

## Evaluation of Antibacterial Properties of Resin Composites Containing Silver Nanoparticles on *Streptococcus Mutans*

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### Abstract

**Background and Objectives:** Studies showed that resin composites are more susceptible to aggregation of biofilm than amalgam and glass ionomers and have lesser antibacterial effects which results in increasing the rate of secondary caries? Therefore, production of antibacterial resin composites was always under investigation. Thus, the aim of this study is evaluating the efficacy of resin composites containing silver nanoparticles against the *Streptococcus Mutans*.

**Methods:** In this experimental study, the antibacterial properties of resin composites incorporating 0, 0/02, 0/03, 0/04, 0/05% w/w of Nano silver particles was investigated using turbidity test. Composites was formed on the walls of 400 ml micro plates and after the curing, they left in contact with bacterial suspension. In times of 3, 24 and 48 hours, the optical density of the broth was read by spectrophotometer. The data was analyzed by Kolmogorov-Smirnov, one-way ANOVA, LSD test and repeated measure ANOVA.

**Results:** The results showed that all composites containing silver nanoparticles had antibacterial effects ( $p \leq 0/05$ ) and by increasing the amount of silver nanoparticles to 0/03% w/w, the anti-bactericidal effect rose and the bacterial growth significantly diminished ( $p=0/001$ ). However, in higher concentrations than 0/03%, this effect decreased. The MBC (minimum bactericidal concentration) were seen in composites with 0/02 percent of silver nanoparticles.

**Conclusion:** While all composite groups containing silver nanoparticles had antibacterial effect, the most efficient group was 0/03% w/w and by increasing the amount of silver nanoparticles over 0/04%, the antibacterial effect didn't increase significantly.

**Keywords:** Silver nanoparticles; *Streptococcus mutans*; Resin composites

## Introduction

Dental caries has become a common oral health problem in many countries including developed nations. Inappropriate oral and dental hygiene causes anaerobic bacterial growth, and proliferation of plaque and dental calculus [1]. Dental caries is caused by prolonged episodes of low PH resulting from acid production of bacterial biofilm. These biofilms are formed by bacteria such as *Streptococcus mutans*, which adhere to the tooth surfaces, producing organic acid metabolites that lead to demineralization of enamel [2]. Secondary caries is a lesion that occurs on a tooth, at the edge of a restored lesion, frequently known as the most common cause of restoration replacement, regardless of the type of restorative material [3,4]. Generally, secondary or recurrent caries is defined as a primary carious lesion at the margin of an existing restoration that has been used for a while. The biofilms have shown different adhesion strengths with different restorative materials [5]. Studies show that composite resins have a tendency of higher accumulation of biofilms and lower antibacterial effects than amalgams and glass ionomers, which in turn increases the rate of secondary caries [6]. Therefore, to prevent secondary caries, it is of utmost importance to repair and prevent the growth of biofilms on tooth surfaces [7]. Since oral hygiene and the durability of restorations is important, much research has been conducted to create antibacterial effects in composite resins by adding compounds such as titanium, gold, and silver particles [8,9]. The effect of silver as an antibacterial agent has been known for many years [10]. Silver nanoparticles not only affect gram-negative bacteria and gram-positive bacteria, but also influence a wide spectrum of antibiotic-resistant microbes. The antibacterial effect depends on the particle size and concentration; so, the greater the concentration and the smaller the particles, the more effective will be its cytotoxic properties [11]. Apart from the size and concentration, the particle shape is also important. Based on research, nanoplates have been found to be more effective than other particle forms [12]. These particles tend to have molecular groups containing phosphorus and sulfur. The silver nanoparticles penetrate the bacterial cell wall, releasing silver ions that bind bacteria's DNA and subsequently disrupt the electron transfer chain of the bacteria [7]. According to studies, fillers containing silver particles exhibited high antibacterial defense against *Streptococcus mutans* [13]. In the present study, we have investigated the antibacterial properties and the minimum concentration of nanoparticles required in composite resin for efficacy against *Streptococcus mutans*, by adding different weight percentages of silver nanoparticles to composite resins.

