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Evaluation of the Major Properties of Composite Resin Containing Zinc-Oxide Nano-Particles

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Abstract

Background: Dental composite resins containing Nano-Particles (NPs) of Zinc-Oxide (ZnO) are reported to exhibit antibacterial properties against cariogenic bacteria; but the important physical and mechanical properties of such materials need more studies. The aim was to evaluate the antibacterial, and some of the important physical and mechanical properties of composite resin containing ZnONPs.

Methods: 0.5% and 1% weight NPs of ZnO were mixed with flowable composite. The composite resins were tested for the antibacterial properties against MutansStreptococcus (MS) and Lactobacillus (L), flexural strength, micro-hardness, degree of conversion, and water sorption. Data for direct contact test were analyzed by two-way ANOVA and Tukey post HOC tests. Data of water sorption were analyzed by Kruskal-Wallis and Mann-Whitney tests. Data analyses for other tests wereOne-way ANOVA and Dunnet tests. Significance level was set at 0.05.

Results: Incorporation of NPs decreased the number of MS and L counts. NPs added to composite resin caused a reduction in all the physical and mechanical properties ($p<0.05$) except water sorption ($p>0.05$). Flexural strength, micro-hardness, and degree of conversion were significantly lower in composite resin with 1% NPs compared to the composite with 0.5% NPs, while 1% NPs indicated higher antibacterial properties.

Conclusions: Although incorporation of ZnO NPs into flowable composite imparted antibacterial properties, it reduced some physical and mechanical properties of composite resin. These properties were lower in composite containing 1% ZnO NP, compared to 0.5% NP. Water sorption of the composite resin did not change significantly by addition of ZnO NPs.

Keywords: Antibacterial Agents;Dental Caries; Nanotechnology; Zinc Oxide

Introduction

Composite resins have limited antibacterial properties.Cariogenic bacteria accumulate more readily on composite resins compared to otherrestorative materials [1,2]. Incorporating antibacterial agents into these restorative materials would increase the longevity of composite resin restorations.

Various antibacterial agents have been studied, such as MDPB monomers [3], chlorhexidine [4], titanium dioxide [5]and metallic nano-particles [6,7]. Nano-Particles (NPs) of Zinc-Oxide (ZnO) incorporated into composite resins are reported aseffective antibacterial agents [6,8,9].

Tavassoli-Hojjati, et al. reported the antibacterial properties of composite resins containing ZnONPs without any significant detrimental effects on some of the important physical and mechanical properties of the restorative material [6].

Antibacterial properties of composites containing ZnONPs are noteworthy if they have acceptable physical and mechanical

properties. Therefore, the present study was performed to evaluate the antibacterial properties, flexural strength, micro-hardness, degree of conversion and water sorption of a composite resin containing ZnO NPs.

Methods and Materials

Sample preparation

0.5% and 1% by Weight (wt) NPs of ZnO (Penta, Czech Republic) with the average size of 50nm were mixed manually with a flowable composite resin (ÆliteFlo, Bisco, USA) for 30 minutes with a plastic spatula on glass slide in dark room.Samples were made in specific molds and cured with an LED light-curing unit (Demi LED Light Curing System, Kerr Corp., Orange, USA) with a light intensity of 800 mW/cm², for 60s. Samples of composite resin without NPs were considered as control group.

Nanoparticle Distribution

To ensure homogenous distribution of the NPs in the composite resin, one disc-shaped sample from each study group was tested for SEM-EDX mapping analysis. Each sample was broken with a chisel-like blade and after gold sputter coating of the broken surface (Sputter Coater, Emitech, K45OX Ashford, Kent, England) in a thin (15 nm) layer, it was observed with a scanning electron microscope (Vega II XMU, Tescan, Czech Republic)(Figure 1).
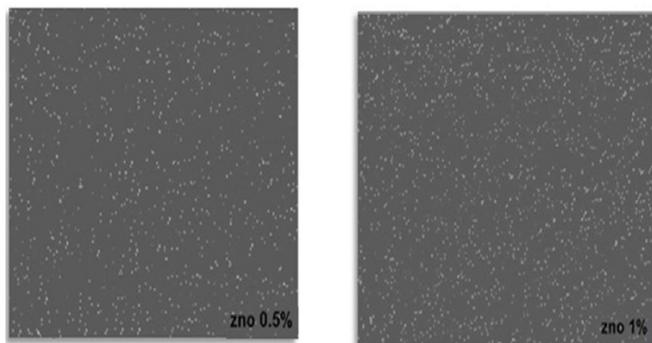

Figure 1: SEM-EDX mapping image of composite resin with 0.5% and 1% nano-particles of zinc-oxide. (×350 magnification).

