10.5005/jp-journals-10024-1442
ORIGINAL RESEARCH

the Journal of CONTEMPORARY DENTAL PRACTICE at www.thejcdp.com

The Antibacterial Properties of Composite Resin Containing Nanosilver against *Streptococcus mutans* and *Lactobacillus*

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ABSTRACT

Aim: The aim was to evaluate the antibacterial properties of composite resin containing nanosilver against *Streptococcus mutans* (SM) and *Lactobacillus* (L).

Materials and methods: Nanosilver was added to Z250 composite at 0.5 and 1% by weight. In order to confirm the homogenous distribution of the nanoparticles in the composite resin, SEM-EDX analysis was performed on one sample in each group. Z250 composite without nanosilver was used as control. Direct contact test was used to test the antibacterial properties of nanoparticle-loaded composites: 0.001 ml of 0.5 Mc Farland suspension of MS and L was placed on composite disks, and incubated for 1 hour in 5 to 10% CO_2 incubator at 37ºC. Samples were placed in 0.5 ml of sterile BHI broth and incubated for 2 hours in CO_2 incubator. Afterwards, 0.001 ml liquid from each medium was distributed on blood agar plates and incubated for 48 hours in CO_2 incubator. The numbers of bacterial colonies were counted visually. Data were analyzed using Two-way ANOVA and Tukey HSD test. Significance level was set at 0.05.

Results: Addition of nanosilver to composite resin had a significant effect on reduction of the number of SM and L colonies ($p = 0.000$). The antibacterial properties of composite resins are different depending on the concentration of nanosilver ($p = 0.014$). Tukey test indicated that increase in the concentration of nanosilver caused the increase in antibacterial properties of composite resin.

Conclusion: Addition of silver nanoparticles to Z250 composite could significantly inhibit the growth of *Streptococcus mutans* and *Lactobacillus* on the surface of this composite.

Clinical significance: The addition of nanosilver to Z250 composite could inhibit the growth of SM and L on the surface of the restoration and therefore prevent the occurrence of secondary caries.

Keywords: Composite resin, Streptococcus mutans, Lactobacillus, Nanotechnology, Laboratory research.

How to cite this article: Azarsina M, Kasraei S, Yousefi-Mashouf R, Dehghani N, Shirinzad M. The Antibacterial Properties of Composite Resin Containing Nanosilver against *Streptococcus mutans* and *Lactobacillus*. J Contemp Dent Pract 2013;14(6):1014-1018.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Although the mechanical properties of methacrylate-based composite resin restorations have been improved substantially, their antibacterial properties are still limited. The bacterial accumulation on the surfaces of these restorative materials can provide the bacterial source leading to the development of secondary caries and periodontal diseases.[1,2] Microorganisms accumulate more readily on composite resins compared to amalgam and glass ionomer restoratives.[3]

Various researchers have attempted to reduce plaque accumulation on the surfaces of dental materials by incorporation of bactericidal agents such as chlorhexidine,[4] antibacterial monomer MDPB,[5,6] antibacterial monomer furanone[7] and other antibacterial fillers.[8]

Silver has a significant antimicrobial activity and is effective against streptococci of the human oral cavity and periodontal pathogens[9] so, it might be useful as an antibacterial agent incorporated into dental restorations, especially when applied in nanometer sizes.

Cytotoxicity of silver nanoparticles on fungi, protozoa, some viruses and bacteria including *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* and *Escherichia coli* is approved in previous studies.[10-13] Considering the fact that silver is effective against the Streptococci of the oral flora and periodontal pathogens, and inhibits bacterial adherence to surfaces and biofilm formation, it can be applied as a useful antibacterial additive to dental restorations.[9,14]

The addition of silver filler particles has been stated to have a very good protective effect against *S. mutans*.[15,16]

Evaluation of the effect of loading the composite resin restorations with nanoparticles might be effective in reduction of plaque accumulation.

