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Antibacterial, physical and mechanical properties of flowable resin composites containing zinc oxide nanoparticles

Sara Tavassoli Hojati[a,b], Homayoon Alaghemand[c], Faeze Hamze[d], Fateme Ahmadian Babaki[e], Ramazan Rajab-Nia[f], Mohammad Bagher Rezvani[g,b], Mehrnoosh Kaviani[h], Mohammad Atai[i,*]

[a] Pediatric Department, Shahed Dental School, Tehran, Iran
[b] Research Center, Shahed Dental School, Tehran, Iran
[c] Operative Department, Dental Material Research Center, Babol University of Medical Science, Babol, Iran
[d] Operative Department, Dental School, Kerman University of Medical Science, Kerman, Iran
[e] Pediatric Department, Dental School, Shahid Beheshty International University of Medical Science, Tehran, Iran
[f] Microbiology Department, Medical School, Babol University of Medical Science, Babol, Iran
[g] Operative Department, Shahed Dental School, Tehran, Iran
[h] General Dentist, Iran
[i] Iran Polymer and Petrochemical Institute (IPPI), P.O. Box 14965/115, Tehran, Iran

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ABSTRACT

Objectives. The aim of this study is evaluating the antibacterial activity of resin composites containing ZnO nanoparticles against *Streptococcus mutans* and examining their physical and mechanical properties.

Methods. The properties of flowable resin composites containing 0–5 wt.% nano-ZnO are investigated using different tests:

A. Antibacterial activity (including agar diffusion test on the cured resins, direct contact test using bacteria in a liquid medium, evaluating the effect of aging while the samples are adjacent to a liquid medium, and scanning electron microscopy (SEM)).
B. Mechanical behavior (including flexural and compressive strength and modulus).
C. Curing aspects (including depth of cure and degree of conversion).
D. Adhesion properties (including micro-shear bond strength).

Results. Although the agar diffusion test reveals no significant difference between the groups, the direct contact test demonstrates that by increasing the nanoparticle content, the bacterial growth is significantly diminished ($p < 0.05$). In the aging test, however, the antibacterial properties reduce significantly ($p < 0.05$). The flexural strength and compressive modulus remains unchanged by incorporation of nanoparticles ($p > 0.05$) while the compressive strength and flexural modulus significantly increase ($p < 0.05$). The ZnO containing resins show significantly lower depth of cure ($p < 0.05$), and higher bond strength ($p < 0.05$). There is

* *Corresponding author*. Tel.: +98 21 48662446; fax: +98 21 44580023.
E-mail addresses: M.Atai@ippi.ac.ir, m.atai@yahoo.com (M. Atai).
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no significant difference between the degrees of conversion, measured by FTIR technique, of the groups ($p > 0.05$).

Significance. Production of a dental resin composite with antibacterial activity without significant sacrificing effect on the mechanical properties is desirable in dental material science.

1. Introduction

Secondary caries are defined as "positively diagnosed carious lesion occurs at the margins of an existing restoration" [1]. This phenomenon remains the most frequent reason leading to shortening the service life of dental restorations [2] and finally results in the need for the replacement of the restorative materials [3].

The main cause of enamel demineralization is adhesion of micro-organisms to the teeth and/or restorations which produces pathogenic plaque. Therefore, one of the most applicable methods for preventing enamel demineralization around the restorations is using dental materials resistant to the bacterial accumulation [4]. On the other hand, as various laboratory and clinical researches have demonstrated, comparing to either other restorative materials or dental hard tissues, more plaque accumulation occurs on resin composites which results in higher prevalence of secondary caries around composite resin restorations [5]. The more biofilm formation on resin composites is related to its surface roughness and free energy, that is the outcome of resin type, filler size, and percentage of filler. Moreover, not only none of the resin composite components have bacteriostatic property, but also they are metabolized or eaten away by microorganisms [6]. Therefore, recent studies pay growing attention to the antibacterial activity of composite resins in order to reduce the risk of recurrent decay around esthetic direct restorative materials. Different approaches have been used to impart the antibacterial activity into the resin based dental composites and adhesives. The first approach is the incorporation of antibacterial agents into the resin matrix which are released over the time inhibiting the bacterial growth. Examples of the agents are fluoride and chlorhexidine [7–9]. Although the agents are initially strong antibacterial agents, their release rates do not last for long periods. In addition, the dental composites containing the agents have shown a higher rate of bond failure because their mechanical properties are diversely affected [10]. The other approach is the inclusion of quaternary ammonium functionality in the resin monomers or as an additive [11–17]. It seems the approach is more promising as the researches have reported higher longevity for the antibacterial activity of the composites containing the materials. The third approach is the incorporation of metal (oxide) particles/ions into the restorative materials [18,19]. From centuries ago, metals such as silver, gold, and zinc have been used as bactericidal and bacteriostatic agents [20–22]. The antibacterial efficacy of metals is directly depends on their total contact surface area. The nano-scale dimensions of nanoparticles allow a considerable broader gamut of interactions with microorganisms increasing their antibacterial activities.

Streptococcus mutans is one of the major species of bacteria responsible for dental caries [23]. Several researches argued that among metallic agents, silver nanoparticles are the most effective metal for inhibiting the growth of *S. mutans* [24]. However, the major drawback of silver in restorative dental materials is the cosmetic changes of tooth colored materials [24]. Hence, insoluble, tooth-colored, or colorless metal oxide powders such as silica, zirconia, alumina and zinc oxide (ZnO) may be more interesting in dental composites. Although the incorporation of ZnO into dental composites may impart antibacterial activity [5], the opacity of the particles against visible light may adversely affect the light curing and, consequently, the mechanical properties of the composites.

The purpose of this study is to evaluate the hypothesis whether the addition of minute amounts of ZnO-NPs into a flowable resin composite would affect its bond strength, physical and mechanical properties, and antimicrobial activity.

2. Materials and methods

2.1. Preparation of test specimens

In this study we had six experimental groups consisting of five resin composites containing ZnO-NPs in different concentrations of 1, 2, 3, 4, and 5 wt.% and one control group with no additive. The tested materials were prepared by the incorporation of the ZnO-NPs (average particle size of 20 nm with hexagonal crystal structure and 99.8% purity) (Nanopars Espadana, Isfahan, Iran) into the dental restorative resin composite Heliomolar Flow (Ivoclar Vivodent AG, FL-9494 Schaan/Liechtenstein). The nanoparticle powder was added to the resin composite and homogeneously mixed in a dark room for 15 min with a glass spatula. These modified composites were stored in completely opaque bottles until each test were performed.

2.2. Anti-bacterial tests

2.2.1. Bacterial strains and growth conditions

S. mutans PTCC 1683 (Persian Type Culture Collection, IROST, Iran) were used in this study. The bacteria were cultured aerobically overnight in 5 ml of brain–heart infusion (BHI) (High Media, India), at 37 °C.