Bacteriologic Test

Direct contact test was applied for evaluation of the antibacterial properties of ZnO NP-loaded composite resins. The samples for bacteriologic test were made in PVC molds with 4mm diameter and 1.5 mm height.All of the samples were polished with 600, 800, 1200 grit SiC papers (991A softlex, Germany) to obtain high polished samples with identical surface roughness (Ra) values. Initially, Mutans*Streptococcus*(MS) bacterial suspension [Persian Type Culture Collection (PTCC) =1683] and *Lactobacillus*(L) bacterial suspension (PTCC=1643) in Brain-Heart Infusion (BHI) with concentration of 0.5 McFarland was prepared (1mL that contains about 1.5×10^8 bacteria). 0.001 mL of 0.5 McFarland sus-

pensions was extracted with a sterile sampler and placed on composite discs which were sterilized with autoclave and incubated for 1 hour in 5-10% CO_2incubator at 37°C. During that period, the suspension liquidevaporated, ensuring direct contact between bacteria and the composite disc surface. Samples were placed in 0.5 mL of sterile BHI broth and incubated for 2 hours in 5-10% CO_2incubator at 37°C.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 5-10% CO_2incubator at 37°C. The numbers of bacterial colonies were counted visually.

Flexural Strength

Three-point bending test was performed on 10 samples in each study groupaccording to ISO 4049. After removing the specimens from the molds, they were polished with 800 grit silicon carbide papers. Three-point bending test was performed with a universal testing machine (STM-20, SANTAM, Iran) at the crosshead speed of 0.5 mm/min. 16±0.5(N) load was applied on the specimens until failure. The flexural strength inMPa was calculated as:

$$\sigma = 3Fl/2bh^2$$

Where F is the load at fracture (N), l is the length of the span (mm), b is the width of the specimen (mm) and h is the thickness of the specimen (mm).

Micro-hardness

Eight samples in each study group were made in stainless steel molds with 6 mm diameter and 2 mm thickness. Samples were polished with 1000, 1500, and 2000 grit silicon carbide papers to remove the surface oxygen inhibited layer of the composite resin. Vicker'smico-hardness test (HVS-1000, LaizhouHuayin Testing Ins, Taiwan) was performed on 3 points in each sample under 200 gr load applied for 15 seconds. The indentation area was measured with ×125 magnification. Mean hardness was calculated in kg/mm²and recorded as:

$$HV=1.8544F/d^2$$

where F is the load applied by the indenter (N), and d is the mean diameter of the diamond indented in the sample (mm).

Degree of Conversion

The degree of conversion was measured with FTIR spectroscopy (EQUINOX55, Bruker, Germany) for 5 samples in each study group. The uncured composites were placed in very thin layers between two polyethylene films and the absorbance peaks were recorded. After curing the samples for 40 seconds, the absorbance peak was measured again. Degree of conversion (DC%) was determined from the ratio of absorbance peaks of aliphatic C=C (peak at 1638 cm^{-1}) against internal reference of the aromatic

C···C (peak at 1608 cm^{-1}) before and after curing of the specimen. DC% was then calculated and recorded as:

DC%=[1- (1638 cm^{-1}/1608 cm^{-1}) peak area after curing] ×100

(1638 cm^{-1}/1608 cm^{-1}) peak area before curing

The mean of 5 specimens in each study group was recorded as the DC% of the group.

Water Sorption

Water sorption was determined according to ISO 4049 on 5 specimens in each study group.

Water sorption of the specimens was calculatedand recorded as:

$$W_{SP}= m_2-m_3/V$$

Where, $m_2$is weight of the specimens after 7 days of storage in water, in microgram;

$m_3$is weight after conditioning the specimens in desiccators, in microgram;

V is the volume of specimens, in cubic millimeter.

Data Analysis

Data for direct contact test were analyzed by two-way ANOVA and Tukey post HOC tests. Data of water sorption were analyzed by Kruskal-Wallis and Mann-Whitney tests. Data analyses for other tests were One-way ANOVA and Dunnet tests. Significance level was set at 0.05.