Since *Streptococcus mutans* and *Lactobacillus* are the main bacteria responsible in tooth carious process,[17] the present study was designed to evaluate the antibacterial effect of composite resin containing nanosilver against *Streptococcus mutans* and *Lactobacillus*.

The null hypothesis of the study is that the addition of nanosilver to composite resin does not have any antibacterial effect against *Streptococcus mutans* and *Lactobacillus*.

MATERIALS AND METHODS

In this *in vitro* study, nanosilver particles (TopNano Tech Co. Taipei, Taiwan) with average size of 50 nm were added mechanically to Z250 (3M ESPE, St Paul, MN, USA) composite at 0.5 and 1% by weight. The nanoparticles were mixed with the composite resin by a plastic spatula continuously for 30 minutes in a dark room. In order to confirm the homogenous distribution of the nanoparticles in the composite resin, SEM-EDX (Scanning electron microscopy with an energy dispersive X-ray analytical system) analysis was performed on one sample in each group. One sample in each group was made in PVC molds of 4 mm diameter and 1.5 mm height. The composite was inserted into the mold and immediately covered with two glass slides from the top and the bottom. The specimen was polymerized using an LED (Demi LED Light Curing System, Kerr Corp, Orange, CA, USA) light-curing unit with a light intensity of 800 mW/cm^2 from both sides. Samples were broken into two pieces with a chisel-like blade and the broken surfaces were gold sputter coated (Sputter coater, EMITECH, K45OX Ashford; Kent, England) in a thin 15 nm layer to prevent the samples' surfaces from burning during SEM observation. The elemental gold was finally eliminated from the diagram by the system software. The broken surfaces of each sample were observed with a scanning electron microscope (TESCAN,VEGAII, XMU, Zech Republic) at ×350 (Fig. 1). Following confirmation of the homogenous distribution of the nanoparticles into the composites, the samples of each study group were made by the same method as described above in PVC molds (N = 12). Z250 composite resin without nanosilver addition was used as control. All of the samples were polished with 600, 800, 1200 grit SiC papers (991A softlex, Germany) to obtain highly polished samples with identical surface roughness (Ra) values.

Direct contact test was used to test the antibacterial properties of nanoparticle-loaded composites: Initially, *Streptococcus mutans* bacterial suspension [Persian type culture collection (PTCC) =1683] and *Lactobacillus* bacterial suspension (PTCC = 1643) in brain-heart infusion (BHI) with concentration of 0.5 Mc Farland were prepared (1 ml that contains about 1.5×10^8 bacteria). 0.001 ml of 0.5 Mc Farland suspension was extracted with a sterile sampler and placed on composite disks which were sterilized in autoclave and incubated for 1 hour in 5 to 10% CO_2 incubator at 37ºC. During that period, the suspension liquid evaporated, ensuring direct contact between bacteria and the composite disk surface. Samples were placed in 0.5 ml of sterile BHI broth and incubated for 2 hours in 5 to 10% CO_2 incubator at 37ºC. Afterwards, 0.001 ml liquid from each medium was extracted with a sampler and distributed on blood agar plates (merch, Damstadt, Germany) and incubated for 48 hours in