2.3. Preparation of the microtiter tubes

A microtiter tube (500 μl, Zimax K.A., Iran) was vertically positioned. Using a flat-ended dental instrument (dental spatula) the side walls of twelve dishes were coated evenly with an equal amount of the tested material (200 μl in each tube). The materials were polymerized for 280 s with an overlapping

regimen by a light curing unit (LED, DEML, SDS Kerr, USA, with an intensity of circa 800 mW cm^{-2}) in seven 40 s cycles from the top and the outside wall of the tubes.

2.4. Direct contact test (DCT)

The direct contact test (DCT) was performed to determine the antibacterial activity of the tested materials [25,26]. A 10 μl of bacterial suspension (~10^6 bacteria) was placed on the surface of each tested material in a set of three tubes for each group. The tubes were then incubated in vertical position for 1 h under a sterile condition. During the incubation period, the suspension liquid was evaporated to obtain a thin layer of bacteria, ensuring direct contact between the bacteria and the tested surface. A 10 μl of the bacterial suspension was placed on the uncoated walls of three tubes, which served as control. Then 300 μl of BHI broth were added to each tube containing the material [17]. After 3, 6, 12 and 24 h, aliquots of 50 μl of the mixture (bacteria + BHI broth) were spread on blood agar plates (High Media, India and defibrinated sheep blood) and incubated at 37 °C for 24 h subsequently. The bacterial colonies were then counted.

2.5. Material aging

Similar microtiter tubes were prepared with the tested materials and aged for 48 h, 1 and 4 weeks. During this period each well was filled with 300 μl PBS (Phosphate Buffered Saline), which was replaced every 48 h, and the plates were incubated at 37 °C. The PBS was aspirated and the plates were dried under sterile condition before the DCT test [17].

2.6. Agar diffusion test

200 μl of bacterial suspension was spread on blood agar. Uniform resin composite test-disks (2 mm thick and 8 mm in diameter) were prepared by pressing the sample resins between two glass slides to obtain smooth surfaces. The disks were photopolymerized for 40 s using the light curing unit from the top side and an extra 40 s cycle was repeated from the bottom side in order to ensure complete photopolymerization. Test-disks of the resin composite Heliomolar Flow with 0, 1, 2, 3, 4 or 5 wt.% ZnO-NPs contents were placed on the surface of the mentioned blood agar plates. The plates were incubated for 24 h at 37 °C and the inhibition zone around each specimen was measured in mm scale [17].

2.7. Scanning electron microscopy

New resin composite disks prepared as previously described. A 10 μl of bacterial suspension (~10^6 bacteria) was placed on the surface of each specimen and incubated for 1 h at 37 °C. Evaporation of the suspension liquid resulted in a thin layer of bacteria, ensuring direct contact between the bacteria and the specimen surface. The samples were fixed with glutaraldehyde and osmium tetroxide solutions, dehydrated in a graded ethanol series, and then gold coated. An additional set of disks was processed as above and incubated for 24 h in 5 ml of BHI broth. The test specimens were then examined by scanning electron microscopy (HITACHI, S-4160, Japan, Field Emission Electron Microscopy (FE-SEM)) [17].

2.8. Measurement of degree-of-conversion

The degree of photopolymerization conversion of specimens containing 0, 1, 2, 3, 4 and 5 wt.% ZnO-NPs was measured by FTIR (EQUINOX 55, Bruker, Germany) spectroscopy. The specimens were placed between two polyethylene films, pressed to form a very thin film and the absorbance peaks of the un-cured samples were obtained. The specimens were then light-cured for 40 s using the light source and the peaks were collected for the cured specimens.

Degree of conversion (DC%) was determined from the ratio of absorbance intensities 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. The degree of conversion was then calculated as follows [27]:

$$DC\% = \left(1 - \frac{(1638\,cm^{-1}/1608\,cm^{-1})\ \text{peak area after curing}}{(1638\,cm^{-1}/1608\,cm^{-1})\ \text{peak area before curing}}\right) \times 100$$

For each group of resin composites the measurement was repeated for three times.

2.9. Measurement of the depth of cure

The depth of cure of resin composite was determined following the ISO 4049 (2000) standardized technique. The composite resins were inserted into a stainless-steel split mold with a cylindrical cavity of 10 mm height and 4 mm diameter while the top of the mold were covered with transparent polymer strips. The specimens were then light-cured for 40 s from the top. Immediately after irradiation, uncured materials were scraped away with a plastic spatula. Subsequently, the height of the cured resin was measured in three different places using a digital micrometer (Mitotoyo, Japan). The measured values were divided by 2 and the average of three measurements was then reported as the depth of cure.

2.10. Flexural strength and modulus

Flexural strength is one of the most important mechanical tests for assessing the performance of dental resins. According to ISO 4049, the resins were inserted in a rectangular stainless-steel mold with 2 mm × 2 mm × 25 mm dimensions, which was placed on a glass slide. Then, the mold was covered with another glass slide and specimens were cured from both top and bottom sides by a light-curing unit irradiated for 40 s in each spot using an overlapping regime. The specimens were removed from the mold and stored in deionized water for 1 day at 37 °C prior to the test. Both surfaces of all specimens were polished using a 600 grit silicon carbide paper in a moist environment. At least 7 specimens were tested for each formulation. A three-point bending test was performed using a universal testing machine (Z20, Zwick Roell, Germany) at a

cross-head speed of 0.5 mm min^{-1}. The flexural strength (FS) in MPa was calculated as:

$$FS = \frac{3PL}{2bd^2}$$

where P stands for load at fracture (N), L is the span length (20 mm), and b and d are, respectively, the width and thickness of the specimens in millimeter. The elastic modulus was also determined from the slope of the initial linear region of stress–strain curve.

2.11. Compressive strength and modulus

According to ISO 9917 for the compressive strength tests, the stainless steel cylindrical molds with diameter of 4 mm and height of 6 mm were placed on a glass slide and then overfilled with the resin composites. After complete filling of the mold, another glass slide was pressed on the top side and the whole materials cured for 40 s from each end. The lateral sides of the cylindrical resin specimens were cured for further 40 s in order to achieve higher polymerization. The specimens of each group were stored in water at 37 °C for 24 h prior to test. The compressive strength was then determined with the universal testing machine at a cross-head speed of 1 mm min^{-1}. The specimens (no. = 5) were placed with their flat ends between the plates of the testing machine so that the progressively increasing compressive load was applied along the long axis of the specimens [28].

2.12. SEM and EDX-mapping

Microstructure of the fracture surfaces obtained from mechanical test was analyzed using SEM (TESCAN, VEGAII, XMU, Czech Republic). The samples were mounted on the aluminum stub using carbon-coated double sided adhesive tape and then coated with gold using sputter coater. In addition, EDX elemental composition analyzer (EDXA, QX2, RONTEC Co., coupled with SEM) was used to map the distribution of ZnO-NPs in resin samples. A probe current of 2.0×10^{-9} A, an accelerating voltage of 30 kV, and spot size of 500 nm with a collection time of 100 s, were used during the mapping.

