Dental Research Journal

Original Article

Antimicrobial effect of different physical and chemical compounds of zinc oxide and graphene oxide added to composite resins

Zohre Farhangian[1], Homayoon Alaghehmand[2], Hamed Tashakkorian[2], Faraneh Mokhtarpour[2], Abolfazl Davoodabadi[3]

[1]Department Operative of Dentistry, Faculty of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, [2]Dental Materials Research Center, Health Research Institute, Babol University of Medical Sciences, [3]Department of Microbiology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran

Received: 19-Dec-2021
Accepted: 03-Mar-2022
Published: 26-Sep-2022

Address for correspondence:
Dr. Homayoon Alaghehmand, Department Esthetic and Restorative Dentistry, Faculty of Dentistry, Babol University of Medical Sciences, Babol, Iran.
E-mail: halagheh@yahoo.com

ABSTRACT

Background: Graphene oxide (GO), a product of graphite, is a candidate for nano-reinforcing cement-based materials due to its good water dispersibility and excellent mechanical properties. On the other hand, zinc oxide (ZnO) is well-known for its antibacterial characteristics as well. Therefore, we aimed to evaluate the impacts of adding ZnO and GO nanoparticles on the antibacterial properties of flowable composites.

Materials and Methods: In this, *in vitro* experimental study was designated into five groups containing: (1) no nanoparticles as control group, (2) 1 wt.% ZnO nanoparticle, (3) 1 wt.% GO, (4) 1 wt.% physical compound of ZnO and GO, and (5) 1 wt.% chemical compound of ZnO and GO. The antibacterial properties of composite resin discs were evaluated by direct contact test. Data were analyzed using a one-way analysis of variance, followed by Tukey's *post hoc* tests ($P = 0.05$).

Results: *Streptococcus mutans* colony counting in the first 24 h showed the least growth rate in the chemical compound group (2.2×10^5). However, in 7 days, the least colony number was observed in the GO group (2×10^3). Moreover, the physical compound showed the least bacterial adhesion.

Conclusion: Adding GO alone to composites, compared to adding ZnO or physical and chemical compounds of GO-ZnO, was more helpful to increase the antimicrobial characteristics.

Key Words: Composite resins, graphite, nanoparticles, zinc oxide

INTRODUCTION

Composite resins are now used extensively in restorative dentistry. Appropriate mechanical properties, low polymerization shrinkage, and high wear resistance, as well as antibacterial activity, are the essential properties for composite resins.

The antibacterial function of composite resins can be effective in controlling secondary caries adjacent to the filling.[1] Therefore, researchers aimed to produce composite resins with antibacterial along with the desired mechanical properties.

There are different approaches for the antibacterial activity of composite resins and adhesives.[2] The first approach was adding different antibacterial agents to the matrix to inhibit bacterial growth over time. Fluoride and chlorhexidine were the most common antimicrobial agents.[3]

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Farhangian Z, Alaghehmand H, Tashakkorian H, Mokhtarpour F, Davoodabadi A. Antimicrobial effect of different physical and chemical compounds of zinc oxide and graphene oxide added to composite resins. Dent Res J 2022;19:81.

The other approach was incorporating quaternary ammonium into resin monomers. The permanent positive charge of these composites helps in electrostatic-based bacterial eradication.

The third approach was mixing metal particles (oxides) or ions into the restorative materials.[4,5] Gold, silver, and zinc are among the most prominent employed metals.[6,7] Antimicrobial properties of metals are directly related to their contact surface. Furthermore, nanoparticles interact widely with microorganisms.[2]

Zinc has an antibacterial effect against many bacteria, including *Streptococcus mutants*.[8,9] Zinc oxide (ZnO), besides the mentioned benefits, is insoluble and white.[10] Furthermore, the ability of nanoparticles to be absorbed in cell membranes results in several intracellular processes that increase reactivity and antibacterial activity.[11] Graphite nanoparticles, which include carbon nanotubes, fluorine, and graphene, due to their innovative features with antimicrobial properties, are considered new and upcoming agents.[12-14] Graphene is a solid two-dimensional and single-atomic substance combining with carbon hybridization SP2, has attracted much attention over the past decade. Its unique properties and prominent features are high electrical conductivity, optimal mechanical properties, large surface area, low thermal expansion coefficient, and very high aspect ratio.[15-17] Graphene is also compatible with the environment and provides a suitable substrate for biological-chemical functions.[18,19] The antimicrobial effect of graphene oxide (GO) particles on both Gram-negatives (e.g., *Pseudomonas aeruginosa*) and Gram-positives (e.g., *S. mutants*) has been investigated in previous studies[20,21] and its low cellular cytotoxicity has been proved in the *in vitro* studies.[20] Furthermore, the ZnO in graphene is more penetrative in the cell wall and consequently more destructive to the bacteria.[20] However, the main limitation of the graphene compounds is its grey color and tendency to aggregate when added to a colloidal suspension.[22] Thus, the composition of GO with composite resins might have antibacterial activity,[10,22] but the opacity of the filler particles prevents visible light from passing through and also affects the mechanical properties of the composites.[23] By combining the antimicrobial properties of GO with the bright color of ZnO, the problem will be solved.[22] The aim of this study was to evaluate the effect of adding ZnO nanoparticles and GO on the antibacterial properties of flowable composites.