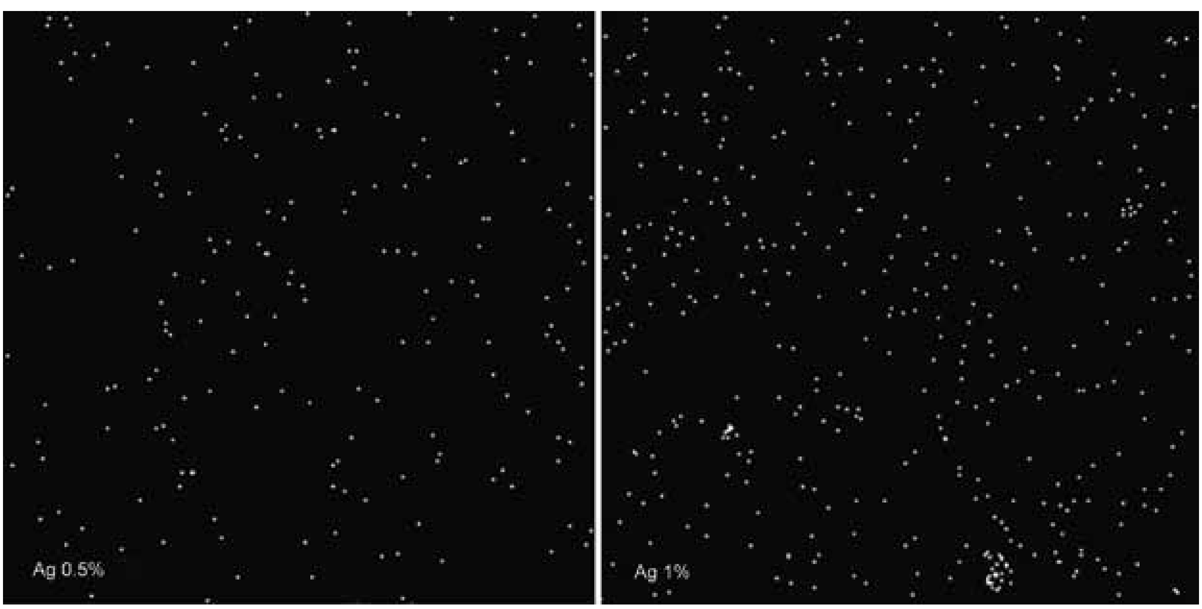

Fig. 1: SEM-EDX image of the surface of composites containing 0.5, 1% nanosilver particles

5 to 10% CO_2 incubator at 37ºC. The numbers of bacterial colonies were counted visually.

Data were analyzed using Two-way ANOVA and Tukey HSD multiple comparisons. Significance level was set at 0.05.

RESULTS

Mean and SD of the studied groups for bacterial counts are summerized in Table 1. The highest amount of bacterial growth was seen in control groups (*Streptococcus mutans* = 209.83 ± 99.50, *Lactobacillus* = 64.58 ± 41.61), whereas the lowest amount of bacteria had grown on samples containing 1% Ag nanoparticles (*Streptococcus mutans* = 127.08 ± 70.35, *Lactobacillus* = 32.16 ± 22.95).

Analysis of variance indicated that addition of silver nanoparticles to composite resin had a significant effect on reduction of the number of SM and L bacterial colonies (p = 0.000). It was also indicated that the antibacterial properties of composite resins are different depending on the concentration of nanosilver particles (p = 0.014). The antibacterial effect of composites containing nanosilver was significant depending on the type of the bacteria (p = 0.001). However, the interaction of the concentration of nanoparticle and the type of bacteria was not statistically significant (p = 0.327).

The comparison of the control and test groups by Tukey HSD test indicated that increase in the concentration of nanosilver causes the increase in antibacterial properties of composite resin (Table 2).

DISCUSSION

Application of nanoparticles of silver is increasingly growing in medicine and industry for their antibacterial properties[18,19] Sorption of microorganisms by the composite resins occurs more often than other restorative materials;[3,20] therefore, we added silver nanoparticles to a composite resin to provide antibacterial properties for this restorative material.

Some studies have used agar disk diffusion to evaluate antibacterial properties of cured composite resins. In this method composite disks are placed on agar plates containing *Streptococcus mutans* and *Lactobacillus*;[21-23] however, considering that mano-posticle containing composites are not supposed to release any antibacterial material and do not create any growth inhibition halo, this method is not suitable for evaluation of bactericidal properties of composite resins.[22-24] Therefore, the present study performed direct contact method and BHI-broth medium to study the antibacterial properties of composite resin disks; and considering the fact that liquid medium contains both live and dead bacteria, in order to trace and count the live and active colonies, 0.001 ml from each bacterial suspension tube was cultured on blood agar medium.

The results of the present study indicated that the amount of bacterial colonies was significantly higher in control groups compared to test groups. The bactericidal potential of Z250 composite containing nanosilver particles was considerably observed.