2.13. Micro-shear bond test

A total of 30 caries-free extracted human premolars were used in this in vitro study. The teeth were washed under running water immediately after extraction and stored in formaldehyde 12% for 1 week. Consequently, they were stored in 0.9% NaCl solution until the micro-shear bond strength test were performed [29]. Using an air water dental turbine, the teeth were cut from the cervical area and then the crown of each tooth was sliced into a buccal and a lingual part. Hence, we had 60 specimens which were randomly divided into six groups. The enamel surfaces were polished with a 280 grit silicon carbide paper (Soft Flex, Germany) to remove the prismless layer. Teeth were etched with a 37% phosphoric acid solution (Total Etch 37%, Ivoclar Vivadent) for 15 s, rinsed with water jet spray for 5 s, and air dried. A commercially available bonding agent (Exite, Ivoclar Vivadent) was applied on the surface of each slice following the manufacturer's instruction. Prior to light-curing, a cylinder tube (internal diameter: 0.75 mm, height: 1.0 mm) were placed on the enamel surface. After 20 s irradiation using a light cure unit, each tube was carefully filled with one of the mentioned six experimental resin composites and then photopolymerized for 40 s from the top of the tube. The tubes around the composite cylinders were removed by gently cutting the tube using a surgery blade, the cylindrical composite were light cured three more 40 s cycles from the right, left and top sides with the purpose of being ensure about the complete polymerization. Having stored for 24 h in water at 37 °C, each specimen was adhered to the testing apparatus with a cyanoacrylate adhesive. A thin wire (diameter 0.2 mm) was looped around each composite cylinder, so that the wire was in contact with the lower half-circle of the cylinder and the tooth surface. Pulling up the wire, a shear force was applied to each specimen at a cross-head speed of 1 mm min^{-1} using the universal testing machine until failure occurred. The micro-shear bond strength was then calculated by dividing the force at break by the composite–enamel interface area [30]. The fracture surfaces were evaluated under the stereo-microscope and SEM in order to determine the mode of bond failure.

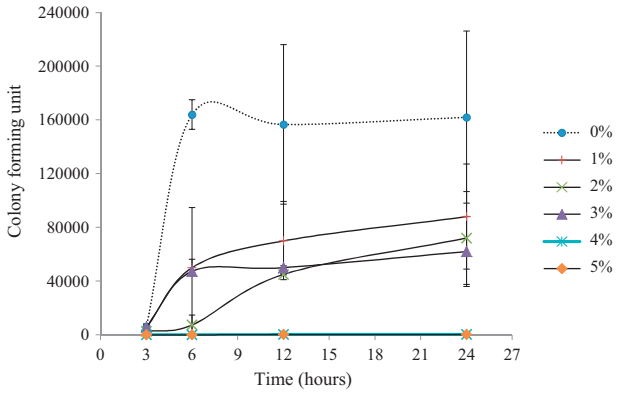

Fig. 1 – Colony forming unit following direct contact between *S. mutans* and resin composites containing 0–5 wt.% ZnO-NPs. The legend shows the weight percents of the loaded nanoparticles. Y-error bars represent standard deviation.

2.14. Data analysis

The data were analyzed using SAS software (version 9.1) by one-way ANOVA, and the Tukey post hoc HSD multiple comparison test. The level of significance was determined as $p = 0.05$.

3. Results

3.1. Direct contact and aging tests

The results of bacterial colony count (Colony Forming Unit, CFU) regarding the direct contact and material aging test after 48 h, 1 week and 4 weeks are depicted in Figs. 1–4, respectively. Each point on the curve represents the mean value measured from three tubes containing the same test material. As can

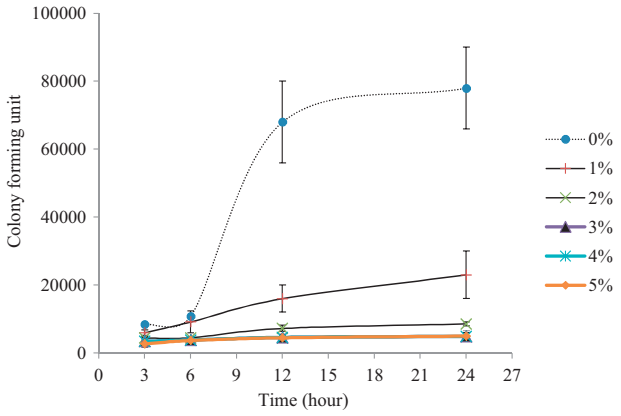

Fig. 2 – Colony forming unit following direct contact between *S. mutans* and 48 h-aged resin composites containing 0–5 wt.% ZnO-NPs. The legend shows the weight percents of the loaded nanoparticles. Y-error bars represent standard deviation.

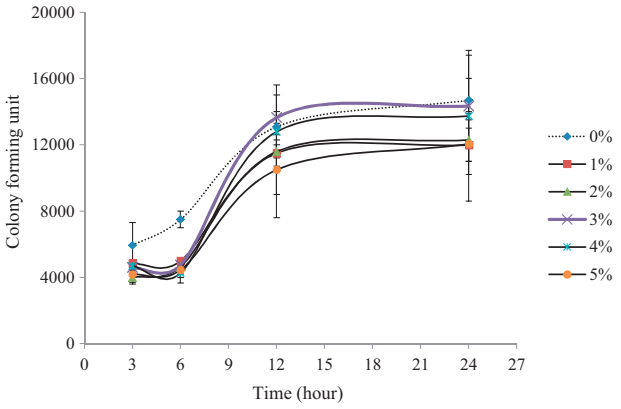

Fig. 3 – Colony forming unit following direct contact between *S. mutans* and 1-week-aged resin composites containing 0–5 wt.% ZnO-NPs. The legend shows the weight percents of the loaded nanoparticles. Y-error bars represent standard deviation.

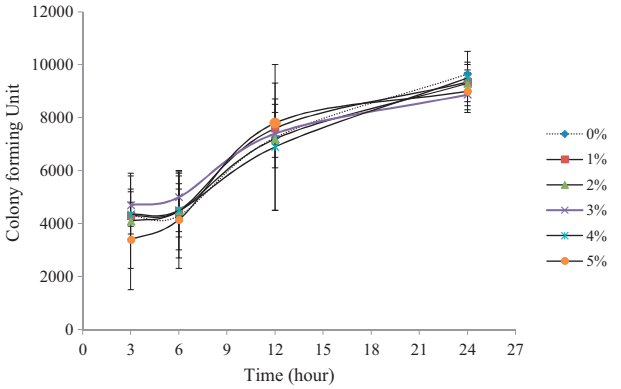

Fig. 4 – Colony forming unit following direct contact between *S. mutans* and 4-week-aged resin composites containing 0–5 wt.% ZnO-NPs. The legend shows the weight percents of the loaded nanoparticles. Y-error bars represent standard deviation.

be seen in Fig. 1, in direct contact test as the NPs increase the CFU decrease and there is significant difference between groups ($p < 0.05$). Meanwhile, both of the 4 and 5 wt.% groups entirely inhibit formation of any bacterial colony.

