RESEARCH PAPER

Antimicrobial Effects of Composite Nanogels at Variable Concentrations on periodontal pathogens: *In Vitro* study

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ABSTRACT

This study aimed to investigate the antimicrobial efficacy of a novel zein-coated magnesium oxide (MgO) nanogel at three concentrations (0.5%, 1%, and 1.5%) against key oral and periodontal pathogens. Two Gram-negative and one Gram-positive bacterium frequently linked to periodontal infections, Escherichia coli and Staphylococcus aureus, were among the species tested. To test antibacterial activity, the agar well diffusion technique was used to measure inhibition zones. Antimicrobial activity increased with concentration. The 1.5% nanogel had the greatest mean inhibition zones against both bacterial strains. The statistical test using one-way ANOVA verified that there were notable variations in the concentrations ($p < 0.001$). Boxplot analysis further illustrated a clear upward trend in inhibition zone diameters with increasing nanogel concentration. The findings suggest that zein-coated MgO nanogel demonstrates promising dose-dependent antimicrobial activity and may serve as an effective adjunct in the management of periodontal pathogens.

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INTRODUCTION

Periodontal disease is still one of the most common chronic inflammatory conditions in the world, affecting up to half of all adults and having a major influence on inflammation throughout the body and tooth loss. [1]. The main pathogens involved in periodontitis are anaerobic Gram-negative bacteria like *Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola*. However, new evidence points to a polymicrobial etiology where facultative pathogens like *Staphylococcus aureus* and *Escherichia coli* are also important, especially in advanced lesions or in people with impaired immune systems [2,3].

The emergence of antibiotic-resistant strains and the limitations associated with traditional antimicrobial agents have necessitated the exploration of alternative therapeutic strategies [4]. In this regard, nanotechnology has grown into a potentially fruitful area of study, due to the novel solutions it provides in the form of antibacterial nanoparticles. The plentiful availability, high biocompatibility, and potent antibacterial capabilities of magnesium oxide nanoparticles (MgO-NPs) have piqued interest in these particles. They are thought to be able to kill a wide range

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of bacteria by disrupting critical intracellular processes, destroying microbial cell membranes, and creating reactive oxygen species (ROS) [5].

Because of its hydrophobic properties, zein has been shown to be an appropriate coating material for MgO-NPs. When applied as a layer, it makes MgO-NPs less prone to assemble in watery settings and increases their stability. In addition to increasing antibacterial action, this change improves sustained release qualities. A stable complex is formed when the non-polar chains of zein interface hydrophobically with the hydrophobic surface of MgO-NPs. This is the mechanism by which the two substances interact with one another [6].

The incorporation of zMgO-NPs into mouthwash formulations has shown significant inhibitory effects against *Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, and Candida albicans*. These results suggest that zMgO-NPs have potential applications in oral healthcare products for preventing and controlling microbial infections [7].

Very little is known about the dose-dependent efficacy of zMgO nanogels against certain periodontal infections. Even though they aren't part of the classic red complex, facultative bacteria like Staphylococcus aureus and Escherichia coli are showing up in subgingival areas at alarming rates, especially in individuals with impaired immune systems or other systemic illnesses. [8,9].

The antimicrobial effectiveness of zMgO nanogel at three concentrations (0.5%, 1%, and 1.5%) will be evaluated against *Escherichia coli and Staphylococcus aureus.* This study aims to elucidate the biologically controlled concentration effects and underlying mechanisms to inform the development of advanced nanotechnology treatments for managing periodontal diseases.

MATERIALS AND METHODS

Ingredients used in the study were Bangkalan dolomite, Zein polymer, 37% HCl, 25% NH3, 99.9% ethanol, 93.7% NaOH, distilled water, and 99.9% ethanol from Merck, 0.9% polyvinyl alcohol (Himedia), bacterium *S. aureus*, and *Escherichia coli*.

Preparation of MgO nanoparticles from dolomite

After being finely mashed with a mortar and pestle, the dolomite powder was sorted through a 200-mesh sieve. After coming to room temperature, the powder was dissolved in HCl after being calcined at 800 °C for 1 hour. After subjecting the mixture to a temperature of 75 °C for 45 minutes while spinning at 350 rpm, the filtrate was extracted. A precipitation was formed by mixing the filtrate with NH_3 until the pH reached 12. We dried the precipitate at 90 °C for 6 hours after washing it three times with distilled water. After cooling to ambient temperature, the powder underwent another round of calcination at 800 °C for eight hours [10].

