00. The nanoparticle diameters were directly read from FE-SEM. To gather an average value, five points were determined by a single FE-SEM image obtained from at least five different spots on dental composite samples. Furthermore, the same device was used to perform EDAX analysis and to detect the elements in the composite.

Minimum Inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were conducted using the CLSI 2015 guidelines for different nanoparticles against *S. mutans* (PTCC® 1683.), at different concentrations (2000–15.62_g/mL) and by broth microdilution assay; in which serial dilution of compounds was done in a sterile 96-well plate. The microorganism strain was cultivated on BHI, and it was grown for 12 h in a liquid BHI at 37°C with agitation at 150 rpm. Suspension of microorganisms was then adjusted to 0.5 McFarland in phosphate-buffered saline.[17,18] Twenty microliters bacteria suspension and 180 µL of the compound solution were added to each well. We used the wells with microorganisms without tested solutions as a negative control. The wells were read for visible turbidity after 48 h of incubation at 37°C. The MIC was defined as the endpoint where no turbidity could be detected with reference to negative, positive, and blank controls. An aliquot of 50 µl from each tested well without turbidity was spread onto BHI plates.[19] After 48 h of incubation, plates that contained no bacterial effects were recorded. The MBC value was determined as the lowest concentration.

Statistical analysis

Data were evaluated using one-way ANOVA and Tukey HSD *post hoc* tests. Significance level was set at 0.05.

RESULTS

FE-SEM images of the fracture surface of the samples were prepared. Figure 1 shows the fracture surface of the sample without nanoparticles is flat and the sample containing nanoparticles is brittle. The AgNPs consist of small grains with mean diameters of 50 ≤ nm. However, Figure 1a shows AgNPs of small grains and some dispersed nanoparticles which are more or less spherical. As shown in Figure 1b, CaF2NPs are spherical and close to the square for the large cases.

The elemental analysis of the AgNPs and CaF2NPs was performed using the EDX on the FE-SEM. Figure 2 shows the EDX spectrum of AgNPs and CaF2NPs. The EDX spectrum revealed strong signals in the AgNPs region of 3 keV [Figure 2b], therefore, confirming the formation of AgNPs and its elemental nature. This signal was formed due to the excitation of surface plasmon resonance of AgNPs. The peaks around 3.20 keV correspond to the binding energies of AgLα and AgLβ, respectively. Furthermore, a peak near 3.90 keV corresponding to CaF2NPs was observed [Figure 2c]. Therefore, the EDX profile of the sample [Figure 2] indicates that the dental composite resin sample contains AgNPs and CaF2NPs.

The results of the disk agar diffusion test are summarized in Table 1. Nanoparticles added to the composite produce a bacterial inhibition zone. The highest amount of bacterial growth inhibition was found in the third group that contains both nanoparticles ($P < 0.05$). According

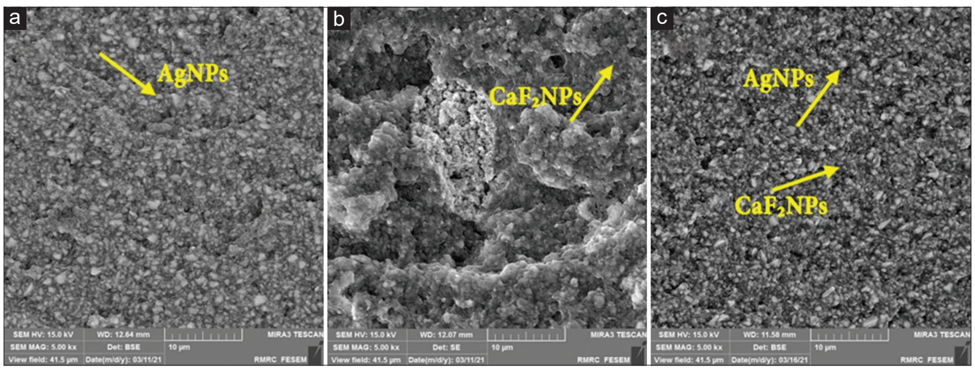

Figure 1: FE-SEM (field emission scanning electron microscopy) images, (a) dental composite + Silver nanoparticles (AgNPs), (b) dental composite + Calcium fluoride nanoparticles (CaF2NPs) and (c) dental composite + AgNPs and CaF2NPs. Magnification: 5.00 k× .Accelerating voltage (Kv):15.0Kv.