Fig. 2 demonstrates a strong antibacterial effect after an aging period of 48 h in all the resin composite specimens in which the ZnO-NPs were incorporated, compared with the control specimen ($p < 0.05$). However, there is no significant difference between either of the 1, 2, 3, 4 or 5 wt.% groups ($p > 0.05$). Moreover, as it is evident in Figs. 3 and 4, after 1 week or 4 weeks incubation all the six tested groups are roughly similar with each other against *S. mutans* and there is no significant difference between them ($p > 0.05$).

3.2. *Agar diffusion test*

There were no inhibition zones around the specimens of different groups on the agar medium containing *S. mutans* strain (data not shown).

3.3. *Scanning electron microscopy*

Surface view of specimens following direct contact of *S. mutans* with the resin composite after 1 h incubation at 37 °C are shown in Fig. 5. Microorganisms are visible in all SEM micrographs, whilst low quantities of colonies and smaller ones are observed in higher NPs contents.

SEM micrographs of the specimens incubated at 37 °C for 24 h were somehow similar to the 1 h incubated ones (data not shown).

3.4. *Degree of conversion*

The data which are obtained from FTIR spectroscopy are displayed in Fig. 6 as the mean values of DC% in six groups. Tukey post hoc test revealed that there is no significant difference between the DC% of the groups ($p > 0.05$).

3.5. *Depth of cure*

The mean depth of cure values showed significant differences ($p < 0.05$). It is seen in Fig. 7 that as the NPs content increase, the depth of cure notably decreases. Accordingly, the curing depth of the 5 wt.% group is about the half of the unmodified resin group.

3.6. *Flexural strength and modulus*

As Fig. 8 illustrates there is no significant difference between flexural strength of the specimens containing different ZnO-NPs content ($p > 0.05$). Incorporation of the nanoparticles into the resin composite, however, resulted in an increase in the flexural modulus of the composites with a maximum corresponding to 4 wt.% ZnO-NPs content ($p < 0.05$) (Fig. 9).

3.7. *Compressive strength and modulus*

Comparison of compressive strength, presented in Fig. 10, showed an increase in the compressive strength of the sample containing 1 wt.% nanoparticle ($p < 0.05$).

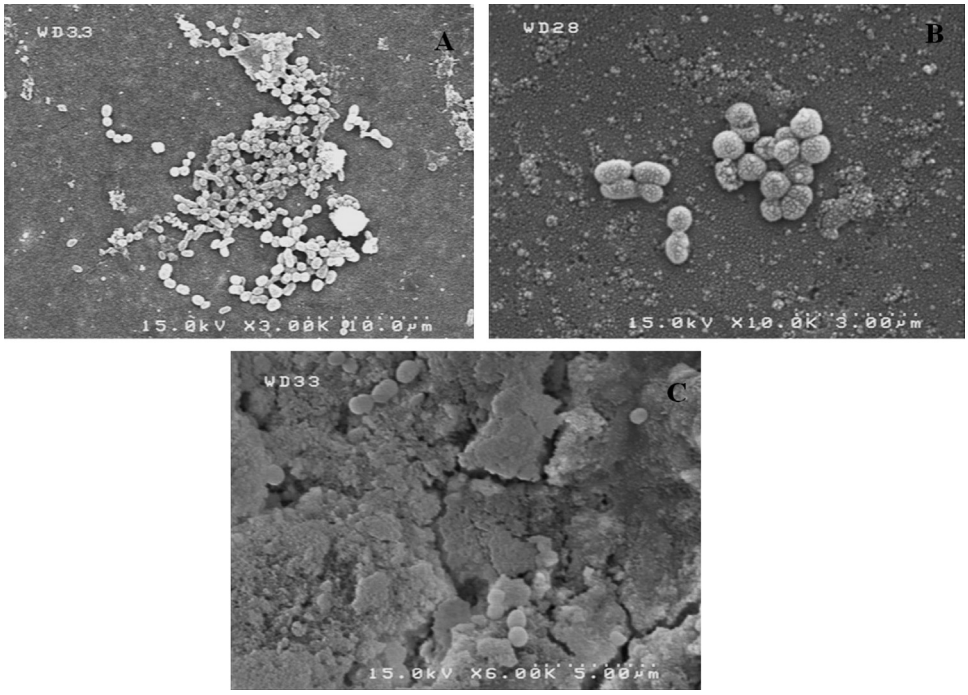

Fig. 5 – SEM micrograph of *S. mutans* cells in direct contact with resin composite after 1 h incubation at 37 °C, showing bacterial attachment and growth (A, B and C, respectively, represent composites containing 0, 3 and 5 wt.% ZnO-NPs).

Statistical comparison also revealed that there is no significant difference between the composites of different groups ($p > 0.05$), shown in Fig. 11.

3.8. *SEM and EDX-mapping*

EDX mapping performed on the fracture surface of the 5 wt.% ZnO-NPs specimen (Fig. 12) demonstrates homogenous distribution of Zn element in the specimen which proves a good distribution of NPs in the resin matrix.

3.9. *Micro shear bond strength*

As Fig. 13 illustrates, the highest and lowest bond strengths is related to 1 wt.% and 5 wt.%, respectively ($p < 0.05$). There was no significant difference between the bond strengths of the other groups ($p > 0.05$).

Stereo-microscope observation (not shown here) and SEM evaluation (Fig. 14) revealed that the failure mode in the test was mostly adhesive in the adhesive–enamel interface.

4. Discussion

The results of the current study show that incorporation of ZnO-NPs into the resin composites could significantly inhibit the *S. mutans* strains, without sacrificing the mechanical properties of the resins except that it would diminish the depth of cure of the resin at high NPs contents.

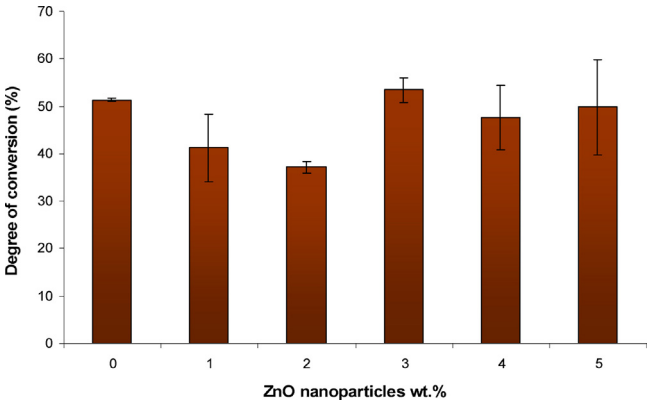

Fig. 6 – Degree of conversions of resin composites containing 0–5 wt.% ZnO-NPs. Y-error bars represent standard deviation.

