Original Article

Effect of zinc oxide nanoparticles on physical and antimicrobial properties of resin-modified glass ionomer cement

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ABSTRACT

Background: To improve the limitations, many modifications in the resin-modified glass ionomer (RMGI) composition have been proposed. In this study, we evaluated the effect of different concentrations of zinc oxide (ZnO) nanoparticles incorporated into RMGI cement on its physical and antimicrobial properties.

Materials and Methods: In this in vitro study, ZnO nanoparticles with 0–4 wt.% concentrations were incorporated into RMGI. The following tests were carried out: (a) Antibacterial activity against Streptococcus mutans tested by disc diffusion method, (b) mechanical behavior assessment by measuring flexural strength (FS) and flexural modulus (FM), (c) micro-shear bond strength (μ-SBS), and (d) fluoride and zinc release. Data were analyzed using the statistical tests of ANOVA, t-test, and Tukey’s HSD post hoc in SPSS V22. The level of significance was 0.05.

Results: In the disc diffusion method, specimens with 2 wt.% ZnO nanoparticles showed the highest antimicrobial efficacy (P < 0.05). After 1 month of water storage, no significant difference was observed in FS and FM of the samples (P > 0.05). In 2 wt.% ZnO nanoparticles group, μ-SBS increased in the first 7 days but decreased by 17% after one month, which showed a significant difference with that of the control group. The fluoride release did no change in the ZnO nanoparticle-containing group compared with the control group at all time intervals.

Conclusion: Incorporation of 2 wt.% ZnO nanoparticles into the RMGI cement adds antimicrobial activity to the cement without sacrificing FS and fluoride release properties, while decreased μ-SBS.

Key Words: Flexural strength, fluorides, glass ionomer cements, microbial sensitivity tests, zinc oxide

INTRODUCTION

The restorative approaches do not eliminate all microorganisms in residual dental tissues. Recurrent caries is the primary reason for the failure of tooth-colored restorations.¹,²

Glass ionomer cement (GIC) is one of the types of dental cement with favorable characteristics such as chemical bonding to the tooth structure.
and anti-cariogenic potential by releasing fluoride.\[^{1,3}\] Resin-modified glass ionomer (RMGI) also has antibacterial activity due to the release of metallic ions and fluoride and the low initial pH.\[^{4,5}\] Despite the anticarious properties of RMGI, recurrent decay is the most common cause of failure of glass ionomer restorations, which indicates that released fluoride is not strong enough to inhibit bacterial growth and protect tooth structures.\[^{1,2}\] Streptococcus mutans is one of the most common bacterial strains responsible for tooth decay.\[^{2,5,6}\]

Several studies have been done on developing GIC with enhanced antibacterial activities by incorporation of antimicrobial agents such as chlorhexidine.\[^{2,4,7}\] Some metal oxides, such as titanium, silver, and zinc, have proven antimicrobial properties. It has been shown that they can even improve such properties when the oxides are in nanoscales.\[^{2,4,6}\] The most suitable choice of antibacterial agents to combine with GICs would be nanoantibiotics (metal oxide nanoparticles with antimicrobial activity), which do not compromise the physical properties.\[^{4,7-11}\]

Currently, the field of nanotechnology has rapidly grown and made a colossal impact on dental material science.\[^{1,7,10,13}\] Owing to the high surface area-to-volume ratio, metal oxide nanoparticles work as better antimicrobial agents compared with traditional antibacterial agents.\[^{6,11,13}\] Zinc oxide (ZnO) nanoparticles are multifunctional inorganic nanoparticles with effective antimicrobial properties against a wide range of Gram-negative and Gram-positive bacteria and inhibit the formation of biofilm, even at a very low concentration. Change in color, as seen with silver nanoparticles, acts as a cosmetic restricting issue.\[^{1,2,6,7,14}\] Moreover, ZnO nanoparticles are safer compared with silver nanoparticles, and the production cost is less than silver nanoparticles.\[^{7,12,13}\]

According to the literature, the incorporation of ZnO nanoparticles in dental materials has been proved to be very promising.\[^{7-16}\] While the effect of ZnO remained for 3 weeks, most studies only confirm the short-term effects of the antibacterial activity of the material.\[^{4,13,17}\] To the authors’ knowledge, there is no published study investigating the effect of incorporating ZnO nanoparticles to RMGI on the mechanical and antibacterial properties of RMGI cement. Therefore, the present study seeks to assess the effect of incorporation of different concentrations of ZnO nanoparticles into RMGI on its bond strength, and physical and antimicrobial properties.