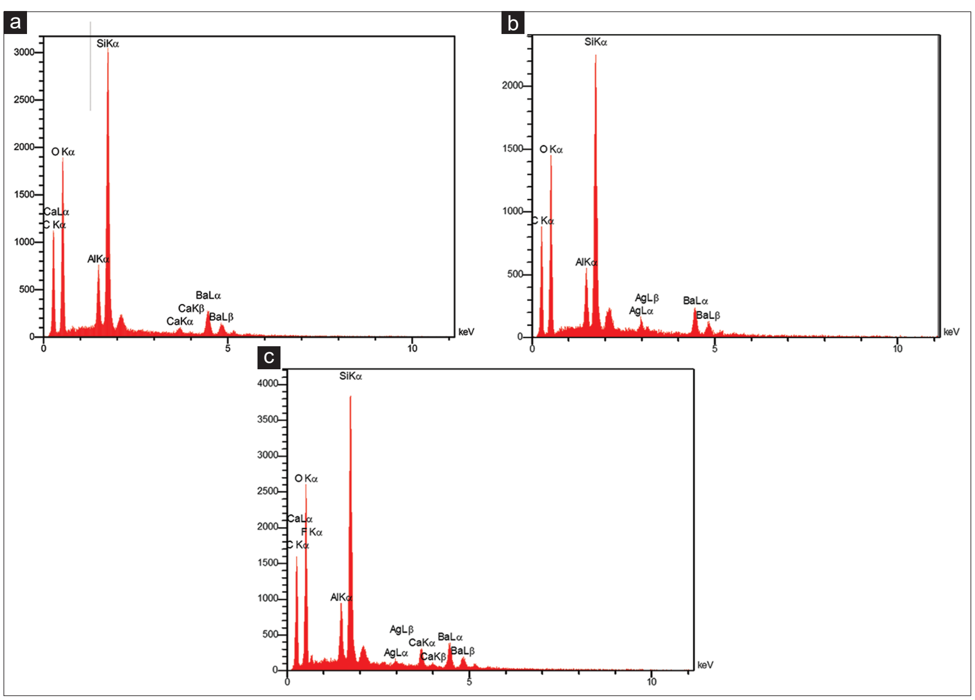

Figure 2: EDX (energy dispersive X-ray microanalysis) spectra of dental composite (a), dental composite + Silver nanoparticles (AgNPs) (b) and dental composite + AgNPs and Calcium fluoride nanoparticles (c).

Table 2: Minimum inhibitory concentration and minimum bactericidal concentration values of composite resin in each group

Percentage of nanoparticles in composite resin		MIC/MBC
AgNPs	CaF2NPs	
0	0	-
0	5	125/500
0	10	62.5/500
0	15	62.5/125
0.5	0	125/125
1	0	62.5/125
1.5	0	62.5/125
0.5	5	62.5/125
0.5	10	62.5/125
0.5	15	62.5/62.5
1	5	31.25/62.5
1	10	31.25/62.5
1	15	31.25/31.25
1.5	5	62.5/62.5
1.5	10	31.25/31.25
1.5	15	31.25/31.25

AgNPs: Silver nanoparticles; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; CaF2NPs: Calcium fluoride nanoparticles

to the Tukey test results, composite resins containing 0.5% of AgNPs and 15% of CaF2NPs exhibited a significantly lower antibacterial activity compared to the 1.5% and 1% of AgNPs with 15% of CaF2NPs. There was no significant difference in bacterial inhibition zone between composite resins with 0.5%, 1%, and 1.5% of AgNPs (group 1) and 5%, 10%, and 15% of CaF2NPs (group 2) and groups 3 (composite resins with AgNPs and CaF2NPs) by Tukey HSD test ($P > 0.05$).

Results of the MIC and MBC confirm the presence of optimal antibacterial properties in the composite that contains both Ag and CaF2 NPs. MIC values decreased significantly after adding CaF2 NPs to the AgNPs-containing composite ($P < 0.001$). MBC and MIC values are recorded in Table 2.