## Materials and Methods

This is a laboratory study, wherein the study population consisted of five groups of flowable resin composites (3M, ESPE®, USA), containing zero-, two-, three-, four-, and five-hundredths by weight of silver nanoparticles (NANOSAV, IRAN), respectively, with an average particle size of 40 nm in direct contact with *Streptococcus mutans* (*Streptococcus mutans* PTCC 1683, IROST®, Tehran). In the first stage, the groups to be studied were prepared by weighing the silver nanoparticles on digital scale (Sartorius CAP 225, Cubis®, Germany) and then adding these, in the specified percentages, to the resin composites. The reactions were conducted in a special darkroom, under red protective lighting (15 w, Imaging warehouse, UK), stirring for 20 minutes with a plastic spatula. Since the direct contact test required floating bacteria in the liquid medium to be in contact with the resin material, sterile 96-well microplates (Biooff®, Iran) with a capacity of 400 µL volume, were selected to prevent possible contamination of the culture medium. Based on the bacterial culture duration, a total of 90 microplates were prepared. Then, using an insulin syringe, 100 µL of each composite material group was introduced into the wells, and the degree of polymerization shrinkage of composite was reduced using a light-curing instrument (Ultradent®- VALO.USA). Thus, a certain volume of the wells was occupied by the composite materials (100 µL) and the remaining 300 µL volume was subsequently filled with the liquid culture, where the said culture in contact with the composite was tested. In this experiment, six samples were prepared from each group and 90 microplates were prepared according to the time of culture of the bacteria. The spectrophotometric (synergy HT, BioTek®) test was performed to study antibacterial properties at the surface of composites containing silver nanoparticles. First, the bacteria were cultured on a solid culture medium (sigmaaldrich®). The cultivated colonies were then used to prepare suspensions required as the McFarland standard solution as well as serial dilutions. The entire process is based on the CLSI 2015 guidelines. A standard half-McFarland solution of 10 µL was poured into each dish along with 150 µL of Tryptic soy broth (TSB, Merck®, Germany). The dishes were sealed and kept Incubated (EMS MyTemp™ 65HC) at 37°C. After each time interval of 3, 24, and 48 hours, the optical density of the culture media was recorded by a spectrophotometer at a wavelength of 600 nm. To determine the amount of bacteria, an optical density-specific bacterial counting chart was used to prepare a standard half-McFarland solution (containing  $10.5 \times 10^8$  *Streptococcus mutans* per mL) and the spectrophotometer

device was adjusted to the wavelength of 600 nm. The optical density was determined and its value recorded on the vertical axis of the graph. Then, using serial dilution, the optical densities were recorded for different bacterial counts, and a curve was plotted. By obtaining the linear regression equation, the number of *Streptococcus mutans* bacteria was determined. In addition, the optical density values up to a maximum of 0.3 were allowed and higher values were considered invalid.

### Statistical Analysis

To determine the normal distribution of variables in each group, the Kolmogorov–Smirnov test was used. Further, to determine the difference in the mean optical density of composite wells containing different weight percentages of silver nanoparticles in the time periods of the measurement, repeated-measures ANOVA was used. To determine the difference between the mean optical density and the number of bacteria in the wells containing different percentages of silver nanoparticles at each time interval of 3, 24, and 48 hours, one-way ANOVA and post hoc test (LSD) were used. The level of significance was less than 0.05.

### Results

The mean and standard deviations of optical density and bacterial count are shown in Table 1. The average number of bacteria and optical density of composite wells with different percentages of silver nanoparticles for different hours are shown in Tables 2 and 3. The comparison between the mean number of bacteria and different time durations at maximum value of 0.03% of silver nanoparticles with time, showed significant reduction of bacterial count, but at higher concentrations this difference was not significant.

### Discussion

Due to its antimicrobial properties, the industrial and medical applications of silver nanoparticles are constantly increasing. Among restorative materials, composite resins are the best substances for surface absorption of microorganisms [14,15]. In this research, the effect of anti-mutant composite resin, supplemented with silver nanoparticles of values 0.02, 0.03, 0.04, and 0.05%, was investigated. The results show that composites containing silver nanoparticles, in contrast to non-nanoparticle composites, significantly restricted the growth of *Streptococcus mutans* in their vicinity, which is consistent with the results of all other studies. The antibacterial effect increased with an increase in the concentration of silver nanoparticles up to a value of 0.03%, but in higher concentrations this effect then decreased. However, this is not consistent with the results of studies by Shafiei et al [4], Nam et al. [15], and Azarsina et al. [13], which show a direct relationship between increasing the concentration of silver nanoparticles and antibacterial effects. Meanwhile, the study by Patrícia Bolzan Agnelli das Neves et al. stated that an increase in concentration is not indicative of an increase in antibacterial activity as is suggested by our research, too [7]. In this study, direct contact was used to evaluate the antibacterial properties of composite resins. Nam et al. in their study showed that tissue regenerative material containing silver nanoparticles has a significant antibacterial effect in comparison with the control (with no nanoparticles), and this effect intensifies with the increase in the concentration of nanoparticles. Thus, while demonstrating the antibacterial properties of silver nanoparticles, they pointed to the unknown mechanism of the silver-containing compounds, and explained that it cannot be concluded that silver nanoparticles are released from the specimens, or that they show their antibacterial effect in direct contact with the microbial cells. They continued to emphasize that the function of silver as a catalyst turns oxygen into activated oxygen (including hydroxyl radicals), which is facilitated by light energy and/or H<sub>2</sub>O in air or water at polar surfaces. As a result, these active oxygen radicals prevent the growth of bacteria. Sondi et al. [16] in their study, pointed to an unknown mechanism of inhibiting silver nanoparticles, and emphasized that when exposed to silver nanoparticles, cellular proteins are inactivated and microorganisms lose their ability to replicate. Patrícia Bolzan Agnelli das Neves et al. [7] added 0.3 and 0.6 wt% silver nanoparticles to resin composites in the laboratory. The microscopic analysis showed no difference in surface roughness between the two groups and the non-nanoparticle group. The antibacterial effect of the 0.3% group was more than the other group and, in general, more than the

