WITHDRAWN: Antimicrobial activity of mouth rinses incorporating zein coated magnesium oxide nanoparticles

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Research article

Keywords: MgO, nanoparticles, Zein polymer, antibacterial, antifungal, mouthwash

Posted Date: August 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-49257/v1

EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Background The addition of antibacterial agents to oral prophylaxis materials showed inhibition of plaque accumulation and bacterial acid production. This study aimed to test the antimicrobial effect of distinctive oral mouth washes on an exemplary of oral bacteria after the addition of zein-coated MgO nanoparticles.

Materials and Methods Three hundred and twelve groups were used in this study. MgO nanoparticles in five concentrations with zein and without zein-coating, were incorporated in three oral mouth washes: Listerine Zero, Listerine Total Control and Oral B in the mass percentages of 0.3%, 0.5%, 1%, 2%, 5% and 10%, in addition to controls with no MgO nanoparticles. The three mouthwashes with variable concentrations were studied in opposition of *Staphylococcus aureus*, *Streptococcus mutans, Enterococcus faecalis* and *Candida albicans* using two different tests, the Disc Diffusion Test (DDT) and Direct Contact Test (DCT). Data was analyzed with Kruskall-Wallis and Mann-Whitney *U* tests.

Results Results showed a highly significant statistical difference of antimicrobial activity for all tested mouth washes with Zein coated MgO nanoparticles on *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans* in the disc diffusion test. While in the DCT, all tested mouth washes with MgO nanoparticles with and without zein coating showed antimicrobial activity on all tested microorganisms.

Conclusion Zein coated MgO nanoparticles is a potent antimicrobial agent when added to oral mouth washes. It is recommended to incorporate Zein MgO nanoparticles to Oral mouth washes to improve its antibacterial property.

Background:

Dental diseases such as caries, periodontal inflammation and candidal infection are the most common diseases affecting the oral environment. The main causative factors are bacteria and fungi like *Streptococcus mutans*, *Porphyromonousgingivalis* and *Candida albicans.* Mouth washes have been used for prevention of plaque accumulation to decrease the incidence of these diseases [1].

Metal oxide nanoparticles have been used in medicine for their bactericidal and bacteriostatic properties [2–4].These nanoparticles are considered as promising novel antibacterial agents, being harmless to mammalian cells and the oral environment [5].

As the antibacterial properties of metal ions depend on their surface contact area, the use of nanoparticles leads to increase surface areas and thus increased interaction with organic and inorganic molecules. ZnO and TiO_2 were recently introduced in the field of dental materials. They have been added to resin composite restorations for their antibacterial effects [6, 7]. While Mg is an essential metal to the human body, nano-structured MgO has been found to be an essential mineral for optimal human health due to its new physical and chemical characteristics [2]. Nano-MgO was shown to exhibit bacteriocidal

activity that is directly proportional to particle size and concentration [8], and to act against both Gram-positive and Gram -negative bacteria [6, 8, 9].

Because of its antimicrobial property, several studies have been conducted to assess MgO nanoparticles [9]. *Makhluf et al, 2005* synthesized nanocrystalline particles of MgO using microwave radiation in an ethylene glycol solution and showed that MgO has an antibacterial effect on selected bacteria [10].

After testing the antimicrobial effect of nanometal oxides, some researchers demonstrated that nanoparticles of MgO in solution have a bactericidal effect in opposition of variable bacteria [11], while others proved that ZnO behaves more as a growth inhibitor specially on Gram positive organisms [12].

MgO nanoparticles showed antibacterial properties, but when prepared as nanoparticles have aggregation problems that degrade their properties. Their tendency to agglomerate necessitates the use of a surfactant to help make them more soluble [13, 14]. Zein is a natural corn polymer that can be used in the nano form to coat the MgO nanoparticles in order to prevent their aggregation.

Zein has many unique properties that nominate it to be used in the industry of drug delivery and film coating [3]. Several investigators believe that zein might serve as an inexpensive and effective alternative for synthetic and semi-synthetic film coatings currently used for extrusion coatings [15, 16].

The addition of antibacterial agents to mouthrinses and toothpastes preparations showed inhibition of plaque accumulation and bacterial acid production [17].

Mouthwashes containing very low concentrations of zinc had high antimicrobial activity on strains of Streptococcus in the mouth [18, 19].