MATERIALS AND METHODS

Raw materials

In this *in vitro* experimental study, spherical ZnO nanoparticles with an average particle size of 20 nm [Figure 1a] and GO nanoparticles with a size range of 3.4–7 nm and layering structure [Figure 1b] were purchased from Merck Company (Germany). To synthesize the physical compound, 0.5 wt.% of ZnO and 0.5 wt.% of GO were used, the ZnO solution was, initially, dissolved in methanol and chloroform and then mixed with an alcoholic solution of GO for 24 h. The specimen was, then, separated by the solvent propagation method in a centrifuge. After solvent evaporation, the obtained sediments were dried in an oven [Figure 1c].

For the synthesis of the chemical compound, 0.5 wt.% of ZnO nanoparticles and aminopropyl triethoxysilane were mixed in dimethyl sulfoxide solvent and activated through the ultrasonic method. The obtained sediment was rinsed with ethanol and centrifuged. The defined value of the sediment was

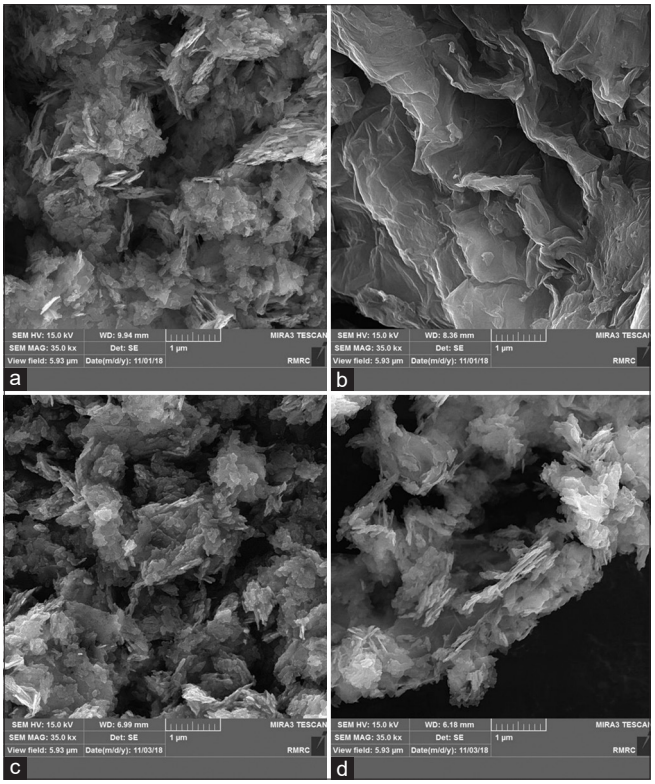

Figure 1: Scanning electron microscopy images of nanoparticle powders (×35K). (a) Zinc oxide powder, (b) Graphene oxide powder, (c) Physical compound of graphene oxide and zinc oxide, (d) Chemical compound of graphene oxide and zinc oxide.

added to 0.5 wt. % of GO in dimethylformamide solvent. With the combination of ultrasonic, alcohol purification method, and drying in oven, the chemical compound was obtained [Figure 1d]. Scanning electron microscopy (SEM) images of nanoparticle powders were taken [Figure 1].

Preparation of the specimen (adding nanoparticles to the composite)

Grandio Flow Composite Shade A2 (VOCO GmbH, Germany) was selected for this study. One percent by weight (1 wt.%) of each nanoparticle was weighed by a digital scale (with the accuracy of 0.0001) (A and D Company, Japan) and mixed manually with a spatula for 15 min under the red light on a vibrator so that homogeneity obtained. Hence, the groups (1) as the control (without adding nanoparticle), (2) containing 1 wt.% of ZnO nanoparticles, (3) containing 1 wt.% of GO, (4) containing 1 wt.% of the physical compound of ZnO-GO, and (5) containing 1 wt.% of the chemical compound of ZnO and GO were formed. SEM images of mixed composites were taken.

