



Effect of Zinc Oxide Nanoparticles on Calcium-enriched Mixture Cement: Physical, Chemical, and Mechanical Properties

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Introduction: Calcium-enriched mixture (CEM) cement is a water-based biomaterial with properties similar to calcium silicate-based cements (CSCs). Zinc oxide (ZnO), a bioactive material widely used for its antimicrobial properties and beneficial biological effects on body tissues, was incorporated into CEM cement in this study. The objective was to evaluate the impact of ZnO nanoparticles (ZON) on the physical, chemical, and mechanical properties of CEM cement. **Materials and Methods:** ZON powder was blended with CEM cement powder at a 10% weight ratio. The mixture was combined with liquid (1 g powder: 0.33 mL liquid) and cast into test-specific molds. Samples of original CEM cement without ZON served as the control group. The samples were evaluated for changes in setting time ($n=6$), compressive strength ($n=6$), solubility ($n=10$), and pH ($n=10$). **Results:** The initial setting time was identical in both groups. ZON incorporation reduced the secondary setting time, though not significantly. Compressive strength remained unchanged. After 7 days, solubility increased significantly in ZON-containing samples. While ZON reduced the pH of CEM cement in deionized water after 5 hours, no significant difference was observed at 24 hours. **Conclusion:** Incorporating ZON into CEM cement caused minor changes, including a reduced secondary setting time, increased solubility, and transient pH changes, without significantly affecting overall performance.

Keywords: Calcium-enriched Mixture; Compressive Strength; Nano Zinc Oxide Particles; pH; Setting Time; Solubility

Introduction

Calcium-enriched mixture (CEM) cement is a bioactive endodontic material introduced in 2008 by Asgary and colleagues [1]. It is a water-based biomaterial with properties akin to calcium silicate-based cements (CSCs), but its chemical composition is more similar to dentin [2]. CEM cement consists of various calcium compounds, such as calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, calcium hydroxide, and calcium chloride, mixed with an alkaline water-based liquid. In an aqueous environment, it releases high concentrations of calcium and phosphate ions, which facilitate the formation of hydroxyapatite during and after setting [3].

CEM cement is recognized for its biological activity, seal ability, antibacterial properties, and the ability to form a complete

dental barrier at the pulp interface [2, 4]. It is comparable to mineral trioxide aggregate (MTA) in terms of cellular compatibility and provides an effective seal, similar to other CSCs [2]. The advantages of CEM cement over white MTA include better handling, thinner film thickness, and shorter setting time [5]. However, its compressive strength is notably lower [6], and it has higher solubility in aqueous environments, sometimes exceeding 3%, which is a limitation [7].

CEM cement has a wide range of clinical applications, including treatments for internal/external root resorptions, repair of root perforations, root-end filling, bio-obturation, vital pulp therapy, and regenerative endodontic procedures [2, 8-11]. Efforts to enhance the physical, mechanical, and chemical properties of CSCs have included adding various substances like glass fibers, bioactive glass nanoparticles, and silver nanoparticles [12-14]. Similarly,

additives such as lidocaine, calcium chloride, and propylene glycol have been explored for improving CEM cement [15-17].

Zinc oxide nanoparticles (ZON), known for their antimicrobial activity, present a potential additive to enhance CEM cement properties [18]. Zinc activates bactericidal enzymes at low concentrations and inhibits plaque growth at higher concentrations [19]. The United States Food and Drug Administration (FDA) considers zinc oxide safe, with no reported carcinogenicity, genotoxicity, or reproductive toxicity [20]. Reducing particle size to the nanoscale increases the surface-to-volume ratio, potentially enhancing certain material properties. Among metal oxide nanoparticles, ZON exhibits strong antimicrobial activity primarily by damaging bacterial cell walls and disrupting cellular metabolism, leading to cell death [21, 22]. Given that few studies have explored the incorporation of ZON into CEM cement [18], this study aims to evaluate the effects of these nanoparticles on their physical, chemical, and mechanical properties.

Materials and Methods

This study received approval from the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.DENTAL.REC. 1402.007).

This study utilized CEM cement (BioniqueDent, Tehran, Iran) and ZON with a purity of over 99% and a particle size of 10–30 nm (Stock#: US3590, CAS#: 1314-143-2, US Research Nanomaterials, Inc., Houston, USA). ZON was incorporated into CEM cement at a 10% weight ratio. The mixture was homogenized using an amalgamator set at 4500 revolutions per minute for 45 seconds. The manufacturer does not specify an exact liquid-to-powder ratio for CEM cement, indicating that a creamy consistency is desired. For consistency with previous studies, a ratio of 1 g of powder to 0.33 g of liquid was used. The prepared paste was placed into specialized molds with a force of 100 g, and excess material was removed with moist cotton pellets. Samples without ZON served as the control.