Most of the previous studies confirm the antibacterial properties of nanosilver incorporated into composite resins; however they have reported different concentrations and effectiveness for these particles.[15,25,26] Hernández-Sierra et al[27] evaluated the sensitivity of *Streptococcus mutans* to nano particles of silver (25 nm), zinc oxide (125 nm) and gold (80 nm) and indicated the higher antibacterial properties of nano silver than nano zinc oxide and nanogold; they attributed this results to smaller particle size of nanosilver than nano zinc oxide particles.

Concerns exist about the adverse effect of nanoparticle additives on the mechanical properties of composite resins; however, previous studies have reported silver compounds added in 10% concentration or greater to dental materials would significantly reduce compressive strength, elastic modulus and tensile strength.[8,28] Therefore, it seems that nanoparticles added in low concentrations as in the present study would not adversely affect mechanical properties of composite resins.

Table 1: Mean and SD of bacterial count of mutans and *Lactobacillus* in 0.001 ml of liquid medium in control and tests groups

Groups	*Streptococcus mutans*					*Lactobacillus*				
	Number	*Mean*	*SD*	*Min.*	*Max.*	*Number*	*Mean*	*SD*	*Min.*	*Max.*
Control	12	209.83	99.50	97	363	12	64.58	41.61	23	128
Ag 0.5%	12	190.00	98.74	68	335	12	44.00	17.48	7	73
Ag 1%	12	127.08	70.35	43	313	12	32.16	22.95	6	74

Table 2: Pairwise comparison of the effect of nanosilver addition to composite resin

Nanosilver percent	*Nanosilver percent*	*Mean difference*	*Significance**
0%	0.5%	40.9271	0.000
0%	1%	79.2396	0.000
0.5%	1%	38.3125	0.000

*:Tukey HSD test

The composites used in this study were not mixed with the nanoparticles by a mechanical mixer, due to their high viscosity. The manual incorporation of the nanoparticles into the composite resin may include air bubbles into the composite, which might affect some of its properties. Although the composites were completely packed into the molds during sample preparation, and despite the observation that these possible porosities did not have any deleterious effect on the antibacterial properties of the studied composites, it might have affected the mechanical properties. Therefore, it is suggested that this step be done in vacuum or by a special machine designed for mixing of such highly viscose materials.

Discoloration and grayish color change is a common problem in all materials containing silver, especially composite resins.[29] It has been reported in a previous study that the 0.3 and 0.6% concentration of silver microparticulates incorporated into the composite resin had the respective antibacterial properties. Therefore, according to previous studies, we chose the 0.5 and 1% concentration of silver nanoparticles to benefit both the antibacterial properties of silver and the less detrimental effect of these nanoparticles on the color of composite resins.

Composites containing nanosilver particles can be applied in restorative and orthodontic treatments and long-term splinting of the teeth.[30,31] In spite of using fluoride and glass ionomer particles in composite restorations, it seems using composite resin cements containing antibacterial materials would be useful for reducing caries activity around orthodontic brackets.[8,32,33] Failure of posterior class II composite restorations due to microleakage and secondary caries can be prevented by using these bactericidal composites as liners under final restorations. In this way, we can benefit the antibacterial properties of these composite resins, while masking their grayish color by conventional composites.

Considering the importance of using antibacterial restorative materials, it is suggested that studies be performed on other antibacterial materials which have less detrimental esthetic effects on composite resins.

CONCLUSION

Within the limitations of the present study, it was concluded that the addition of silver nanoparticles to Z250 composite could significantly inhibit the growth of *Streptococcus mutans* and *Lactobacillus* on the surface of this composite.

Clinical significances

Nanosilver incorporated into composite resins inhibited the growth of *Streptococcus mutans* and *Lactobacillus* on the surface of the restoration and therefore can prevent the occurrence of secondary caries.

ACKNOWLEDGMENTS

The authors thank the Dental Research Center and Vice Chancellor of Research, Hamadan University of Medical Sciences, for supporting this study.

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