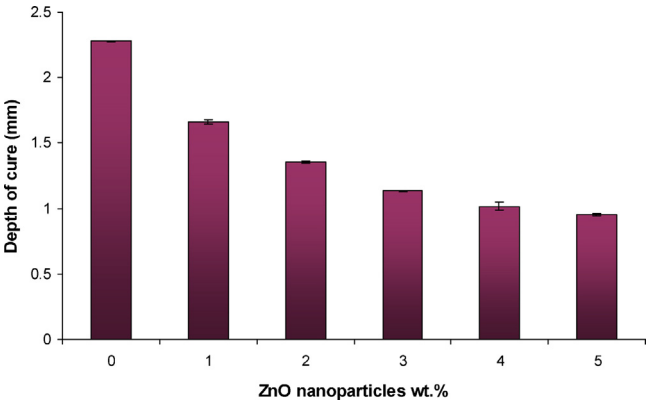

Fig. 7 – Depth of cure (mm) of resin composites containing 0–5 wt.% ZnO-NPs. Y-error bars represent standard deviation.

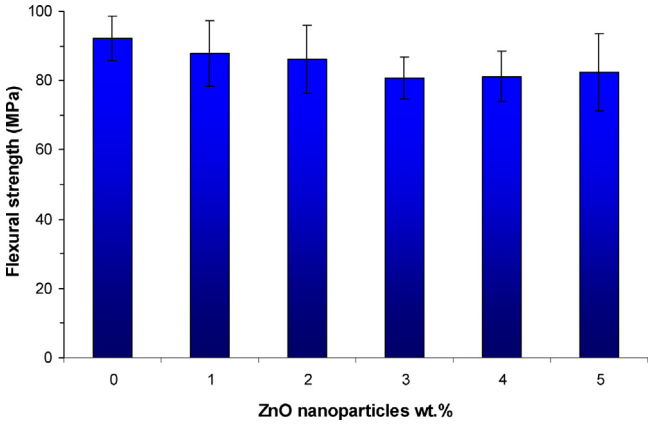

Fig. 8 – Flexural strength (MPa) of resin composites containing 0–5 wt.% ZnO-NPs. Y-error bars represent standard deviation.

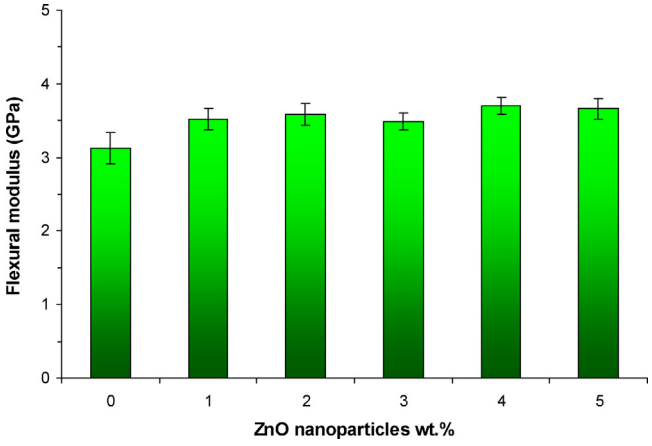

Fig. 9 – Flexural modulus (GPa) of resin composites containing 0–5 wt.% ZnO-NPs. Y-error bars represent standard deviation.

Our findings in the agar diffusion test were in agreement with those of Sevinc et al. as there were no inhibition zones around the test specimens [5]. Although Sevinc et al. did not report any inhibition zone around the specimens containing

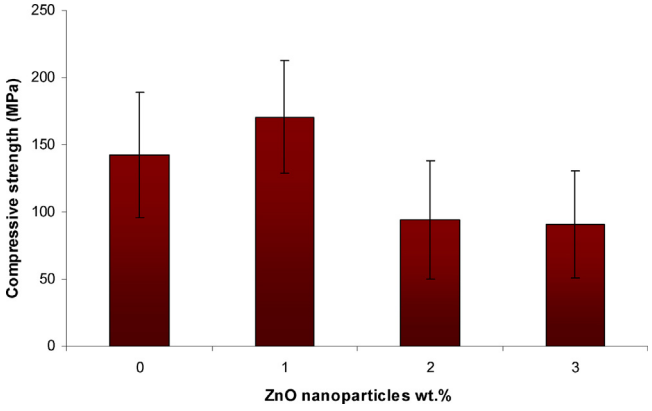

Fig. 10 – Compressive strength of the ZnO-NPs containing resin composites. Y-error bars represent standard deviation.

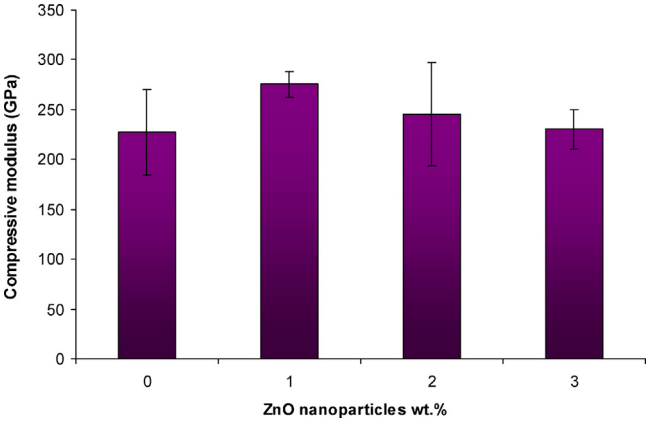

Fig. 11 – Compressive modulus of resin composites containing ZnO-NPs. Y-error bars represent standard deviation.

ZnO-NPs, they showed an inhibition zone on the agar medium around the resin specimens containing Ag-NPs. They concluded that, due to the insolubility of the ZnO-NPs, a sufficient amount of Zn^{2+} could not leach to the surrounding environment to establish an antibiotic efficacy while the Ag^{+} may release and develop the inhibition zone around tested specimens. Therefore, the ZnO-NPs could not be considered as an effective non-contact antibacterial agent [5].

The agar diffusion test and the minimum inhibitory concentration are most important traditional methods for evaluating the antibacterial behavior of many dental and medical materials [31]. The mechanism of these methods, which are frequently used investigating the antibiotics, is based on water-soluble components released from the bulk of the materials. Accordingly, the examination of the antibacterial

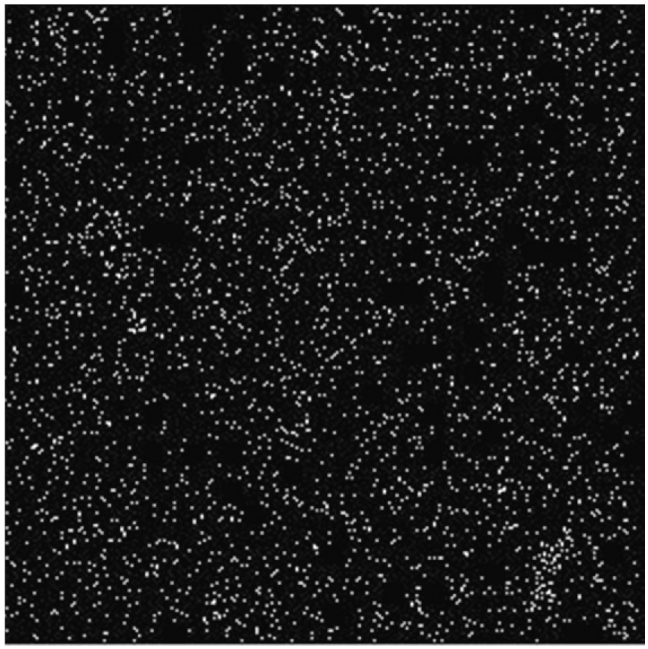

Fig. 12 – EDX Zn map of the resin composite containing 5 wt.% ZnO-NPs. White spots represent Zn element.