Setting time measurement

To assess setting time, Plexiglas rings with an inner diameter of 10 mm and a thickness of 1 mm were used ($n=6$). The samples were condensed in a plastic vial and incubated at 37 °C with 100% humidity for 150±1 sec. Initial and final setting times were evaluated according to ISO-6876 with some modifications and ASTM standards. Measurements were taken every 3 min for the first 30 min, every 5 min up to 90 min, and every 15 min thereafter [23].

For the initial setting time (S1), a Vicat apparatus with a 100±0.5 g weight and a 2±0.1 mm diameter needle was used

until the needle no longer left an impression on the material at three separate points. The final setting time (S2) was determined using a Vicat apparatus with a 456±0.5 g weight and a 1±0.1 mm diameter needle, following the same procedure.

Compressive strength testing

Compressive strength was tested according to the BSI-6039 standard with some modifications ($n=6$) [24]. Samples were prepared in cylinders (4 mm diameter and 6 mm height), incubated at 37 °C with 100% humidity for 3 h, and then kept for 24 h and 21 days by wrapping them in wet pieces of gauze saturated with PBS. Testing was conducted using an Emic DL2000 machine (Zwick/Roell Z020 Zwick, CombH & Co, Germany) with a load of 5 kN and a speed of 0.5 mm/min. The compressive strength was calculated using the formula:

$$(N/mm^2) MPa = \frac{4p}{\pi d^2}$$

Where p is the maximum force applied in Newtons and d is the mean diameter of the specimen in mm.

Solubility testing

Solubility was assessed per the ISO-6876 standard with some modifications ($n=10$) [25]. Samples were prepared with a 7 mm diameter and 1 mm thickness, with a 5 cm nylon thread embedded for handling. They were incubated at 37 °C with 100% humidity for 2 and 7 days. After incubation, samples were desiccated for 1 h in a desiccator, weighed (initial mass), immersed in deionized water at 37 °C for 15 h, and then desiccated again for 2 days. The final mass was recorded, and solubility was calculated using the formula:

$$(\%)solubility = \frac{w1-w2}{w1} \times 100$$

Where $W1$ is the dry weight before immersion, and $W2$ is the dry weight after immersion.

pH measurement

The pH was measured following the method by Faria-Junior *et al.* [26]. Samples ($n=10$) were incubated at 37 °C with 100% humidity for 2 and 7 days, then immersed in 10 mL of deionized water (pH=6.5) in a sealed flask. pH measurements were taken at 5 and 24 h using a pH-meter (S/N:17110805; Clean instruments, Shanghai, China) with 0.01 accuracy. Measurements were averaged for each sample at each time point.

Data analysis

Data were analyzed using SPSS software version 22 (IBM Co., Chicago, IL, USA). Normality tests determined data distribution. A two-way ANOVA test was performed to compare different groups, with a significance level set at 0.05.

Results

As shown in Table 1, the initial setting time for all samples in both groups occurred within the first 11 min, with no statistically significant difference observed. Independent sample t-test showed that mean time setting was not statistically different between the two groups ($P=0.699$).

According to Table 2, incorporating ZON into CEM cement resulted in an increase in compressive strength after 24 h of setting. Nonetheless, this increase was not statistically significant ($P=0.986$). Similarly, after 21 days, the compressive strength of the CEM cement did not show a statistically significant difference between the groups ($P=0.607$).

As depicted in Table 3, the solubility of CEM cement did not show a statistically significant change after 48 h with the addition of ZON ($P=0.216$). However, after one week, there was a significant increase in solubility in the group containing ZON ($P<0.05$).

The pH measurements, presented in Table 4, indicated that the addition of ZON to CEM cement did not result in statistically significant differences in pH after 24 h across all groups.

Discussion

This study examined the effects of incorporating 10% ZON into CEM cement, focusing on initial and secondary setting times, compressive strength, solubility, and pH levels. The results indicated that ZON slightly reduced the secondary setting time, though this reduction was not statistically significant, and had no notable impact on the initial setting time or compressive strength. Solubility increased significantly after seven days with ZON, despite showing no significant difference after two days. The pH of the cement was slightly lower with ZON after two days, but this difference diminished after 24 h.