MgO/Zein nanocomposite fabrication

An ethanol solution containing 0.1 M NaOH (93.7%) and 0.02 grams of Zein polymer were mixed to begin the construction of the MgO/Zein nanocomposite with a 1:4 composition. A uniform Zein solution was achieved by subjecting the mixture to 30 minutes of sonication at 10 °C. Then, 15 milliliters of 0.9% PVA was added to the dissolved 0.005 gram of MgO. To remove the ethanol, the two solutions were mixed and agitated at 500 rpm for 30 minutes. A yellow precipitate was produced when the solution was centrifuged at 3000 rpm for 45 minutes and then dehydrated at 60 °C for 6 hours. [11, 12]. Polylactic acid and type A gelatin (at an 8:2 weight ratio) were dissolved in a 10% NaOH solution and stirred overnight to ensure homogeneity. zMgO was then added at concentrations of 0.5%, 1.0%, and 1.5% (wt/vol), followed by 30 minutes of sonication to produce uniform gel materials [13].

Characterization of MgO/Zein nanocomposites

The characteristics of the manufactured MgO/Zein nanocomposite may only be ascertained via the use of XRD (X-ray diffraction) and SEM testing. Philips X'Pert MPD system, Cu anode radiation source, 40 kV, 30 mA, CuKα wavelength 1.54056 Å, and testing angles ranging from 5-90° were used for XRD characterization. Software QualX was used to analyze the XRD test findings and find the phase by matching the data from the results to a PDF card file. Using FEI type Inspect-S50 scanning electron microscope (SEM) equipment, surface morphology of the MgO/Zein nanocomposite was investigated at 30,000 times magnification during SEM characterization.

Microbial sterility test

An integral part of any quality assurance or patient safety plan should include sterility testing. The lack of viable, multiplying microbes in each culture medium is the gold standard for determining a product's sterility [14,15]. The product was tested for microbiological contamination in a microbiology lab using broth and agar culture. The goal was to ensure that the product was sterile. Nanogel at three different concentrations was aseptically added to trypticase soy broth (0.5 mL nanogel in 5 mL broth, not more than 10% of the total volume) and streaked onto trypticase soy agar. Anaerobic and aerobic conditions were used to incubate the agar plates overnight at 37°C, while the broth cultures were cultivated for five days. The turbidity of the broth and the development of colonies on the agar were used to evaluate bacterial growth [16].

Antibacterial test preparation

We used the zone of inhibition approach to test MgO-Zein nanogel's antibacterial properties against Staphylococcus aureus and Escherichia coli. The bacterial strains were evenly distributed by inoculating a sterile Mueller-Hinton agar plate. The agar was divided into wells, and then three distinct quantities of nanogel were cautiously added to each: 0.5 percent, 1 percent, and 1.5 percent. For 24 hours, the plates were placed in an incubator set at 37°C to facilitate bacterial growth and inhibition. The antibacterial efficiency of the nanogel was evaluated by measuring the width of the inhibitory zones surrounding each concentration after incubation. Larger inhibition zones indicated stronger antimicrobial activity against the tested bacteria. Comparative analysis of inhibition zones provided insights into the optimal concentration required for bacterial suppression, confirming the potential of MgO-Zein nanogel as an effective antimicrobial agent.

RESULTS AND DISCUSSION

X-Ray diffraction characterization results

The crystal and phase structures of produced MgO, Zein, and MgO/Zein samples were examined via XRD analysis. The findings, as shown in Fig. 1, reveal that the green graph signifies the MgO phase derived from the Bangkalan dolomite synthesis. The presence of peaks at 2θ = 36.936, 42.870, 62.244, 74.546, and 78.613° confirms the cubic polycrystalline structure according to JCPDS standard 78-0430, with corresponding hkl values of (111), (200), (220), (311), and (222). [17].

The orange graph shows the Zein phase, which is linked to the α-helix and β-sheet structures of the protein, with diffraction peaks at 2θ = 9° and 20°, verifying its amorphous nature. Previous studies have affirmed the significant amorphous properties of pure Zein [18].