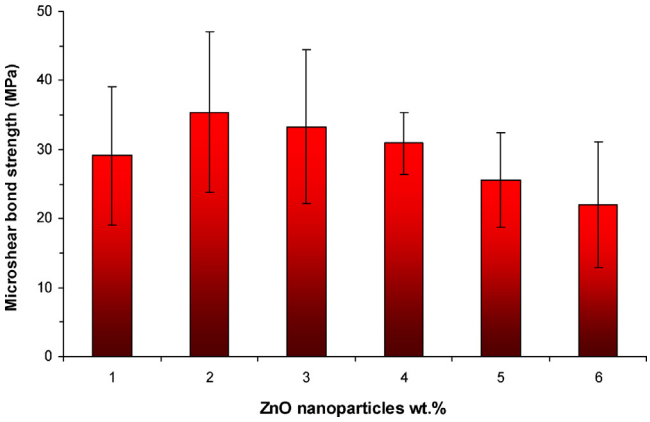

Fig. 13 – Micro-shear bond strength of resin composites containing 0–5 wt.% ZnO-NPs. Y-error bars represent standard deviation.

materials with low solubility by these methods is not valid [26]. However, one of the most important requirements of dental restorative materials is that they should have quite low solubility in an aqueous environment accomplishing their task in the oral cavity. The DCT is a test for evaluation of solid materials that has extremely low soluble components. In DCT method, bacteria have a controlled direct contact with the desired material, after which the amount of the bacterial growth is quantified and analyzed [17].

As can be seen in Fig. 1, the DCT results revealed that ZnO-NPs could endow the resin with antibacterial activity, which was significantly enhanced as the concentration increased.

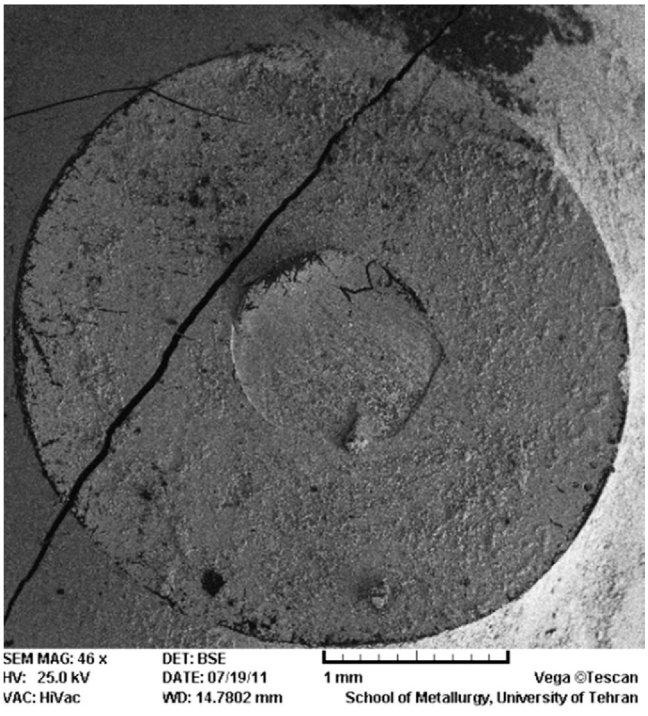

Fig. 14 – SEM of the composite-bonding-enamel interface showing the failure mode of the adhesive layer. The failure was mostly adhesive between enamel and bonding.

This finding is similar to the results of Niu et al. [32] and Sevinc et al. [5] whom resin test specimens contained up to 10% ZnO-NPs and also some of the other previous studies [33]. Nevertheless, Niu et al. also confirm that the incorporation of up to 5% ZnO-NPs into the resin is preferred in comparison to 10% ZnO-NPs to give appropriate antibacterial activity [32].

In order to estimate the sustainability of the ZnO-NPs efficacy in the resin matrix the aging test was performed. The results, demonstrated in Fig. 2, showed that after 48 h immersing in PBS the ZnO-NPs containing groups exhibited significantly lower CFU in comparison to the unmodified flowable composites ($p < 0.05$). After more than 1 week, however, there was no significant difference between the six groups (Figs. 3 and 4) ($p > 0.05$). Although the prior studies proposed that ZnO-NPs are insoluble and the antibacterial effect of ZnO-NP composites would not diminish over time [5], our aging test displayed that the efficacy of antibacterial activity of ZnO-NPs in the resin matrix in a liquid environment is questionable. Sevinc et al., who investigated the biofilm growth on the surface of 10% ZnO-NP-composites after multiple cycles, reported that although in the second cycle the ZnO-NP-composites showed considerable lower bacterial growth comparing to their control unmodified resins, in the third cycle they did not differ significantly with each other [5]. Their finding is close to ours. The 48 h aged group received only one cycle of immersing in the PBS while the 1 week group received three cycles because the PBS was refreshed every 48 h. Nonetheless, our specimens were immersed in a new environment while Sevinc et al. just scraped the surface of their test specimens between each cycle [5].

Turbidity or optical density absorbance has been used for the evaluation of antibacterial properties of resin composites containing different antibacterial particles [17]. The turbidity measurements, however, have an important limitation as they consider both dead and vital bacteria in liquid environment [34]. In the present study, as we used the colony count method, only the number of the live bacterial cells is ranked at the end of the mentioned time intervals.

One of the mechanisms explaining the antimicrobial behavior of ZnO-NPs is that they produce different active oxygen species like H_2O_2 which inhibit growth of viable microbes. Some metal oxide NPs such as TiO_2 demonstrate antimicrobial activities based on the same mechanism [35]. However, the production of the active oxygen is just possible in the presence of ultraviolet light, so, in the absence of light, TiO_2-NPs are apparently unable to significantly inhibit the bacterial growth. ZnO-NPs may also exhibit photocatalytic behavior under ultraviolet light, leading to the production of antimicrobial active oxygen species [36]. But, the antibacterial character of ZnO-NPs also could be detected without exposing to any light source [36,37].

Another mechanism for the antimicrobial activity of ZnO-NPs could be attributed to the possible interaction between the nanoparticles and bacteria caused by electrostatic forces which are promoted by exposure to the light [38].

Zn^{2+} ions interfere with the bacterial enzyme systems by displacing with magnesium ions which is essential for enzymatic activity of the dental plaque. Although the possible leaching of Zn^{2+} into the growth media has been reported as an

alternate antimicrobial mechanism of ZnO-NP [39]. The lack of inhibition zone in the agar diffusion test revealed that, at least in the present study, direct contact is of higher importance.