Results

Direct Contact Test

Mean and SD of the studied groups for bacterial counts are listed in Table 1.The highest amount of bacterial growth for mutans streptococcus was observed in the control group (N=209.83 ± 99.50), and the lowest amount was in the group containing 1% ZnONPs (N=85.58 ± 28.20). The result was the same for Lactobacillus.

Groups	Mutans *Streptococcus*					*Lactobacillus*				
	Number	Mean	SD	Min	Max	Number	Mean	SD	Min	Max
control	12	209.83	99.5	97	363	12	64.58	41.61	23	128
ZnO 0.5%	12	179.16	79.31	76	304	12	40.91	14.9	16	66
ZnO 1%	12	85.58	28.2	49	154	12	26	17.88	2	51

Table 1: Mean and SD of bacterial count of mutants and lactobacillus in 0.001 mL of liquid medium in study groups.

The analysis of variance indicated that the antibacterial properties of NP-loaded compositesagainstmutans streptococcus and lactobacillus varied significantly depending on the NP concentration (p=.000) and bacterial type (p=.000). The interaction of NP concentration and bacterial type was statistically significant (p=.000).

Tukey HSD tests also revealed that 1% concentration of NPswas significantly more effective against MS and L bacteria than 0.5% concentration (p=.000).

Flexural Strength

Mean and SD of flexural strength (MPa) in study groups are listed in Table 2.

Test Group	Flexural Strength (MPa)	Surface Hardness (Kg/mm^2)	Degree of Conversion (%)
Control	124.71 ± 6.92[a]	24.39 ± 0.44[b]	64.75 ± 3.40[c]
ZnO 0.5%	102.11 ± 10.72[a]	20.74 ± 0.27[b]	60.18 ± 0.16[c]
ZnO 1%	79.13 ± 8.44[a]	17.13 ± 0.65[b]	57.26 ± 1.74[c]
Groups with similar letters in superscripts had significant differences in Dunnet test (p<0.05). Number of samples in each test: Flexural Strength: 10; Surface Hardness: 8; Degree of Conversion: 5			

Table 2: Comparison of the Mean ± SD of flexural strength, surface hardness and degree of conversion in study groups.

One-way ANOVArevealed significant decrease in flexural strength of composite resin by incorporation of ZnO NPs (p=0.001).Dunnet test indicated that increase in the concentration of ZnONPs from 0.5% to 1% caused a decrease in flexural strength of the composite resin (p<0.05).

Surface Hardness

Mean and SD of surface hardness (kg/mm^2) in study groups are listed in Table 2.

The results of One-way ANOVAindicated significant decrease in surface hardness of the composite resin after incorporation of 0.5 and 1% ZnO NPs (p=0.001).Dunnet test revealed significant differences between the test groups and the control group (0.001), and also between 0.5% and 1% ZnO NPs containing composite resins (p=0.001).

Degree of Conversion

The mean and SD of the study groups for degree of conversion are listed in Table 2.

One-way ANOVA analysis indicated that degree of conversion had a significant difference among the study groups (p=0.002). Dunnet test indicated significant differences between control and 0.5% ZnO groups (p=0.008); and between control and 1% ZnO

groups (p=0.008); and also between 0.5% and 1% ZnO groups (p=0.015).

Water Sorption

Table 3 is indicative of the mean and SD of water sorption in study groups.

	Number	Mean	SD
Control	5	6.2	0.6[a]
ZnO 0.5%	5	5.7	1.3
ZnO 1%	5	5.2	0.5[a]
Groups with the same letter in superscript were significantly different by Mann-Whitney U test.			

Table 3: Mean ± SD of water sorption ($\mu g/mm^3$).

Since Kolmogorov-Smirnov test did not indicate a normal data distribution, non-parametric tests were selected for data analysis. Kruskal-Wallis test revealed no significant differences in water sorption among the study groups (p=0.18).

Mann-Whitney test indicated no significant difference between the 0.5% ZnO and control group (p=0.46), and also between the 0.5% and 1% ZnO group (p=0.6). A significant difference was observed between the control and 1% ZnO group (p=0.03).

Discussion

The results of the current study showed that incorporation of ZnO NPsinto composite resin imparted antibacterial properties against the main bacteria responsible for caries initiation and progression, butcaused a decrease in the flexural strength, micro-hardness and degree of conversion of the composite resin. However,water sorption was not affected by loading the composite resin with ZnO NPs.