**MATERIALS AND METHODS**

**Fabrication of test specimens**

In this *in vitro* study, ZnO nanoparticles (Nanoshel LLC, USA) measuring 20–40 nm in diameter with >99.7% purity were used in this study. Desired amounts of ZnO nanoparticles were precisely weighed by a digital scale (Mettler Toledo-AB 204) and added to a light-cured, radiopaque, RMGI restorative material (Fuji II LC, GC Corporation, Tokyo, Japan) until the nanoparticles reached the weight percentages of 1, 2, 3, and 4 wt.% in the RMGI powder. The RMGI group with 0% ZnO was considered as the control group. To achieve a homogenous distribution of particles, the obtained powder was blended by mortar and pestle for 20 min, then mixed with liquid by P/L ratio of 3.2/1 on a glass slab according to the manufacturer’s instructions.\[^{18}\] All procedures were carried out under aseptic conditions in a laminar airflow cabinet.

**Antibacterial tests**

**Bacterial strains and growth conditions**

*S. mutans* PTCC 1601 (Persian Type Culture Collection, IROST, Iran) was used in this study. The bacteria were cultured in Mueller-Hinton agar culture medium (Liofilchem, Italy) and stored in an incubator at 37°C for 24–48 h to form the colonies.

**Disc diffusion testing**

The most commonly used method described in microbiology is the agar or disc diffusion test.\[^{4-6,19}\]

Fifty specimens containing 0% (negative control), 1, 2, 3, and 4 wt.% ZnO nanoparticles (10 specimens for each concentration) were fabricated using a two-piece stainless-steel mold (6 mm in diameter and 2 mm in height), which was placed on Mylar strip and a glass plate. The paste was packed into the mold from one side to eliminate voids, then another Mylar strip and lass plate were placed on the top surface. The specimens were fabricated under a sterile airflow cabinet and cured with a light cure unit (Demetron LC, SDS Kerr, USA) for 20 s from the top and the bottom of the mold (total 40 s) to ensure complete cure. After setting, the mold assembly was disassembled, the specimens were demolded and numbered. A sterile swab was soaked in bacterial suspension, and 40 µl of the suspension was spread on blood agar (High Media,
India, and defibrinated sheep blood). Fabricated RMGI discs were placed on the plates and adhered to the surface of the culture medium with mild pressure applied by a hemostat. The plates were then stored in an incubator at 37°C for 24 h.\(^{[18,19]}\)

The diameter for the growth inhibition zone was measured daily in millimeter-scale using a specific ruler (Diameter ruler UK). The antimicrobial test disc of amoxicillin-clavulanic acid (Padtan Teb Co. Iran) was used as a positive control group.

**Physical tests**

*Flexural strength and modulus*

Flexural strength (FS) testing is one of the most important mechanical tests for assessing the performance of dental materials.\(^{[1,8,13]}\) One-hundred and fifty bar specimens were prepared (10 in each group) according to ISO 4049. The powders were mixed with the liquid following the manufacturer’s recommendations. The obtained paste was inserted in a two-piece rectangular stainless-steel mold (2 mm × 2 mm × 25 mm dimensions), which was placed on Mylar strip and a glass plate. Another Mylar strip and a glass plate were placed on the top of the mold to compress the paste and eliminate voids. The specimens were cured at both the top and the bottom sides for 200 s using the overlapping technique. Subsequently, the specimens were removed from the mold and immersed in deionized water, and stored in an incubator at 37°C until the time of testing (1 day, 1 week, and 1 month). Both surfaces of all specimens were polished by a 600-grit silicon carbide paper in a moist environment. A three-point bending test was performed using a universal testing machine (Z2.5, Zwick, Germany) at a crosshead speed of 0.5 mm/min. The FS was calculated based on the obtained data and according to the equation:

\[
FS = \frac{3PL}{2bd^2}, \quad \text{Where } P \text{ stands for load at fracture (N), } L \text{ is the span length (20 mm), } b \text{ and } d \text{ are the width and thickness of the specimens (mm), respectively.}
\]

The flexural modulus (FM) was also determined from the slope of the initial linear region of the stress-strain curve.\(^{[8,13]}\)

Based on disc diffusion, FS, and spectrometry tests, the optimum concentration of ZnO nanoparticle for incorporation in RMGI was determined, and by using optimum concentration, micro shear bond strength (μ-SBS) and potentiometry tests were performed.