Characterization

SEM (JSM 6701F, JEOL) was used to observe the morphology of nanoparticles-contained cured composites discs and GO, ZnO, physical, and chemical compounds of nanoparticle powder. X-ray photoelectron spectroscopy (XPS, PHI-5702, Physical Electronics) was used to analyze homogeneity, purity percentage, and level percent of materials on the surface of specimens by Al-Ka radiation as the excitation source and the bonding energy of Au (Au 4f7/2: 84.00 eV) as reference.

Antibacterial test

The antibacterial activities of composite resins containing different nanoparticles were evaluated using the direct contact test. Initially, three 500 µL sterile microtubes were selected for each group. Then, 200 µL of prepared resin composite was added to each microtube. A predesigned Teflon jig was pressed into microtubes, and composites were cured by light cure unit (Bluephase 8, 800 mW/cm^2, Ivoclar Vivadent, Lichtenstein) for 20 s. 10 µL of standard *S. mutans* suspension ATCC 35668 (purchased from Pasteur Institute, Iran) (1.5×10^8) was added to the microtubes and shaken. Microtubes were incubated for an hour for solution evaporation. Then, 300 µL of brain–heart infusion broth was added to them. Ten µL from each solution was collected after incubation in 37°C for 24 h and 1 week, 10 µL from each solution was selected, and bacterial colonies in the blood agar culture were counted. The number and average of colonies in three microtubes were reported.

Bacterial adhesion to composite

One piece of each composite group (2 mm × 2 mm × 1 mm) was light-cured for 20 s and placed in the phosphate-buffered saline (137 mM NaCl, 207 mM Na_2HPO_4, 2Mm KH_2PO_4) containing the standard *S. mutans* suspension ATCC 35668 (1.5×10^8 bacteria) and was shaken in 37°C for 24 h. At that moment, the specimens were dried and fixed with methanol. FE-SEM was used to observe the bacterial adhesion to the composite specimens.

Statistical analysis

One-way analysis of variance followed by Tukey's *post hoc* comparison test was used to test the differences between the control and experimental groups at the level of significance of $P < 0.05$ with SPSS (SPSS, IBM Corp, IBM, USA).

RESULTS

Scanning electron microscopy evaluation of the powder of nanoparticle

The SEM was used to observe the morphology of nanoparticles and composite discs. In Figure 1a, it can be seen the thickness and length of ZnO nanoparticles for almost 20 nm and 40–200 nm, respectively. Most of the nanoparticles are seen in irregular plate forms. In Figure 1b, GO nanoparticles are seen in very thin (almost 4–7 nm) but very wide (10–50 µ) sheets. In Figure 1c, physical compound of ZnO + GO is seen in agglomerated great amounts of ZnO on GO sheets. In Figure 1d, chemical compound of ZnO + GO can be seen in irregular plates of ZnO connected to GO sheets.

Figure 2 shows SEM images of composites discs (Figure 2a shows powder-free composite). Black arrows show ZnO nanoparticles. None of Figure 2c-e is not showing graphene oxide nanoparticles. A little of porosities are observed in Figure 2b-e.

Energy dispersive X experiment and X-ray diffraction sample chart

Figure 3 shows X-ray diffraction (XRD) analysis of four types of powders that were used in this study. Vertical axis is intensity (a.u.), and horizontal axis is energy (eV). Zn is being observed in Figure 3a, c, and d. Amount of Zn in Figure 3a is very more. In Figure 3b, carbon is being seen very more. Figure 4 shows XRD