However, no studies tested the effect of adding MgO nanoparticles specially coated with zein polymer Hence, we decided to test the antimicrobial effect of the zein-coated MgO nanoparticles when added to distinctive mouth washes on an exemplary of oral bacteria.

Methods:

MgO nanoparticles, Zein polymer (Sigma Aldrich, Misouri 63103, USA), Listerine Total care mouth wash, Listerine Zero mouth wash (Johnson & Johnson, New Jersey 08933, USA) and Oral B mouth wash (Procter & Gamble, Ohio 45202, USA) were used in this study. Agar plates and the sterile paper discs were purchased from Becton, Dickinson and company (New jersey 07417, USA).

Synthesis of MgO nanoparticles:

MgO nanoparticles were synthesized by directly reacting magnesium acetate and urea in the microwave hydrothermal technique as follows [2]: A solution of 6.44 g of magnesium acetate in 75 ml of distilled water was stirred magnetically at 25ºC for 30 min. Then, drops of a mixture of urea and water (1.2 g / 25 ml) were added under vigorous magnetic stirring for 5 min. An autoclave lined with Teflon was

charged with the mixture, secured and kept on a 1000W power microwave at 180 °C for 15 min. After cooling of the autoclave and resolution of the reaction, the products were centrifuged for 5 min, filtered with distilled water, then ethanol, then dried at 60 °C for 24 h. Finally, the white-colored material that resulted was calcinated at 600 °C for 1 h [2].

Preparation of MgO-Zein Nanoparticles Using pH-Controlled Nanoprecipitation

The zein polymer was mixed with polyvinyl alcohol (PVA); then MgO nanoparticles were added at a weight ratio of 4:1. The MgO-zein- PVA mélange was then stirred for 30 minutes. The nanoparticles mix of MgO-zein nanoparticles develop by phase separation. After evaporation of the PVA content, the final product was then centrifuged and dehydrated.

Incorporation of Mg/zein nanoparticles in mouthwash

MgO nanoparticles and zein coated MgO nanoparticles were incorporated in three mouth washes: Listerine Zero, Listerine Total Control (Johnson & Johnson, NJ 08933) and Oral B (P&G, Ohio 45202) in the mass percentages of 0.3%, 0.5% 1%, 2% 5% and 10%. In our previous study [20], on the antimicrobial properties of MgO coated with zein polymer the percentage of 1% showed an antimicrobial effect so we wanted to increase the dose and see its antimicrobial effect. The solution was kept on a magnetic stirrer at 500 rpm at room temperature for 15 minutes for complete dissolution.

Antimicrobial assay

Two antimicrobial activity tests were carried out: the Kirby-Bauer agar diffusion test [21], and the direct contact test.

Development of bacterial cultures

The test organisms used in the study were acquired from the American Type Culture Collection (ATCC, VA 20110, USA). They are as follows: *Streptococcus mutans* (ATCC 25175; gram-positive coccus), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC 10231 fungus).

The bacteria and fungi concentration were prepared from stock and cultured on agar and sabareaud plates.

Determination of antimicrobial effect of the mouth wash mix

1- Agar diffusion test

156 groups were prepared. Freshly prepared inoculums of microorganisms were cultured on blood agar and sabareaud plates. Sterile paper discs of 6 mm in diameter were filled with 100 µl of MgO nanoparticles or Zein coated MgO nanoparticles in solution of different concentrations. Bacteria and

fungi were allowed to grow for one day in the 37 °C incubator, then inhibition zones were measured [6]. Bacterial inhibition was assessed by measuring the diameter of bacterial inhibition zone (mm) data represents the mean +/- SD [7].

2- Direct contact test

156 groups were prepared. Freshly prepared inoculums of microorganisms were prepared into a 1 Mc Farland dilution.

Equal amount of the bacteria solution and the solution of the different concentrations of MgO nanoparticles or Zein coated MgO were mixed. The tubes were incubated at 37 °C. Solutions then tested for bacteria growth by streaking fresh agar and sabareaud plates at different time points: 1hour and 1, 2, 3, 4,5 6 and 7 days.

Plate count:

Viable bacterial cell concentrations were estimated by counting CFU's before and after exposure to the nanoparticles solutions. This was performed by serial dilution in Broth and then removing 10 µl of the serially diluted culture and spreading with sterile glass beads (5 mm, Sigma, UK) onto an agar plate (Tryptic Soya Agar) in triplicate. The plates were then incubated at 37 °C for 24 hours and CFUs were counted (Fig. 1).