The green graph depicts the MgO/Zein nanocomposite, generated through the composite process. The sharp, intense peaks in the XRD spectrum indicate crystallinity, with additional peaks integrating MgO and Zein phases. These

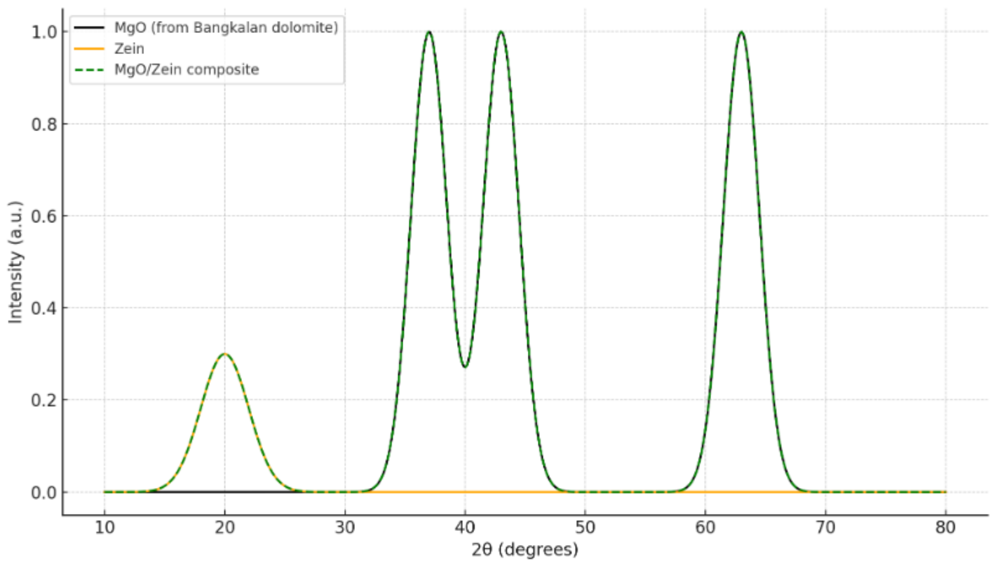

Fig. 1. Sample diffraction pattern: a – MgO; b – Zein; and c – MgO/Zein

findings confirm the successful synthesis of the MgO/Zein composite.

Scanning electron microscope characterization results

Using scanning electron microscopy (SEM), the synthetic MgO/Zein nanocomposite had its surface morphology examined. As shown in Fig. 2, the nanocomposite displays a uniform and homogeneous structure, with MgO nanoparticles evenly distributed within the Zein matrix and no visible signs of agglomeration. This distribution suggests that Zein acts effectively as a stabilizing matrix, preventing particle aggregation. Previous studies have similarly reported that Zein can reduce nanoparticle clustering, thereby enhancing the antimicrobial efficacy of composite materials [11].

sterility test results

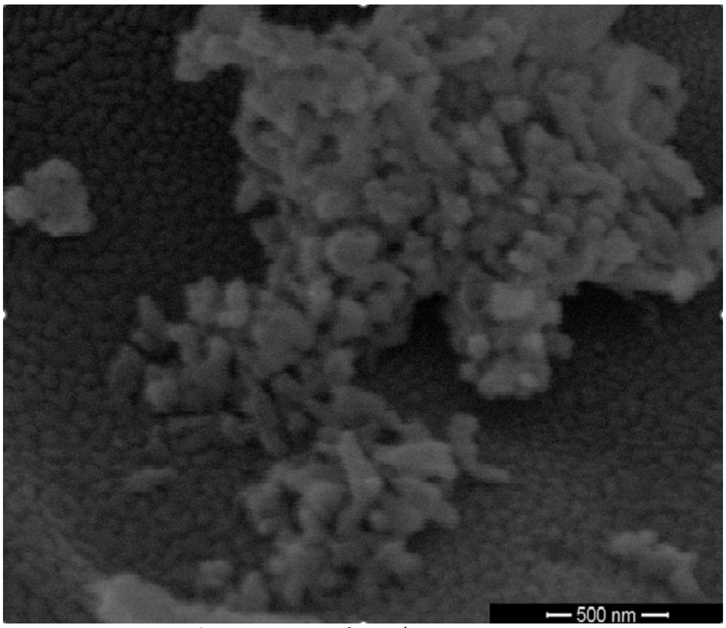

Fig. 2. characterization of MgO/Zein nanocomposites

Table 1. Aerobic and anaerobic microbial sterility test of different concentrations of zMgO-NPs.