**Micro shear bond strength**

Thirty intact human third molars, extracted no longer than 3 months before the study, were mechanically cleaned from debris and soft tissues, and then observed under a stereomicroscope (Carton Optical Industries, Thailand) asking for the teeth free from cracks, wear, caries, or decalcification, subsequently, and were kept in thymol solution for 24 h, and then in normal saline throughout the testing period. The occlusal enamel was removed, and approximately 1 mm of dentin was ground by a diamond bur (Tizkavan, Iran), then the exposed dentin surface was evaluated by a stereomicroscope to ensure no enamel is left on the surface and pulp exposure has not occurred. Then, the teeth were embedded 2 mm below the cementoenamel junction in self-polymerizing acrylic resin in such a way that their occlusal surface was easily accessible and oriented perpendicular to the bottom of the mold. After polymerization of the resin, the dentin surface was polished with 600, 1000, and 1200 grit silicon carbide abrasive paper (3M, USA) until a smooth, uniform surface was achieved. All specimens were conditioned by a cavity conditioner (GC Corporation, Tokyo, Japan) using a micro brush for 10 s, then rinsed thoroughly with water, and finally dried by clean cotton pellets to achieve glistening appearance. The specimens were then randomly divided into two groups; group 1: RMGI (control) and group 2: RMGI + optimum percentage (2 wt.% of ZnO nanoparticle. Silicon molds with an internal diameter of 0.9 mm and a height of 1 mm were placed on a glass slide by sticky wax. The cement paste was packed from one side to eliminate the void. Then, the specimens were placed on the dentin surface and cured for 20 s. Three molds were placed on each tooth in each group (\(n = 15\)). After light curing, bonded samples in each group were randomly subdivided into three subgroups to be stored in an incubator at 37°C under 100% humidity for 1 day, 1 week, and 1 month. Just before testing, the mold was removed using a #11 scalpel and evaluated to exclude cracked or defective specimens. The specimens were tested by the universal testing machine equipped with a ligature wire (0.2 mm diameter), which was in touch with the lower half-circle of the cylindrical specimens, at a crosshead speed of 1 mm/min. The bond strength values were calculated as a ratio of failure load to the bonded surface area and reported in Megapascal (MPa).\(^{[18]}\) After bond strength testing, the fractured specimens were evaluated under a
Fluoride and zinc release testing
Specimens required for fluoride (F⁻) and zinc (Zn²⁺) release testing were fabricated similar to disc diffusion testing specimens. According to the pilot study, for spectrometry, a total of 100 disc specimens of RMGI containing 0%, 1 wt.%, 2 wt.%, 3 wt.%, and 4 wt.% ZnO nanoparticles were prepared for F⁻ and Zn²⁺ release test, for each ion 50 specimens (n = 10).

The potentiometer was utilized for testing the fluoride release of RMGI containing an optimum concentration of ZnO nanoparticles determined in antibacterial and flexural tests. Spectrometry test for F⁻ and Zn²⁺ release: mixed cement was poured into the stainless steel mold (6 mm in diameter and 2 mm in height) and stored for an hour in 100% moisture. The specimens were then removed from the mold, placed in a single polyethylene vial containing artificial saliva (with no fluoride-releasing ingredient), and incubated at 37°C. Artificial saliva was refreshed daily, and specimens were washed with deionized water for 4 s and dried by an absorbent paper, then immersed again in artificial saliva at 37°C. Fluoride and zinc release was measured at day 2 using spectrophotometry (ultraviolet visible) and ICP (inductively coupled argon plasma atomic emission spectrometry), respectively.

Potentiometer test for F⁻ release: 20 disc specimens of RMGI containing 0 and 2 wt.% ZnO nanoparticles were prepared (n = 10). Each specimen was placed in a polyethylene vial containing 7 mL of deionized water that was changed daily until 7 days. F⁻ release was determined daily until the 7th day and after 7 days (at 14th day) by a fluoride ion-specific electrode and potentiometer (HACH Company, Germany, UUSEPA Spands). Before testing, the specimens were washed by 1 mL deionized water, which was added to previous water and buffered by 4 mL of Total Ionic Strength Adjustment Buffer.

Statistical analysis
Data were statistically analyzed using SPSS software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). The repeated measures ANOVA, t-test, and Tukey post hoc honestly significant difference were used as appropriate. The level of significance was set at 0.05.