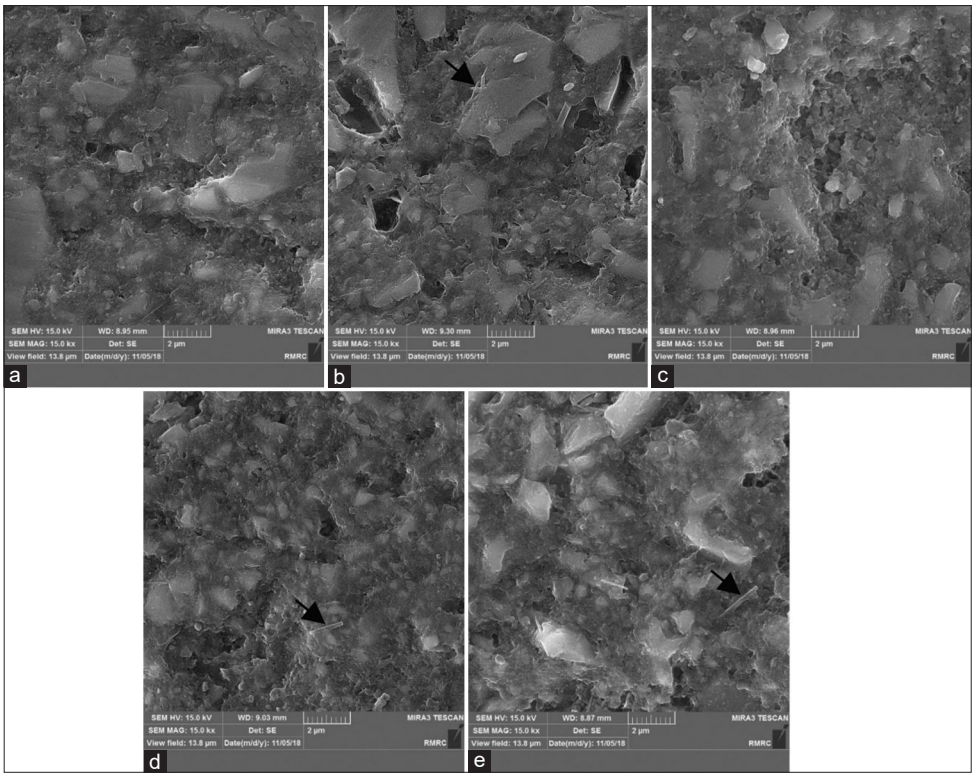

Figure 2: Scanning electron microscopy images of composites discs (×15K). (a) Without powder, (b) With zinc oxide powder, (c) With graphene oxide powder, (d) With physical compound of graphene oxide and zinc oxide, (e) With chemical compound of graphene oxide and zinc oxide.

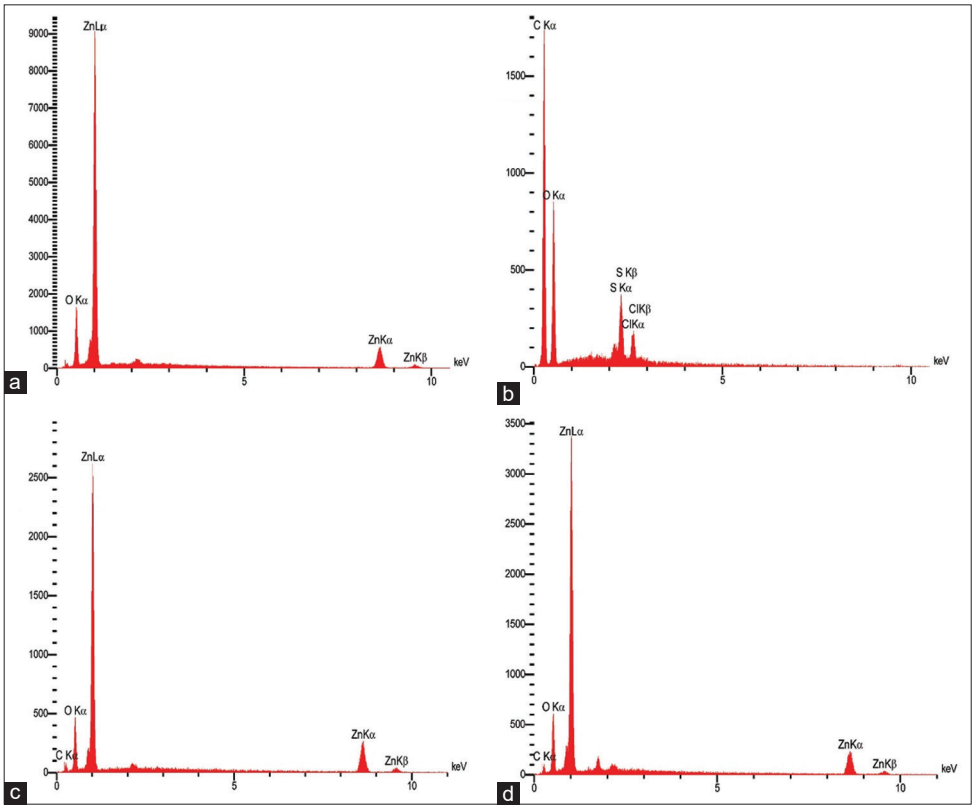

Figure 3: X-ray diffraction analysis. Zinc oxide nanoparticles powder (a), Graphene oxide nanoparticles powder (b), Physical compound of zinc oxide and graphene oxide powder (c), Chemical compound of zinc oxide and graphene oxide powder (d).