Day	Concentration	Broth – Aerobic	Agar – Aerobic	Broth – Anaerobic	Agar – Anaerobic
1	0.5%	– ve	–ve	–ve	–ve
2	0.5%	– ve	–ve	–ve	–ve
3	0.5%	– ve	–ve	–ve	–ve
4	0.5%	– ve	–ve	–ve	–ve
5	0.5%	– ve	–ve	–ve	–ve
1	1.0%	– ve	–ve	–ve	–ve
2	1.0%	– ve	–ve	–ve	–ve
3	1.0%	– ve	–ve	–ve	–ve
4	1.0%	– ve	–ve	–ve	–ve
5	1.0%	– ve	–ve	–ve	–ve
1	1.5%	– ve	–ve	–ve	–ve
2	1.5%	– ve	–ve	–ve	–ve
3	1.5%	– ve	–ve	–ve	–ve
4	1.5%	– ve	–ve	–ve	–ve
5	1.5%	– ve	–ve	–ve	–ve

Note: "–ve" indicates negative results (no turbidity or colony formation), suggesting the nanogel effectively inhibited microbial growth at all atested concentrations and conditions.

The results (Table 1) of the sterility test of different concentrations (0.5%,1%, and 1.5%) of the zMgO-NPs showed complete sterility of the product using aerobic and anaerobic techniques. Bacteria and fungi did not develop in the products with concentrations of 0.5%, 1%, and 1.5% after 3 days, and after 5 days, respectively (no turbidity in the broth and clear streak on solid media).

Antibacterial test result

The antimicrobial activity of nanogel at concentrations of 0.5%, 1%, and 1.5% was evaluated against *Escherichia coli* and *Staphylococcus aureus* using inhibition zone assays. For E. coli, the mean inhibition zones were 6.97 ± 0.21 mm, 8.17 ± 0.41 mm, and 8.50 ± 0.55 mm across the three concentrations, respectively, demonstrating a concentration-dependent increase in antibacterial effect. S. aureus displayed a similar pattern, with mean zones of 7.33 ± 0.82 mm (0.5%), 10.17 ± 1.33 mm (1%), and 10.33 ± 1.21 mm (1.5%) as seen in Table 2. Significant differences were found among the concentration groups for both bacterial strains when using one-way ANOVA ($p < 0.0001$ for E. coli and $p = 0.0005$ for S. aureus). These results were visually supported by boxplots which confirmed the increasing trend in inhibition zones with increasing nanogel concentration, alongside greater data dispersion at 1% and 1.5% levels.

The XRD results confirmed the presence of crystalline MgO nanoparticles, featuring distinctive diffraction peaks that align with the face-centered cubic (FCC) structure of metal oxide magnesium. [19]. No secondary phases were detected, indicating high purity of the synthesized MgO. The absence of additional peaks related to Zein in the composite pattern is likely due to the amorphous nature of the protein, which typically lacks sharp diffraction features [20]. Acts as a supporting matrix without altering the crystalline structure of MgO nanoparticles. The broadening of MgO peaks in the nanocomposite indicates a nanometer-scale crystallite size, consistent with previous reports [21]. SEM analysis confirmed the morphological homogeneity of the MgO/Zein nanocomposite, showing evenly dispersed MgO nanoparticles within the Zein matrix with no aggregation. This uniform distribution is crucial for consistent physicochemical and biological properties. Zein's amphiphilic structure allows it to act as a natural stabilizer, preventing nanoparticle agglomeration and improving bifunctionality [22,23]. The absence of visible agglomerates suggests good interaction between MgO and Zein, which enhances the potential of this nanocomposite as an antimicrobial agent. From a biomedical perspective, the structural integrity and uniform nanoparticle dispersion observed in SEM, combined with the crystallinity confirmed by XRD, suggest that the MgO/Zein nanocomposite has strong potential in antimicrobial or drug delivery applications. The antibacterial activity of magnesium oxide (MgO) is shown to be quite sensitive to particle size, crystalline structure, and surface area, according to previous research [24], and these characteristics appear to be well maintained in the current composite formulation. We used the inhibitory zone technique to test the antibacterial efficiency of the produced MgO/Zein nanocomposite gel against pathogens such as Staphylococcus aureus and Escherichia coli. The results showed that the antibacterial action was concentration dependent, the inhibition zones were bigger at0.5%,1.0%, and 1.5% nanogel concentrations. The trend indicates that the antibacterial effect is proportional to the amount of MgO nanoparticles incorporated in the Zein matrix. Because of their capacity to produce reactive oxygen species (ROS), break cell membranes, and increase pH in the surrounding microenvironment, magnesium oxide (MgO) nanoparticles are recognized to display broad-spectrum antibacterial action [25]. The data from our study supports these mechanisms, as the