RESULTS

Disc diffusion test
The mean diameters of the growth inhibition zones for all of the experimental groups are demonstrated in Figure 1. Intergroup comparison of disc diffusion tests revealed that the growth inhibition zone in RMGI with 2 wt.% ZnO nanoparticles demonstrated the highest value, which was similar to the positive control group, but significantly higher than that of the negative control group (P < 0.05). However, the size of inhibition zones was dependent on the amount of ZnO nanoparticles incorporated into RMGI. No statistical difference was seen between the sizes of inhibition zones of S. mutans in groups with 2, 3, and 4 wt.% ZnO nanoparticles.

Flexural strength and modulus
Figures 2 and 3 show the FS and FM of RMGI containing different weight percentages of ZnO nanoparticles (0–4 wt.%) at different periods (days).

The highest value of FS and FM of RMGI with 0–2 wt.% ZnO nanoparticles was observed at 1 week that was significantly higher than those at 1 day (P < 0.05). In RMGI with 0–3 wt.% ZnO nanoparticles, FS and FM significantly increased at 1 month compared with 1 day (P < 0.05); but no significant difference was found in the mean FS or FM at 1 week and 1 month (P > 0.05). No statistically significant difference was noted in FS and FM of RMGI with 4 wt.% ZnO nanoparticles between the three time-points of 1 day, 1 week, and 1 month (P > 0.05).

At 1 day, mean FS of RMGI containing 3–4 wt.% ZnO nanoparticles was significantly higher than...
those of the control group ($P < 0.05$). There was no significant difference in the mean FS of RMGI containing 1–3 wt.% ZnO nanoparticles ($P > 0.05$).

At 1 week, mean FS of RMGI containing 0–1 wt.% ZnO nanoparticles was significantly higher than those of RMGI containing 3–4 wt.% ZnO nanoparticles ($P < 0.05$). No significant difference was observed between the mean FS of RMGI containing 1 and 2 wt.% ZnO nanoparticles ($P > 0.05$).

At 1 month, no statistically significant difference was detected in mean FS and FM between the understudy groups ($P > 0.05$).

**Micro shear bond strength**

As demonstrated in Table 1, the $\mu$-SBS of RMGI containing 2 wt.% nanoparticles has no significant increase from 1 day to 1 month ($P > 0.4$). The $\mu$-SBS at 1 day and 1 week, increased by 3.6 MPa or 33% ($P < 0.01$). A comparison of $\mu$-SBS between the two groups at 1 day and 1 week showed that the difference was not significant ($P > 0.9$). A comparison of the mean $\mu$-SBS between the groups at 1 month by $t$-test revealed that RMGI containing 2 wt.% ZnO nanoparticles had a significantly lower bond strength than the control group ($P < 0.01$).

**Fluoride and zinc release**

The lowest F$^-$ release belonged to RMGI containing 3 wt.% nanoparticles (34.6 ppm), while maximum release occurred in the control group (35 ppm). Data analysis by one-way ANOVA test demonstrated that the differences between groups were not statistically significant ($P > 0.05$).

The lowest Zn$^{2+}$ release belonged to the control group (0.43 ppm), while maximum release occurred in RMGI containing 4 wt.% nanoparticles (5.71 ppm). Data analysis by one-way ANOVA test showed that the differences between groups were statistically significant ($P < 0.05$).

Figure 4 shows the results of the potentiometer that the released fluoride in the group containing 2 wt.% nanoparticles at day 1 was significantly higher than that in the control group. The amount of released F$^-$ at all understudy time points was higher in RMGI containing 2 wt.% nanoparticles than in the control group. On the 14th day, the amount of released F$^-$ in RMGI containing 2 wt.% nanoparticles was 15.2% higher than that in the control group ($P < 0.001$).

**DISCUSSION**

This study aimed to evaluate the effect of incorporation of various concentrations of ZnO nanoparticles to RMGI cement on its antimicrobial activity, FS and modulus, $\mu$-SBS, and F$^-$ and Zn$^{2+}$ release properties at three time-intervals.