p-value	Bacteria count	Optical density	Time (Hour)	Silver nanoparticles Concentration (percent)
0/001	9199166±21872/820	0/089 ± 0/0005	3	0/00
	9787083±21872/420	0/094 ± 0/0005	24	
	1048750±37884/40	0/101± 0/0009	48	0/02
0/73	8300000±37884	0/080± 0/0009	3	0/02
	8334583±87489	0/080±0/0020	24	
	8334583±21872	0/080±0/0005	48	
0/003	8300000±92796	0/080± 0/0009	3	0/03
	8127083±14149	0/079±0/0013	24	
	8196250±92796	0/079±0/0008	48	

Table 1. The mean and standard deviation of optical density and bacterial count

% 0/05	% 0/04	% 0/03	% 0/02	0 %	Silver Nanoparticles	
0/001	0/001	0/001	0/001		%	0
0/001	0/001	0/001			%	0/02
0/001	0/001				%	0/03
0/144					%	0/04

Table 2 . Post hoc test Comparison of the average number of bacteria and optical density of wells containing composite with different percentages of silver nanoparticles at hour 3

% 0/05	% 0/04	% 0/03	% 0/02	0 %	Silver nanoparticles
0/001	0/001	0/001	0/001		%0
0/657	0/378	0/001			%0/02
0/004	0/001				%0/03
0/657					%0/04

Table 3 . Post hoc test Comparison of the average number of bacteria and optical density of wells containing composite with different percentages of silver nanoparticles at hour 24

control group. The present study also points to the existence of such a phenomenon. In their study, Durner et al. added silver nanoparticles of percentages 0.125, 0.225, 0.05, 0.1, 0.03, and 0.4, to composites to investigate the amount of monomer release or washing and other compounds. The rate of washes in samples with 0.1 and 0.3% weight were significantly higher than the other groups, and it was proved that nanoparticles in these percentages affect the polymerization process of dental materials [17]. By increasing the release of materials from polymerized composites, the adaptability of silver - containing materials decreases; hence, we used nanoparticles in lower weight percentages for the present study [15]. The study of Lee et al. showed that the binding of streptococci to the resin adhesives was significantly less than for non-composite types such as resin-modified glass ionomer, due to this polarization phenomenon and the surface properties of glass ionomer modified with resin, which has a more rigid surface than conventional composites [18]. Resin groups containing silver nanoparticles were rigorously reported to be the least affected by the effect of inhibiting silver nanoparticles [19]. In this study, the antibacterial properties of the resins are due to direct contact with bacteria. It is argued that the silver nanoparticles are not released from the composite mass [3], and therefore are not effective against adjacent bacteria in the liquid medium. This, as an issue in maintaining mechanical and bond strength of the composites is very useful, because it prevents the formation of bubbles within the composite masses and, as a result, prevents weaknesses in the composite. In other studies, the antibacterial effects of additives in composites have been investigated by the turbidity method [20]. In our study, there was a significant difference in the bacterial growth rate. The mechanism by which silver nanoparticles exhibit their antibacterial properties state that silver ions react with living bacterial enzymes and inactivate them, causing bacteria to lose their ability to replicate and then die [14]. In the present study, the growth process of bacteria at 3, 24, and 48 hours at concentrations of two-hundredths of weight percent is not significant, while in the four- and five-hundredth concentrations, this trend decreases. This means that 100% inhibition of bacterial growth does not occur, and hence, they continue to proliferate. On the other hand, in higher concentrations, due to the rapid and effective effect of the silver nanoparticles, this growth preclusion is quite clear. However, at a concentration of three-hundredths of a percent, this process initially decreased at 24 hours, in comparison with three hours, and then increased. Ghasempour et al. [20] stated that it's because of bacterial log phase that the

number of bacterial cells doubles at a constant, exponential rate. Another important advantage of silver is that it does not create bacterial resistance, which should be between 1–10 nm for direct contact of nanoparticles with bacteria. In this study, the average size of nanoparticles was 40 nm, which had the optimum antibacterial effect [22].

## Conclusion

In this study, all the groups containing silver nanoparticles had antibacterial effects which were statistically significant in all groups and minimum bactericidal concentration was 0/02 % ww of Nano silver.



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