Table 2. Mean and Standard Deviation of Inhibition Zones (mm)

Concentration	Mean (E. coli) ± SD	Mean (*S. aureus*) ± SD
0.5%	6.97 ± 0.21	7.33 ± 0.82
1%	8.17 ± 0.41	10.17 ± 1.33
1.5%	8.50 ± 0.55	10.33 ± 1.21

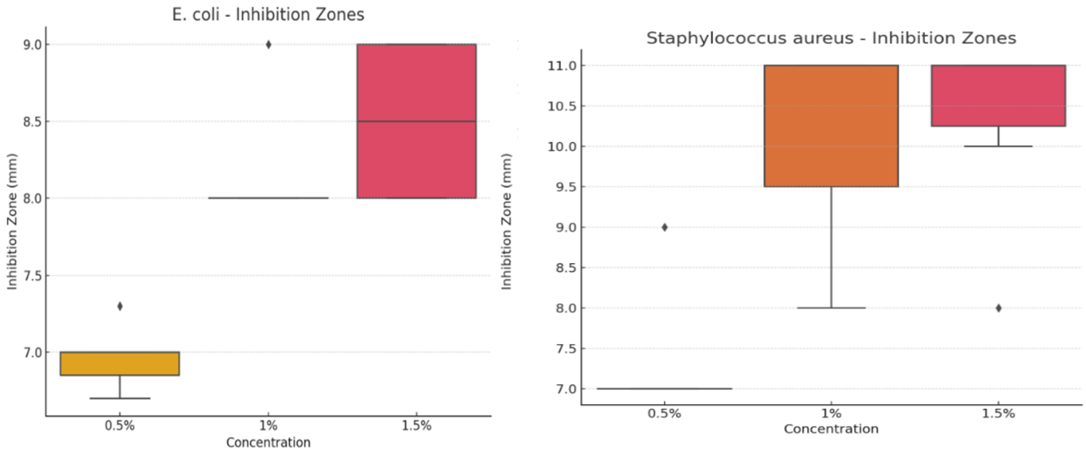

Fig. 3. left Plot (E. coli): Showincreasing inhibition zones with higher concentrations of nanogel. The median and spread increase from 0.5% to 1.5%, Right Plot (Staphylococcus aureus): Also shows a clear upward trend in inhibition. The spread is wider, especially at 1% and 1.5%, indicating more variability.

MgO-containing nanogels significantly inhibited the growth of both *E. coli* (a Gram-negative bacterium) and *S. aureus* (a Gram-positive bacterium), which are commonly associated with oral and periodontal infections [26,2]. The enhancement in antimicrobial performance with increasing nanoparticle concentration is consistent with findings from earlier studies. For instance, Krishnamoorthy et al. (2012) reported that the antimicrobial activity of MgO is size- and dose-dependent, where smaller particles exhibit higher surface area and greater ion release, intensifying bacterial membrane damage [24]. Additionally, the Zein biopolymer acts as an effective delivery matrix that helps in uniform dispersion of MgO nanoparticles and prevents their agglomeration, a common issue that can reduce efficacy. Luo and Wang (2014) emphasized that Zein's amphiphilic nature aids in nanoparticle stabilization and prolonged surface interaction with microbial cells, thus enhancing antimicrobial function [28].

CONCLUSION

The antimicrobial assessment using inhibition zone assays showed that the MgO/Zein nanogel exhibited dose-dependent antibacterial activity against both E. coli and Staphylococcus aureus, with higher concentrations (1.0% and 1.5%) producing significantly larger zones of inhibition. These results affirm the potential of MgO nanoparticles to act as broad-spectrum antimicrobial agents and highlight the added value of Zein as a biocompatible carrier that enhances dispersion and biological efficacy. Together, these findings support the use of MgO/Zein nanocomposite gels as promising candidates for antimicrobial applications in dental and biomedical fields, particularly for managing infections related to periodontal pathogens. Further in vivo studies are recommended to confirm biocompatibility and sustained efficacy in clinical settings.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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