According to the acquired data, from the 3rd day, the diameter of the growth inhibition zone decreased. This finding was in agreement with Aydin Sevinç and Hanley[15] and may be explained by the fact that due
to bacterial growth, nutrients are depleted over time, resulting in the death of some bacteria. Subsequently, bacteria at the periphery of the growth inhibition zone grow and migrate toward the center of the disc resulting in a subsequent decrease of the diameter of the zone.\textsuperscript{15,19}

The obtained results showed that by adding ZnO nanoparticles to RMGI cement, Zn\textsuperscript{2+} release increased, but this increase had no significant effect on F\textsuperscript{−} release. Furthermore, a significant increase of the growth inhibition zone in RMGI with 2 wt.\% ZnO nanoparticles compared with the negative control group, indicates the dissolution of Zn\textsuperscript{2+} ions from cement and diffusion into the medium surrounding the discs (microbial environment). These results are comparable with those of Hammouda.\textsuperscript{23} who reported Zn enhanced the antimicrobial activity of fluoride against \textit{S. mutans}. It may be related to the synergistic action of the release of F\textsuperscript{−} and Zn\textsuperscript{2+}, in which Zn\textsuperscript{2+} release can enhance the inhibitory activity of F. In agreement with our results, Vanajassun \textit{et al.}\textsuperscript{[3]} showed that the addition of ZnO nanoparticles significantly increased the antibacterial properties of GIC. Esmi \textit{et al.}\textsuperscript{[19]} reported that the antibacterial activity was dependent upon the concentration of ZnO nanoparticles, and the addition of ZnO nanoparticles increased the growth inhibition zone diameter. Zhang \textit{et al.}\textsuperscript{[24]} showed that the antibacterial activity of ZnO nanoparticles increased with an increase in nanoparticle concentration and a decrease in the size of nanoparticles. The results of this study showed that by increasing the concentration of ZnO nanoparticles from 2 to 3 wt.\% and 4 wt.\%, there was a gradual nonsignificant reduction in the antibacterial activity. This may be due to a higher tendency to agglomeration, which may have affected the bactericidal efficiency as supported by Ruparelia \textit{et al.}\textsuperscript{[24]} Most of the previous studies confirm the antibacterial activity of ZnO nanoparticles added to dental materials; however, they have reported different concentrations and effectiveness of these nanoparticles.\textsuperscript{2,5,7-9,19} The optimum concentration of nanoparticles must be carefully selected because they must not adversely affect the mechanical and handling properties of RMGI.\textsuperscript{[4,7]}

At 1 day, by increasing the percentage of nanoparticles, FS and FM increased, which is probably due to the reinforcing effect of ZnO nanoparticles. This finding is comparable to those of Tian \textit{et al.}\textsuperscript{[26]} and Al Badr,\textsuperscript{[13]} who reported that the addition of nanoparticles along with their uniform distribution could enhance the mechanical properties of the material. Significant increased FS and FM of the control group over time are similar to other studies and probably due to the higher number of cross-links and formation of silica-gel phase.\textsuperscript{[13,23,27]} Similar to Al Badr\textsuperscript{[13]} findings, FS and FM of groups with 1, 2, and 3 wt.\% ZnO nanoparticles also significantly increased at 1 month compared with 1 day. According to the literature, it was proved that a high content of F and Zn in the glass powder induced a higher flexural and compressive strength since enhanced network connectivity would occur. The increase in the amount of Zn is directly related to an enhanced reactivity.\textsuperscript{[13,23,27,28]} In agreement with our findings, Al Badr\textsuperscript{[13]} concluded that the addition of ZnO nanoparticles up to 3 wt.\% acted as a reinforcing material and improved FS of GIC. However, agglomeration of nanoparticles leads to the fragility in the matrix of GIC, which adversely affects the FS and FM. Also, Panahandeh \textit{et al.}\textsuperscript{[8]} and Agarwal \textit{et al.}\textsuperscript{[10]} reported that the addition of nanospherical ZnO to GIC caused no change in FS.

The additives should be added in an optimum concentration to improve the different properties of RMGI so that it should not interfere with the normal matrix of the RMGI cement. With the increase in nanoparticle concentration of the cement, there may have been a deficiency of powder. Thus, the normal matrix formation could have been hindered. Hence for an increase in the FS, there should be an optimum concentration of RMGI cement matrix and ZnO nanoparticles.\textsuperscript{[29]} According to the obtained results, there is no significant difference between in flexural and antibacterial properties of RMGI containing 2