A dental composite should have satisfactory mechanical properties besides the antibacterial activity to be accepted as a successful restorative dental material, especially in positions with heavily occlusal stresses. The physical and mechanical properties which are evaluated in the current study include the flexural and compressive strength, flexural and compressive modulus, degree of conversion, depth of cure and the micro-shear bond strength.

Although there are several methods for assessing the DC% of resin composites, the most sensitive method is the FTIR spectroscopy [40]. No significant difference was observed between the DC% of the tested group. It means that the addition of up to 5% of ZnO-NPs does not affect the DC% of flowable resin composites in the specimens with low thicknesses which were tested in the FTIR technique.

In light-curing systems the curing process depends on different factors including the irradiation energy, the wavelength and penetration of light beam in filled systems. The amount and size of filler particles also affect this phenomenon [41]. As an important aspect, the penetration of light is influenced by transparency of the whole resin and filler of composite [29]. ZnO is opaque against visible light and when incorporated into the resin composite it would adversely affects the curing process. To determine the effect of the ZnO-NPs content on the curing in thick specimens, the depth of cure of the composites were tested according to ISO 4049. The results demonstrate a dramatic decrease in depth of cure ($p < 0.05$) with the increase of ZnO-NPs content (Fig. 7). The curing depth of 5% ZnO-NPs containing group is less than half of the control one, which is free of ZnO-NPs. This could be attributed to the high opacity of the ZnO-NPs.

Since the resin samples containing 4% or 5% ZnO-NPs have a very low depth of cure, we omitted these two groups from the compressive strength test because the prepared test was performed according to ISO 9917 in which the cylinder mold has 4 mm × 6 mm dimensions. Meanwhile, the flexural strength of the test groups was measured using the three point bending test according to ISO 4049 standard. As can be seen in Figs. 8 and 10, both the flexural and compressive strength diminish as the percentage of ZnO-NPs increase. Although there is no statistically significant difference between the flexural strength of the six groups ($p > 0.05$), the compressive strength of the group incorporating 1% of ZnO-NPs is significantly higher than unmodified control group. It could be concluded that the incorporation of ZnO-NPs into the resin matrix may enhance its mechanical property in low percentages. Previous studies also reported that incorporation of various NPs into resin composite would leads to mechanical improvement up to a threshold beyond which more NPs will no further increase the mechanical properties [42]. The enhanced compressive strength at low NPs content is due to their relatively good dispersion, while higher mass fraction of NPs would lead to the formation of bundles and agglomerations leading to defects and flaws which in turn deteriorate mechanical properties of the composite [29,42]. The EDX-mapping results (Fig. 12), however, show a good distribution, at least in the resolution of the mapping, in the group containing up to 5 wt.% ZnO-NPs. Therefore, it could be concluded that the decrease in the mechanical properties is probably more related to the effect of the NPs on the curing of the composite rather than the formation of structural defects due to the agglomeration of the particles.

Normally, the fillers in the resin matrix have the responsibility of mechanical reinforcement and improvement of the modulus of matrix [29]. As Figs. 9 and 11 illustrate the flexural and compressive modulus of the groups increase incorporating up to 1 wt.% nanoparticles ($p < 0.05$) with no further increase in the higher ZnO-NPs contents ($p > 0.05$). The observations could be related to the role of ZnO-NPs in the incomplete curing of system [29].

The most important goal of any tooth bonding system is establishing a strong and durable bond between the restorative material and the tooth tissue. Microshear bond strength test is one of the methods for evaluating the bond strength in dentistry researches [43]. As can be seen in Fig. 13, incorporation of ZnO-NPs into resin matrix would significantly increase the bond strength ($p < 0.05$). This is in consistent with the previous studies reporting that the addition of filler to resins leads to increase in mechanical properties and bond strength [44]. Fig. 13, however, shows that the bond strength tends to decrease at higher ZnO-NPs loadings which might be the result of agglomeration phenomenon [30] and/or increase in the opacity of the composite and incomplete curing. Primary analyzing of the shear bond test area by the stereo-microscope demonstrated that most of the failure modes were adhesive. Further analysis by scanning electron microscopy confirmed the adhesive failure mode (Fig. 14).

Overwhelmingly, as it is represented in Figs. 1–4, our results showed that the group with 1 wt.% ZnO-NPs has a great difference with the 0 wt.% besides that in the aging test the 1% is similar to 4 and 5%. So, we may conclude that incorporation of 1% ZnO-NPs provides beneficial antibacterial properties while the mechanical properties are maintained.

5. Conclusion

With the limitations of this study, we may conclude that:

1. The addition of ZnO-NPs into flowable resin composite would significantly inhibit the growth of *S. mutans*.
2. Incorporation of the NP in minute amounts of about 1 wt.% would not adversely affect the mechanical properties of the composite.

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REFERENCES

[1] McComb D, Erickson RL, Maxymiw WG, Wood RE. A clinical comparison of glass ionomer, resin-modified glass ionomer and resin composite restorations in the treatment of cervical caries in xerostomic head and neck radiation patients. Operative Dentistry 2002;27:430–7.

[2] Burke FJ, Crisp RJ, Bell TJ, Healy A, Mark B, McBirnie R, et al. One-year retrospective clinical evaluation of hybrid composite restorations placed in United Kingdom general practices. Quintessence International 2001;32:293–8.

[3] Mjor IA. The reasons for replacement and the age of failed restorations in general dental practice. Acta Odontologica Scandinavica 1997;55:58–63.

[4] Ahn SJ, Lee SJ, Kook JK, Lim BS. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dental Materials 2009;25:206–13.

[5] Aydin Sevinç B, Hanley L. Antibacterial activity of dental composites containing zinc oxide nanoparticles. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2010;94:22–31.

[6] Sehgal V, Shetty VS, Mogra S, Bhat G, Eipe M, Jacob S, et al. Evaluation of antimicrobial and physical properties of orthodontic composite resin modified by addition of antimicrobial agents – an in-vitro study. American Journal of Orthodontics and Dentofacial Orthopedics 2007;131:525–9.

[7] Burke F, Ray N, McConnell R. Fluoride-containing restorative materials. International Dental Journal 2006;56:33–43.

[8] Wiegand A, Buchalla W, Attin T. Review on fluoride-releasing restorative materials – fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. Dental Materials 2007;23:343–62.

[9] Leung D, Spratt DA, Pratten J, Gulabivala K, Mordan NJ, Young AM. Chlorhexidine-releasing methacrylate dental composite materials. Biomaterials 2005;26:7145–53.

[10] Jedrychowski JR, Caputo AA, Kerper S. Antibacterial and mechanical properties of restorative materials combined with chlorhexidines. Journal of Oral Rehabilitation 1983;10:373–81.