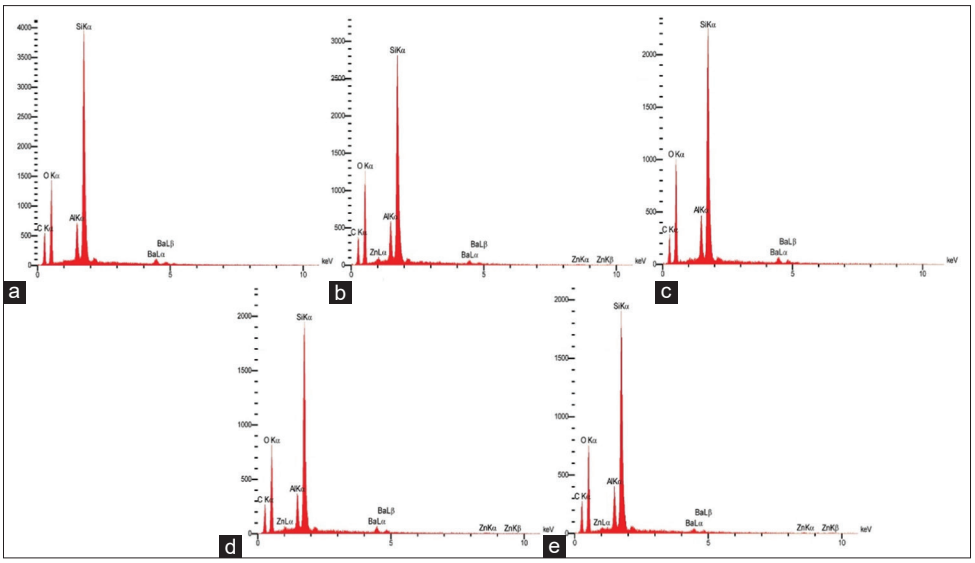

Figure 4: X-ray diffraction charts of composites discs. (a) Without powder, (b) With zinc oxide powder, (c) With graphene oxide, (d) With physical compound of graphene oxide and zinc oxide, (e) With chemical compound of graphene oxide and zinc oxide.

analysis of composite discs (Figure 4a shows powder-free composite). Zn is being observed in Figure 4b, d, and e, and is not being observed in figure 4c.

Elemental analysis in energy dispersive spectroscopy X [Tables 1 and 2] is being seen. According to Table 2, in Groups 2,4 and 5, Zn is considerably more than the others.

ANTIMICROBIAL TEST RESULTS

According to Table 3, the number of colonies in each group had approximately the same range in 24 h. The number of colonies in Groups 3 and 5 was lower than that of the other groups, but this difference was not significant.

The number of colonies in all groups decreased in a week in comparison to 24 h. This decrease was particularly significant in Group 3 (GO), followed by Group 2 (ZnO).

Scanning electron microscopy images of bacterial adhesion

Figure 5 shows SEM images of microbial adherence to composite discs after 24 h. Black arrows are showing *S. mutants* coccies. The least amounts of adhesion were observed in Groups 4 and 3, respectively.

Table 1: Energy dispersive spectroscopy elemental analysis to weight percentage of four types of powders that used in study

	ZnO powder	GO powder	Physical compound	Chemical compound
C	-	60.12	12.21	14.53
O	31.65	36.04	22.92	28.61
S	-	2.43	-	-
Cl	-	1.41	-	-
Zn	68.35	-	64.87	56.86

Unit: Weight percentage. ZnO: Zinc oxide, GO: Graphene oxide

Table 2: Energy dispersive spectroscopy elemental analysis to weight percentage of composite discs: Group 1 (control), Group 2 (with zinc oxide), Group 3 (with graphene oxide), Group 4 (physical compound of zinc oxide and graphene oxide), Group 5 (chemical compound of zinc oxide and graphene oxide)

	Group 1	Group 2	Group 3	Group 4	Group 5
C	36.02	32.38	31.33	33.16	35.46
O	36.3	40.99	40.96	39.92	39.89
Al	3.51	3.79	3.9	3.32	3.5
Si	18.94	19.13	19.02	18.55	17.89
Ba	5.23	3.29	4.78	4.16	2.69
Zn	-	0.42	-	0.89	0.57

Unit: Weight percentage

DISCUSSION

According to Dias *et al.*, *S. mutans* is the main cause of dental caries around the world and was also recognized as the most cariogenic streptococcal species in that study.[24] Furthermore, *S. mutans* is the first agent involved in bacterial colonization and biofilm growth.[25] This was the rationale behind using a single *S. mutans* in the present study.