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**Table 1: Mean and standard deviation of micro shear bond strength (MPa) of two tested groups**

| Groups (n=15) | 1 day          | 7 days         | 30 days        |
|--------------|----------------|----------------|----------------|
| RMGI         | 10.8±2.2\textsuperscript{A} | 12.36±3.9\textsuperscript{B} | 16.76±5.62\textsuperscript{C} |
| RMGI- 2wt.% ZnO nanoparticles | 10.96±3.72\textsuperscript{A} | 14.63±2.56\textsuperscript{B} | 12.1±2.78\textsuperscript{C} |

Differences in lower case letters indicate statistically significant differences within rows and differences in capital letters indicate statistically significant differences within columns (P<0.05). SD: Standard deviation; RMGI: Resin-modified glass ionomer; ZnO: Zinc oxide
wt.% of ZnO nanoparticles and higher concentration. Although ZnO is generally considered as a safe and low toxicity compound, because of the unique physicochemical properties of nanoparticles, they are more reactive and responsive with higher absorption, which may cause hazardous effects. Therefore, in this study 2 wt.% was selected as the optimum concentration.

The µ-SBS testing is one method to evaluate the bond strength of dental materials and could be a viable test when evaluating brittle materials such as GICs. In the current study, the obtained results from the control group are similar to previous studies at day one and other time intervals that showed an increasing trend over time. There was no significant difference in µ-SBS of RMGI containing ZnO nanoparticle and the control group at 1-day and 1-week assessment. This finding is in agreement with the results of Nuri Sari et al. and Vanajassun et al. who reported that the addition of ZnO nanoparticles to GICs had no statistically significant difference over the bond strength of GIC. A nonsignificant increase occurred in the µ-SBS within the first 7 days, which seems to be related to an increase in the FS of the cement, but this value decreased at 1 month by 17% and showed a significant difference with that of the control group. This finding may be due to the release of nanoparticles over time that leave empty spaces in the dentin-cement interface and decrease µ-SBS. Similar results were obtained by Moshaverinia et al. A mechanism of adhesion between RMGI cement and dentin depends on the formation of hydrogen bonds originating from the free carboxyl groups in the cement interacting with tightly bound water in the mineral phase of dentin. Gradually, the replacement of these hydrogen bonds by true ionic bonds leads to enhance the bond strength of cement, which showed in the control group. The lower µ-SBS of RMGI containing 2 wt.% nanoparticles might be due to the presence of fewer amounts of free carboxylic groups that can chemically bond with dentin. In our study, the specimens were evaluated under a stereomicroscope following µ-SBS testing to assess the mode of fracture, and it was revealed that all fractures were the adhesive type. This finding is similar to that of Banomyong et al. and Rezvani et al. Furthermore, the results of the stereomicroscopic assessment confirmed the results of µ-SBS tests. It appears that the early adhesive strength of the cement remained unchanged. Over time, we noticed a reduction in bond strength due to the further dissolution and release of ZnO nanoparticles that proofed by ICP spectrometry and their weaker bond to dentin. According to the results of the disc diffusion test and improvement of antibacterial activity of cement, this is not unexpected. The µ-SBS of the RMGI containing ZnO nanoparticle in 1, 7, and 30 days were in the range of shear bond strength recommended for RMGI.

Three mechanisms were suggested for F- release from GICs; surface loss, diffusion through pores and cracks, and bulk diffusion. Similar to Hammouda findings, the results of the potentiometer test demonstrated that RMGI with 2 wt.% ZnO nanoparticles released a higher amount of fluoride compared with the control group. While the results of the spectrometry test revealed no significant difference between all groups, these different results may be attributed to different storage environments and testing methods, and effects of existing Zn2+ ions in the testing solution. Differences in the amount of F- released attributed to storage medium have been reported. It is important to point out that the F- release pattern overtime is the most important finding in fluoride release assessment. The pattern of F-release from both groups in daily assessments in the 1st week shows a drastic decrease on the 1st day, which is similar to other studies.

According to the above-mentioned points, ZnO nanoparticles can be considered as promising fillers for RMGI to be used as a restorative dental material in areas of high-risk caries. Further work should be focused on investigating other properties that are required for any clinically utilized dental material (for example, the setting time and biological effects of adding such amounts of nanoparticles to RMGI, etc.).

CONCLUSION

Within the limitations of this study and based on the obtained results, it can be concluded that incorporation of 2 wt.% ZnO nanoparticles to RMGI significantly increases its fluoride release and antibacterial activity while it does not change its FS and modulus. However, the addition of 2 wt.% ZnO nanoparticles reduces the µ-SBS but is within the clinically acceptable range.

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

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