Similar to the results of the present study, some of the previous studies confirm the antibacterial properties of ZnO NPs incorporated into composite resins, however different concentrations and effectiveness of these particles have been reported [10-13]. Tavassoli-Hojati, et al. reported antibacterial properties of composite resins containing various concentrations of ZnO NPs by direct contact test [6]. Hernández-Sierra, et al.[12] evaluated the sensitivity of MS to nano particles of silver, zinc oxide and gold and indicated the higher antibacterial properties of nano silver; they suggested this result can be related to smaller particle size of nano silver (25 nm) than ZnO NPs (125nm).

The antibacterial properties of 1.23% and 13% zinc oxide in orthodontic adhesives were reported by Spencer et al.They observed that zinc oxide was able to reduce decalcification that happens during orthodontic treatments [13].

Our findings on the flexural strength are in contrast with those of Tavassoli-Hojjati, et al. [6]. They suggested that due to the limited concentration and the small size of ZnO NPs, it can be assumed that these particles had played the role of fillers in the composite resin, and by filling the free spaces and defects inside the resin matrix, they had improved the mechanical properties of the restoration. However, the average size of ZnO NPs in the current study (50nm) was two times larger than the particles used in the study of Tavassoli-Hojjati, et al.(20nm) b [6]. On the other hand, we think that due to the opacity of ZnO NPs, they can prevent adequate light penetration into the mass of composite resin.

Neves, et al.[14]. Have also reported better compressive strength and modulus by addition of 0.3% silver NPs to composite resin compared to control group; while these properties were decreased by incorporation of 0.6% wt silver NPs. The size of NPs used in the study of Neves et al is the same as the particle size in the present study. The results obtained for 0.6% NPs of silver is in concurrence with the results for the mechanical properties of NP containing composite resin in the present study.

In spite of the decrease in flexural strength of the composite resins mixed with nano-particles of zinc-oxide, these groups had still a clinically acceptable flexural strength, reported for flowable composites (minimum 70 MPa)[15] and determined by ISO 4049 for composite resins.Therefore, considering that both 0.5% and 1% nano-particles are reported to have antibacterial properties, using composite resins with 0.5% zinc-oxide nano-particles is recommended as antibacterial composites to have less detrimental effects on the mechanical properties of the restoration compared to 1%nano-particles.

Degree of conversion was lower in composites containing ZnO NPs compared to the control group. This can be due to the interference of the unbounded NPs dispersed within the composite resin matrix, which prevent monomers from correct activation, attachment and polymerization. The results of the present study on degree of conversion are in contrast with Tavassoli-Hojati, et al. [6]; which is assumed to be related to the difference in size of the studied NPs between the two studies.

Water sorption did not have a significant difference between the study groups, while a slight decrease in sorption was observed by increasing the amount of ZnO NPs.Although ZnO is hydrophilic in nature, the NPs could have played the role of fillers in the matrix of the flowable composite and decreased the percent of methacrylate resin monomers, causing a slight reduction in water sorption of the antibacterial composite resin.

ZnO NPs mixed with composite resin increase the opacity of the restorative material and impair its esthetic appearance; which

prevents the usage of these antibacterial composites in esthetic applications. However, smaller sizes of NPs might cause less opacity which should be studied more.ZnO NP-loaded composites can be most useful in situations with higher risk of caries incidence, such as under orthodontic brackets, or as a layer in the gingival portion of posterior composite restorations.

Future studies should focus on methods to bond these nano-particles to monomers of the composite resin. In this way, not only the decrease in mechanical and physical properties of the material would minimize, also the nano-particles would not release quickly from the composite resin and the antibacterial properties of the composite resin would be retained for a longer period. Recent studies that have bonded other antibacterial agents such as quaternary ammonium and chlorhexidine to composite resin components, have reported better physical properties compared to the composites with directly mixed antibacterial agents[9,16].

Within the limitations of the present study, it was concluded that incorporation of 0.5 and 1% wtZnO NPs with 50nm diameter particle size into flowable composite resin,imparted antibacterial properties against MS and L; However, some physical and mechanical properties such as flexural strength, micro-hardness, and degree of conversion were lower in ZnO NP-loaded composites compared to the control group. These properties were lower in composite containing 1% ZnO NP than 0.5% NP. ZnO NPs incorporated into composite resin did not cause any significant changes in water sorption of the studied composite resin.

Acknowledgements

Conflict of interests

The Authors of this manuscript declare that they had no conflict of interests.

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