Although carbon-based nanomaterials such as GO potentially fight against multidrug-resistant bacteria.

Table 3: Colony count of microbial culture after 24 h and 7 days

	24 h	7 days
Group 1 (control)	2.5×10^5	2.5×10^4
Group 2 (ZnO)	2.4×10^5	2.5×10^3
Group 3 (GO)	2.3×10^5	2×10^3
Group 4 (ZnO + GO physical)	2.4×10^5	4×10^2
Group 5 (ZnO + GO chemical)	2.2×10^5	3.1×10^3

ZnO: Zinc oxide, GO: Graphene oxide

The antibacterial activity and mechanism are far from explicit molecular views.[26] GO is synthesized by oxidation of graphite through the Hummers method. It was believed earlier that graphene nanoparticles were not small enough to be released and perfused into surroundings and in turn, reducing the effects of fluoride and other ions on the bacteria. However, our study indicated that GO could reduce bacterial adhesion and growth on the composite surface. This can be important because the bacterial colonization on composite surfaces is significantly more than on other surfaces such as dental amalgam and glass ionomers resulting in early recurrent caries.

In the present study, a concentration of 1% of GO was used to avoid the toxicity of zinc.[27] Furthermore, previous studies showed that incorporation of ZnO nanoparticles for up to 1% into the composite resins could significantly inhibit the colonization of cariogenic bacteria without sacrificing the mechanical properties such as flexural strength, flexural modulus, compressive strength, and micro-shear bond strength of the composite resins.[2]

Another study by Brandão *et al*. showed that the incorporation of 2–5 wt.% of ZnO-NP could endow antibacterial activity to composite resins without jeopardizing their physicochemical properties.[25]

Zhang *et al*. reported the concentration and time dependency of antibacterial activity of GO. They evaluated the effect of GO on *Escherichia coli* and reported tension in the membrane of *E. coli* as soon as GO nanorods contacted with the cells. By interacting with the phospholipid membrane of *E. coli*, the membrane was damaged due to the increase in the amount of reactive oxygen species followed by the glutathione reduction. This can lead to bacterial death.[26]

In the present study, there was no significant difference in the antibacterial activity between the nanoZnO and control groups, which could be due to the spherical form of nanoZnO.

ZnO has a broad-spectrum antibacterial activity and has a wide range of nanoscale forms, such as nanowires, nanoparticles, nanobelts, nanosprings, nanopencils, nanocomposites, nanoboxes, and nanorings. The morphology of ZnO is determined by the condition and method of synthesis. Some parameters such as pH, temperature, solvents, various precursors, and physicochemical settings can be controlled to obtain the best antibacterial properties. It has been shown that the shape of ZnO can affect the internalization mechanism which indicates rods and wires can penetrate the bacterial cells more easily than spherical-shaped particles. According to this concept, the properties of the surface of particle are likely to play a crucial role in the production of reactive oxygen species, but the antimicrobial activity of the substances may depend on the shape. Shape-dependent activity is explained by the percentage of active facets on the nanoparticles which can be synthesized as a function of growth parameters. It has been shown that rod structures have more active aspects that increase antibacterial activity compared to spherical nanostructures.[24]

Two pathways have been described as the possible mechanisms for the antibacterial activity of ZnO. The first advocates that ZnO reacts with the water of environment. Releasing Zn^{2+} into the growth media may interfere with the bacterial metabolism by displacing Mg^{2+}, which is extremely necessary to the enzymatic activity of the biofilm. The second advocates that ZnO can also generate reactive peroxides that penetrate the membrane cell, causing damage, and inhibiting bacterial growth. Taking into account that both mechanisms involve the release of active species from ZnO surfaces, it is clear that the high surface area to volume ratio of the 10–50 nm ZnO-NP particles in this study affected the behavior of the experimental composites.[25]

Dias *et al*. reported the antibacterial effect of adding ZnO to resin composites on *S. mutants*.[24]

Tavassoli Hojati *et al*. showed that the added ZnO nanoparticles could effectively prevent the growth of *S. mutans*. By increasing the amount of ZnO nanoparticles, the growth of bacteria significantly decreased, and the composition of these nanoparticles did not show adverse effects on the mechanical properties of composites. The flexural strength and compression modulus with the nanoparticles remained unchanged, while these composites exhibited lessened depth of light penetration and increased bond strength,