DISCUSSION

Composite resins-containing nanoparticles can interfere with the formation of biofilms, due to the general inhibitory effect of cariogenic bacteria.[6,20] They can also prevent secondary caries with significant antimicrobial activity against residual bacteria in the prepared tooth cavity and colonized bacteria in microleakage.[10]

The present study attempted to evaluate the antibacterial properties of composite resins-containing CaF_2NPs and AgNPs against *S. mutans*. In this research, the mechanical stirring method was used to combine the composite with CaF_2NPs and AgNPs. Most previous studies demonstrated the antibacterial activity of nanosilver and calcium fluoride incorporated into dental material. They have reported that different performance of composite resins containing these particles in different concentrations is observed.[3,10,21] However, there has been no report of the composite-containing CaF_2NPs and AgNPs to achieve antibacterial capabilities.

The antibacterial effect of nanoparticles depends on their size.[22] In the present study, the bactericidal properties of composite resins containing 1.5% silver were higher against *S. mutans* in comparison to 15% of CaF2 nanoparticles. Through this study, Espinosa–Cristóbal *et al.*[23] demonstrated the significant anti-adherence and antibacterial activity against *S. mutans* by Ag nanoparticle in ≤50 nm size. Wojnarowska *et al.*[24] doping fluorapatite nanoparticles with silver ion nanoparticles and showed a 30% inhibition of bacterial growth after 4 h of incubation while maintaining the natural morphology of fluorapatite. Discoloration due to silver is a major problem with this composite resin. The use of low concentrations of metallic nanoparticles can prevent the severe color change of composite resins.[25]

The addition of CaF2 nanoparticles can provide composite resins with antibacterial activity and reduce the caries-causing composition of dental biofilms. Pandit[26] showed that the concentration of fluoride released from restorations can help reduce the cariogenic composition of *S. mutans* biofilms when sufficient fluoride is released in the early stages of biofilm formation.

According to Weir *et al.*,[27] the fluorine release concentration in NCaF2-composite resin is similar to or even superior to that of commercially modified glass ionomer. CaF_2NPs are promising to inhibit enamel demineralization, white spot lesions, and caries.[28] Xu *et al.*[12] incorporated CaF_2NPs (10%, 20%, and 30%) into composite resin and showed that increasing the nano-CaF_2 content will increase the F release. In addition, a lower pH increases the F release from the CaF_2 particles.

Remineralization is a natural reparative process to restore the minerals to ionic forms that develop at a near-neutral physiological pH. The calcium ions in the nanoparticles that precipitate from saliva and dental plaque fluid to form new hydroxyapatite crystals are larger and more resistant to attack by acids.[29] Xu *et al*.[12] pointed out that high F-release composite resin can help reduce secondary caries. The results of the present study showed that the growth inhibitory halo in the plates of composite resin-containing AgNPs and CaF_2NPs together was significantly higher than that in the other two groups, demonstrating the synergy in the bactericidal potential of the composite containing CaF_2 and AgNPs against *S. mutans*.

There are concerns about the adverse effects of adding nanoparticles on the mechanical properties of composite resins. Previous studies indicated that a concentration of 1% AgNPs can significantly inhibit caries-causing bacteria without reducing the mechanical properties.[30] However, silver compounds added in a concentration of 10% or greater to composite resins would reduce compressive strength, elastic modulus, and tensile strength.[31] There are few studies on the effects of adding CaF_2NPs to resin composites. Mitwalli *et al*.[32] investigated the effects of incorporating 15% CaF_2NPs into composites on the mechanical performance and reported the new composite resin had antibacterial activity against biofilms derived from humans without deteriorating the mechanical properties.

The production and application of composite resins with antibacterial activity can help improve the clinical outcomes of restorative treatments with composite resins. The use of composite resins-containing Ag and CaF_2 nanoparticles could be effective in reducing the risk of recurrent tooth decay by bringing notable antimicrobial properties. It is suggested that the physical and chemical properties of composite resins with different Ag and CaF_2 nanoparticles be investigated to prevent problems in relation to mechanical strength, discoloration, or other possible changes.

CONCLUSION

In summary, the presence of silver and CaF2NPs in the dental composite resin was determined by FE-SEM and EDX. In addition, the antibacterial activity of the dental composite resin was evaluated by the disk agar diffusion test. The results of this study clearly showed that composite resins containing the AgNPs and CaF_2NPs inhibited the proliferation and growth of the important oral cavity microorganism: *S. mutans*.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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