Statistical analysis

Data was analyzed using SPSS version 18.0. One Way ANOVA test was performed followed by LSD to detect significance between groups at $p < 0.05$

Results:

Results showed a statistical significant increase of the antibacterial activity when Zein coated MgO nanoparticles are added to all tested oral mouthwashes rather than MgO nanoparticles alone at ($p < 0.05$).

For S. mutans

The addition of different concentrations of Zein coated MgO nanoparticles to Listerine Total care showed a statistical significant increase in the antibacterial activity against *S. mutans*, than the control and the one with MgO nanoparticles alone ($P = 0.0001$) (Fig. 2).

The same results were seen with Listerine Zero and Oral B mouth washes. There was a significant difference in the antibacterial activity against *S. mutans* with the addition of different concentrations of Zein coated MgO nanoparticles compared to the control and the regular MgO nanoparticles ($P = 0.0001$) (Fig. 2).

For S. aureus

All concentrations added of Zein coated MgO nanoparticles to Listerine Total care showed a statistical significant increase in the antibacterial activity against *S. aureus*, than the control and the one with MgO nanoparticles alone (*P = 0.0001*) (Fig. 3).

Also, the addition of all concentrations of Zein coated MgO nanoparticles to Listerine Zero and Oral B showed a statistical significant increase in the antibacterial activity against *S. aureus*, than the control and the one with MgO nanoparticles alone (*p = 0.0001*) (Fig. 3).

For E. faecalis

The antibacterial effect of all the three mouthwashes: Listerine Total Care, Listerine zero and Oral B after the addition of Zein coated MgO nanoparticles showed a significant increase from the control and the ones with MgO nanoparticles alone. (p = 0.0001) (Fig. 4)

For C. albicans

The addition of different concentrations of Zein coated MgO nanoparticles to Oral B showed a statistical significant increase in the antifungal activity against *C. albicans*, than the control and the one with MgO nanoparticles alone (*p = 0.0001*) (Fig. 5).

While all concentrations added of Zein coated MgO nanoparticles to Listerine Total care showed a statistical significant increase in the antifungal activity against *C. albicans*, than the control and the one with MgO nanoparticles alone (*p = 0.0001*) except at the 10%, there was no significant difference in the antifungal activity of coated or noncoated MgO nanoparticles (*p = 0.286*) (Fig. 5).

Meanwhile there was a statistically significant increase in the antifungal activity of Listerine Zero after the addition of 0.3% and 0.5% zein coated MgO nanoparticles than the control and the MgO nanoparticles alone (p = 0.002). But there was no significant difference in the antifungal activity between the effect of adding coated or non-coated MgO nanoparticles in 1,2,5 or 10% (*p = 0.177, p = 0.242, p = 0.242, p = 1.00)* (Fig. 5).

Complete inhibition of all bacteria (*S. mutans, S aureus, E. faecalis* and for the fungus *C. albicans)* with all concentrations was observed for the three mouth washes after 24 hours, 2, 3, 4,5,6 and 7 days.

Discussion:

Mouth rinses often contain several antimicrobial agents. They also contain methylparaben (methyl p-hydroxybenzoic acid methyl ester) as a preservative that has antimicrobial activity. Listerine mouth rinses contain “essential oils” like eugenol, eucalyptol, camphor, etc. that are probably responsible for the observed antimicrobial activity in the mouth rinses alone. However, addition of 0.3% -10% by weight MgO/Zein nanoparticles enhanced the antimicrobial effect of the studied mouth rinses. Several

investigators studied the addition of bactericidal to oral hygiene products to inhibit plaque accumulation and reduce bacterial acids [18, 22, 23]. Some of them added copper and zinc to enhance the antimicrobial activity of mouth washes [17, 19, 24].These studies also reported that mouth rinses with concentration less than 1% of bactericidal had good antibacterial effects [19, 24].

In our study we added different concentrations of zein coated and non-coated MgO nanoparticles to three most popular mouth washes, and tested the mixtures on gram positive *Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis* and on the fungus, *Candida albicans*.

Results showed that the incorporation of MgO/zein nanoparticles to all tested mouth washes had significant antimicrobial activity on all tested organisms.