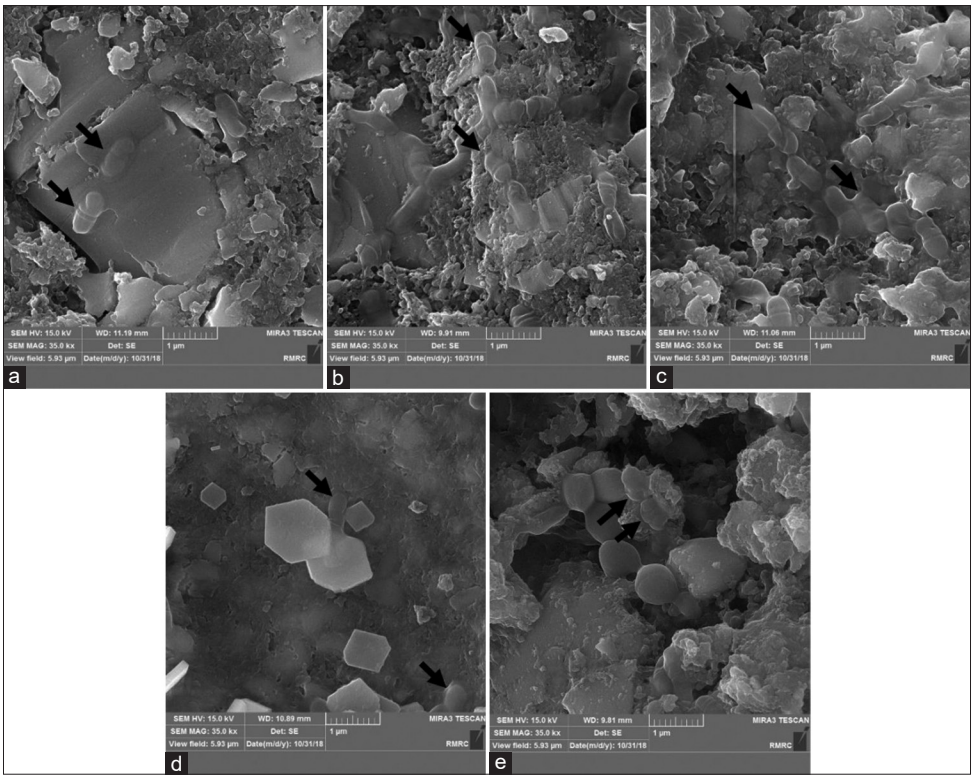

Figure 5: Scanning electron microscopy images of microbial adherence to composite discs (×35K). (a) Without powder, (b) With Zinc oxide powder, (c) With graphene oxide, (d) With physical compound of graphene oxide and Zinc oxide, (e) With chemical compound of graphene oxide and zinc oxide.

with no significant change in the degree of conversion between the groups. The results of this study indicated that adding a low concentration of nanoparticles led to homogeneous distribution, while the higher mass fraction of nanoparticles resulted in heterogeneous distribution which reduced the mechanical properties of the composite. The reduced mechanical properties in this study were probably related to the effect of nanoparticles on composite polymerization, rather than the formation of structural defects due to the heterogeneous distribution of particles.[2]

Kasraei *et al*. found that the composites containing nanoZnO particles exhibited higher antibacterial activity against *S. mutans* and *Lactobacillus* compared to the control group.[27]

CONCLUSION

Based on our results, adding GO alone to composites, rather than ZnO, or the physical and chemical compounds of GO-ZnO was more helpful to increase the antimicrobial characteristics.

Financial support and sponsorship

Nil.

Conflicts of interest

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

REFERENCES

1. Addy M, Handley R. The effects of the incorporation of chlorhexidine acetate on some physical properties of polymerized and plasticized acrylics. J Oral Rehabil 1981;8:155-63.
2. Tavassoli Hojati S, Alaghemand H, Hamze F, Ahmadian Babaki F, Rajab-Nia R, Rezvani MB, *et al.* Antibacterial, physical and mechanical properties of flowable resin composites containing zinc oxide nanoparticles. Dent Mater 2013;29:495-505.
3. Ahn SJ, Lee SJ, Kook JK, Lim BS. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater 2009;25:206-13.
4. 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. J Biomed Mater Res B Appl Biomater 2012;100:1378-86.
5. Niu LN, Fang M, Jiao K, Tang LH, Xiao YH, Shen LJ, *et al.* Tetrapod-like zinc oxide whisker enhancement of resin composite. J Dent Res 2010;89:746-50.
6. 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). J Antimicrob