Table 1. Mean and standard deviation of setting time in minutes

Cement	Initial	Secondary
CEM	11 (0.00)	45 (6.64)
CEM+ZnONps	11 (0.00)	43.5 (13.66)

During the secondary setting time between the two groups, no statistically significant difference was observed

Table 2. Mean and standard deviation of compressive strength in MPa

Cement	24 hours	21 days
CEM	0.76 (0.35) ^b	5.78 (1.89) ^a
CEM+ZON	1.01 (0.25) ^b	4.88 (1.58) ^a

Similar letters in each column indicate no statistically significant difference

Several studies have explored methods to enhance the physical properties of endodontic cements. For instance, Sedigh-Shams *et al.* [27] investigated the addition of 5% chicken eggshell powder to CEM cement, reporting improvements in compressive strength, solubility, and setting time, which could enhance sealing ability, durability, and resistance to chewing forces in endodontic applications. Similarly, Shokouhi *et al.* [28] evaluated the effect of 10% calcium chloride (CaCl_2) on the push-out bond strength of CEM cement and MTA to root canal dentin, finding that CaCl_2 enhanced CEM cement's bond strength over time while negatively affecting MTA.

The use of ZON in this study was inspired by its established ability to improve properties of dental materials, such as glass ionomer cements and other dental cements [29-31]. However, prior research on ZON reveals inconsistent effects on setting time and mechanical properties, influenced by the base cement's composition. For example, Hemmati *et al.* [32] noted a significant reduction in setting time for zinc phosphate cement but not for zinc polycarboxylate cement with ZON, while da Rosa *et al.* [33] reported shorter setting times for calcium aluminate cement. These discrepancies underscore the role of the base cement's chemistry in its interaction with ZON.

Efforts to optimize CEM cement properties have included adding calcium nitrate to reduce setting time [34] and incorporating propylene glycol to enhance compressive strength and microhardness [17]. ZON, often used as a radiopacifier, has been shown to improve sealing ability but may reduce compressive strength [35], though their addition to MTA does not significantly alter microhardness [36]. To our knowledge, the only prior study incorporating ZON into CEM cement was conducted by Razavi *et al.* [18]. Their *in vitro* analysis of ZnO

Table 3. Mean and standard deviation of solubility as a percentage of the initial mass

Cement	2 days	7 days
CEM	0.0186 (0.006) ^a	0.0124 (0.008) ^a
CEM+ZON	0.0290 (0.002) ^a	0.0764 (0.020) ^b

Similar letters in each column indicate no statistically significant difference

Table 4. Mean and standard deviation of pH

	2 days after setting		7 days after setting	
Time of immersion cement	5 hours	24 hours	5 hours	24 hours
CEM	11.31 (0.26) ^{bB}	9.75 (0.32) ^{aA}	11.34 (0.10) ^{bB}	9.67 (0.35) ^{aA}
CEM+ZON	10.93 (0.19) ^{cC}	9.42 (0.12) ^{aA}	11.24 (0.12) ^{bB}	9.53 (0.09) ^{aA}

For data comparison, small letters are used within each row, and capital letters are used within each column. Similar letters indicate no statistically significant difference

and zirconium oxide (ZrO₂) nanoparticles revealed that ZnO slightly reduced setting time, consistent with our findings, while ZrO₂ prolonged it. Interestingly, combining both nanoparticles yielded a setting time similar to the control.

Higher compressive strength is critical for withstanding occlusal forces and enabling immediate restoration. Various studies have investigated the incorporation of different nanoparticles to improve compressive strength [37]. Although Razavi *et al.* [18] found that ZnO and ZrO₂ significantly improved microhardness and compressive strength, our study observed no significant effect of ZON on compressive strength. Regarding solubility, the significant increase after seven days with ZON may stem from their high surface area, which enhances water interaction and dissolution, or from alterations to the cement matrix that increase reactive sites.

The slight pH reduction with ZON after two days, which normalized by 24 h, suggests a transient interaction requiring further exploration. Previous studies by Forghani *et al.* and Amini Ghazvini *et al.* [38, 39] noted variable pH trends in CEM cement, aligning with our findings. Given ZON promising biological properties, future research should investigate their effects at varying concentrations on properties like color stability, flow, shear strength, and radiopacity, while also assessing optimal concentrations to mitigate potential toxicity risks.

Conclusion

The addition of zinc oxide nanoparticles to CEM cement led to alterations in the material's properties, yet these modifications were within the acceptable standard range.

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Conflict of interest

None.

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Authors' contributions

Methodology: MN/AA/FZ; Formal analysis and investigation: MN/FZ; Writing-original draft preparation: AA/FZ; Writing-review and editing: MN/AA; Supervision: MN/AA. All authors read and approved the final manuscript

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