[11] Imazato S, Imai T, Russell R, Torii M, Ebisu S. Antibacterial activity of cured dental resin incorporating the antibacterial monomer MDPB and an adhesion promoting monomer. Journal of Biomedical Materials Research 1998;39:511–5.

[12] Beyth N, Yudovin-Farber I, Bahir R, Domb AJ, Weiss EI. Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against *Streptococcus mutans*. Biomaterials 2006;27:3995–4002.

[13] Weng Y, Guo X, Gregory RL, Xie D. Preparation and evaluation of an antibacterial dental cement containing quaternary ammonium salts. Journal of Applied Polymer Science 2011;122:2542–51.

[14] Xu X, Wang Y, Liao S, Wen ZT, Fan Y. Synthesis and characterization of antibacterial dental monomers and composites. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2012;100:1151–62.

[15] Melo MAS, Cheng L, Weir MD, Hsia RC, Rodrigues LK, Xu HH. Novel dental adhesive containing antibacterial agents and calcium phosphate nanoparticles. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2012, http://dx.doi.org/10.1002/jbm.b.32864.

[16] He J, Söderling E, Lassila LV, Vallittu PK. Incorporation of an antibacterial and radiopaque monomer in to dental resin system. Dental Materials 2012;28:e110–7.

[17] Beyth N, Houri-Haddad Y, Baraness-Hadar L, Yudovin-Farber I, Domb AJ, Weiss EI. Surface antimicrobial activity and biocompatibility of incorporated polyethylenimine nanoparticles. Biomaterials 2008;29:4157–63.

[18] Cheng L, Weir MD, Xu HH, Antonucci JM, Lin NJ, Lin Gibson S, et al. Effect of amorphous calcium phosphate and silver nanocomposites on dental plaque microcosm biofilms. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2012;100:1378–86.

[19] Niu L, Fang M, Jiao K, Tang L, Xiao Y, Shen L, et al. Tetrapod-like zinc oxide whisker enhancement of resin composite. Journal of Dental Research 2010;89:746–50.

[20] Elsome AM, Hamilton-Miller JM, Brumfitt W, Noble WC. Antimicrobial activities in vitro and in vivo of transition element complexes containing gold(I) and osmium(VI). Journal of Antimicrobial Chemotherapy 1996;37:911–8.

[21] Lansdown AB. Silver in health care: antimicrobial effects and safety in use. Current Problems in Dermatology 2006;33:17–34.

[22] Phan TN, Buckner T, Sheng J, Baldeck JD, Marquis RE. Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. Oral Microbiology and Immunology 2004;19:31–8.

[23] Burne R. Oral streptococci . . . products of their environment. Journal of Dental Research 1998;77:445–52.

[24] Hernandez-Sierra JF, Ruiz F, Pena DC, Martinez-Gutierrez F, Martinez AE, Guillen Ade J, et al. The antimicrobial sensitivity of *Streptococcus mutans* to nanoparticles of silver, zinc oxide, and gold. Nanomedicine 2008;4:237–40.

[25] Beyth N, Domb AJ, Weiss EI. An in vitro quantitative antibacterial analysis of amalgam and composite resins. Journal of Dentistry 2007;35:201–6.

[26] Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. Endodontics and Dental Traumatology 1996;12: 179–84.

[27] Atai M, Ahmadi M, Babanzadeh S, Watts DC. Synthesis, characterization, shrinkage and curing kinetics of a new low-shrinkage urethane dimethacrylate monomer for dental applications. Dental Materials 2007;23:1030–41.

[28] Yli-Urpo H, Lassila LV, Narhi T, Vallittu PK. Compressive strength and surface characterization of glass ionomer cements modified by particles of bioactive glass. Dental Materials 2005;21:201–9.

[29] Sadat-Shojai M, Atai M, Nodehi A, Khanlar LN. Hydroxyapatite nanorods as novel fillers for improving the properties of dental adhesives: synthesis and application. Dental Materials 2010;26:471–82.

[30] Atai M, Solhi L, Nodehi A, Mirabedini SM, Kasraei S, Akbari K, et al. PMMA-grafted nanoclay as novel filler for dental adhesives. Dental Materials 2009;25:339–47.

[31] Karanika-Kouma A, Dionysopoulos P, Koliniotou-Koubia E, Kolokotronis A. Antibacterial properties of dentin bonding systems, polyacid-modified composite resins and composite resins. Journal of Oral Rehabilitation 2001;28:157–60.

[32] Niu LN, Fang M, Jiao K, Tang LH, Xiao YH, Shen LJ, et al. Tetrapod-like zinc oxide whisker enhancement of resin composite. Journal of Dental Research 2010;89:746–50.

[33] Fang M, Chen JH, Xu XL, Yang PH, Hildebrand HF. Antibacterial activities of inorganic agents on six bacteria associated with oral infections by two susceptibility tests. International Journal of Antimicrobial Agents 2006;27: 513–7.

[34] Guggenheim B, Giertsen E, Schupbach P, Shapiro S. Validation of an in vitro biofilm model of supragingival plaque. Journal of Dental Research 2001;80:363–70.

[35] Sunada K, Watanabe T, Hashimoto K. Bactericidal activity of copper-deposited TiO_2 thin film under weak UV light illumination. Environmental Science and Technology 2003;37:4785–9.

[36] Zhou G, Li Y, Xiao W, Zhang L, Zuo Y, Xue J, et al. Synthesis, characterization, and antibacterial activities of a novel

nanohydroxyapatite/zinc oxide complex. Journal of Biomedical Materials Research Part A 2008;85:929–37.
[37] Zhang L, Jiang Y, Ding Y, Povey M, York D. Investigation into the antibacterial behavior of suspensions of ZnO nanoparticles (ZnO nanofluids). Journal of Nanoparticle Research 2007;9:479–89.
[38] Zhang L, Ding Y, Povey M, York D. ZnO nanofluids – a potential antibacterial agent. Progress in Natural Science 2008;18:939–44.
[39] Paunio IK. The effect of certain cations and anions on the alkaline phosphomonoesterase activities in human dental plaque. Acta Odontologica Scandinavica 1970;28:399–415.
[40] DeWald JP, Ferracane JL. A comparison of four modes of evaluating depth of cure of light-activated composites. Journal of Dental Research 1987;66:727–30.
[41] Powers J, Sakguchi R. Craig's restorative dental materials. 12th ed. Mosby: USA; 2006.
[42] Chen L, Yu Q, Wang Y, Li H. BisGMA/TEGDMA dental composite containing high aspect-ratio hydroxyapatite nanofibers. Dental Materials 2011;27:1187–95.
[43] Placido E, Meira JB, Lima RG, Muench A, de Souza RM, Ballester RY. Shear versus micro-shear bond strength test: a finite element stress analysis. Dental Materials 2007;23:1086–92.
[44] Kim JS, Cho BH, Lee IB, Um CM, Lim BS, Oh MH, et al. Effect of the hydrophilic nanofiller loading on the mechanical properties and the microtensile bond strength of an ethanol-based one-bottle dentin adhesive. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2005;72:284–91.