Chemother 1996;37:911-8.
7. Lansdown AB. Silver in health care: Antimicrobial effects and safety in use. Curr Probl Dermatol 2006;33:17-34.
8. Beyth N, Domb AJ, Weiss EI. An *in vitro* quantitative antibacterial analysis of amalgam and composite resins. J Dent 2007;35:201-6.
9. Hernández-Sierra JF, Ruiz F, Pena DC, Martínez-Gutiérrez F, Martínez AE, Guillén Ade J, *et al.* The antimicrobial sensitivity of *Streptococcus mutans* to nanoparticles of silver, zinc oxide, and gold. Nanomedicine 2008;4:237-40.
10. Aydin Sevinç B, Hanley L. Antibacterial activity of dental composites containing zinc oxide nanoparticles. J Biomed Mater Res B Appl Biomater 2010;94:22-31.
11. Park HJ, Park S, Roh J, Kim S, Choi K, Yi J, *et al*. Biofilm-inactivating activity of silver nanoparticles: A comparison with silver ions. J Ind Eng Chem 2013;19:614-9.
12. Olivi M, Zanni E, De Bellis G, Talora C, Sarto MS, Palleschi C, *et al.* Inhibition of microbial growth by carbon nanotube networks. Nanoscale 2013;5:9023-9.
13. Akhavan O, Gaderi E. Photocatalytic reduction of graphene oxide nanosheets on TiO2 thin film for photoinactivation of bacteria in solar light irradiation. J Phys Chem 2009;113:20214-20.
14. Liu J, Liu L, Bai H, Wang Y, Sun DD. Gram-scale production of graphene oxide–TiO2 nanorod composites: Towards high-activity photocatalytic materials. Appl Catalysis 2011;106:76-82.
15. Huang X, Yin Z, Wu S, Qi X, He Q, Zhang Q, *et al.* Graphene-based materials: Synthesis, characterization, properties, and applications. Small 2011;7:1876-902.
16. Zhu Y, Murali S, Cai W, Li X, Suk JW, Potts JR, *et al.* Graphene and graphene oxide: Synthesis, properties, and applications. Adv Mater 2010;22:3906-24.
17. De Bellis G, Tamburrano A, Dinescu A, Santarelli ML, Sarto MS. Electromagnetic properties of composites containing graphite manipulatable at radio frequency. Carbon 2011;49:4291-300.
18. Guo S, Dong S. Graphene nanosheet: Synthesis, molecular engineering, thin film, hybrids, and energy and analytical applications. Chem Soc Rev 2011;40:2644-72.
19. Jiang H. Chemical preparation of graphene-based nanomaterials and their applications in chemical and biological sensors. Small 2011;7:2413-27.
20. Zanni E, De Bellis G, Bracciale MP, Broggi A, Santarelli ML, Sarto MS, *et al.* Graphite nanoplatelets and *Caenorhabditis elegans*: Insights from an *in vivo* model. Nano Lett 2012;12:2740-4.
21. Rago I, Bregnocchi A, Zanni E, D'Aloia A, De Angelis F, Bossu M, *et al.* Antimicrobial activity of graphene nanoplatelets against *Streptococcus mutants*. In: 15th International Conference on Nanotechnology. IEEE, Rome, Italy; 2015.
22. Zanni E, Chandraiahgari CR, De Bellis G, Montereali MR, Armiento G, Ballirano P, *et al*. Zinc oxide nanorods-decorated graphene nanoplatelets: A promising antimicrobial agent against the cariogenic bacterium *Streptococcus mutants*. Nanomaterials 2016;6:179.
23. Beun S, Glorieux T, Devaux J, Vreven J, Leloup G. Characterization of nanofilled compared to universal and microfilled composites. Dent Mater 2007;23:51-9.
24. Dias HB, Bernardi MI, Ramos MA, Trevisan TC, Bauab TM, Hernandes AC, *et al.* Zinc oxide 3D microstructures as an antimicrobial filler content for composite resins. Microsc Res Tech 2017;80:634-43.
25. Brandão NL, Portela MB, Maia LC, Antônio A, Silva VL, Silva EM. Model resin composites incorporating ZnO-NP: Activity against *S. mutans* and physicochemical properties characterization. J Appl Oral Sci 2018;26:e20170270.
26. Zhang N, Hou J, Chen S, Xiong C, Liu H, Jin Y, *et al.* Rapidly probing antibacterial activity of graphene oxide by mass spectrometry-based metabolite fingerprinting. Sci Rep 2016;6:28045.
27. Kasraei S, Sami L, Hendi S, Alikhani MY, Rezaei-Soufi L, Khamverdi Z. Antibacterial properties of composite resins incorporating silver and zinc oxide nanoparticles on *Streptococcus mutans* and *Lactobacillus*. Restor Dent Endod 2014;39:109-14.