For *Streptococcus mutans*, *staphylococcus aureus* and *Enterococcus faecalis*, all three mouth washes showed the best antimicrobial activity results with a concentration of 1% of zein coated MgO nanoparticles. This is in accordance with our previous study [20] and other studies that showed that the use of bactericidal of less than 1% had good antimicrobial effect. [19, 24]

For *Candida albicans*, results were different with different mouthwashes. For Listerine Total care, the 1% MgO/zein concentration showed significant antifungal effects. While for Listerine zero the 0.5% MgO/zein concentration showed significant antifungal effects. And for Oral B, the 2% MgO/zein concentration showed significant antifungal effects. These results are also in close accordance with previous studies [20, 25] that nanoparticles of 0.5%-1% concentration were able to remove almost 100% of bacteria.

In summary, in this study, the addition of MgO/zein nanoparticles showed significant higher antibacterial and antifungal effect than the control and the addition of MgO nanoparticles alone.

Conclusion:

Since MgO is beneficial for human health, and has antibacterial and antifungal effects, we recommend that MgO/zein nanoparticles be added to these mouth washes to reduce the amount of bacteria in the mouth and prevent plaque accumulation.

Abbreviations

DDT: Disc Diffusion Test; DCT:Direct Contact Test; PVA:polyvinyl alcohol; KAU:King Abdulaziz University; ANOVA:analysis of variance; LSD:least significant difference.

Declarations

Acknowledgment

Not applicable.

Authors contributions

GHN and MTH proposed the idea and designed the study. GHN, MTH, SMB and AMA performed the experiment, MTH, AMA and SMB contributed to the collection of data. introduction, methods, and discussion. MTH performed statistical analysis. GHN, MTH, AAH and AHH wrote the manuscript and proof read the manuscript. The authors read and approved the final manuscript.

Funding

The authors report no funding sources.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding authors upon reasonable request.

Ethics approval and consent to participate

The present study has been done and followed by the Ethical Guidelines in the Faculty of Dentistry at King Abdulaziz University (reference number #094-10-17)

Consent for publication

The authors have given their consent for their data to be published in the report.

Competing interests

The authors certify that they have no affiliation with or involvement in any financial interest (such as honoraria, educational grants, participation in speaker's bureau, membership, employment, consultancies, stock ownership, or other equity interest, and expert testimony or patent licensing arrangements) or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Figures

Figure 1

The three mouth washes used with different concentrations of zein coated or non-coated MgO nanoparticles concentrations on 4 types of organisms (S. mutans, S. aureus, E. faecalis and C. albicans).

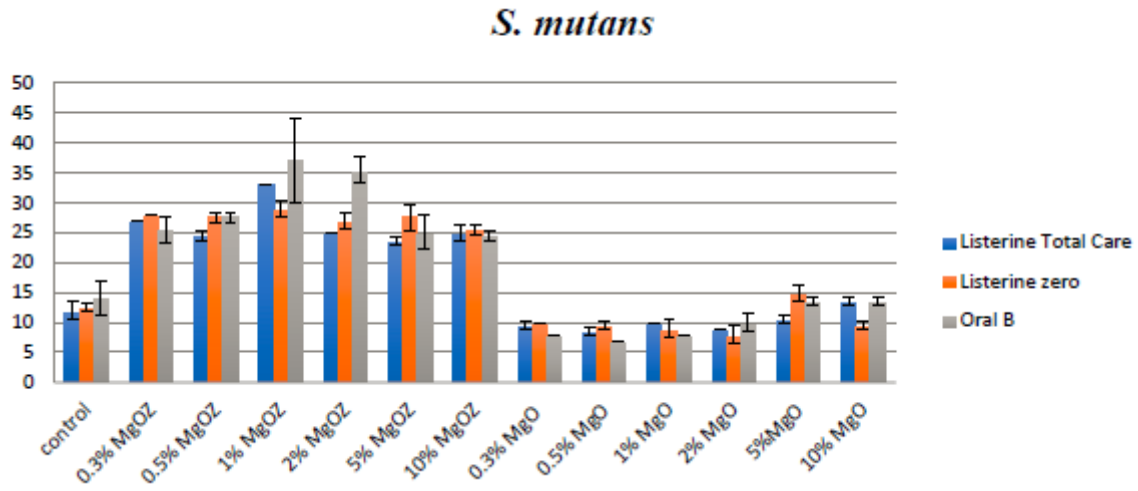

Figure 2

Zones of inhibition zones (in mm) for Oral B, Listerine Zero and Listerine Total Control with and without zein coated MgO nanoparticles with S. mutans.

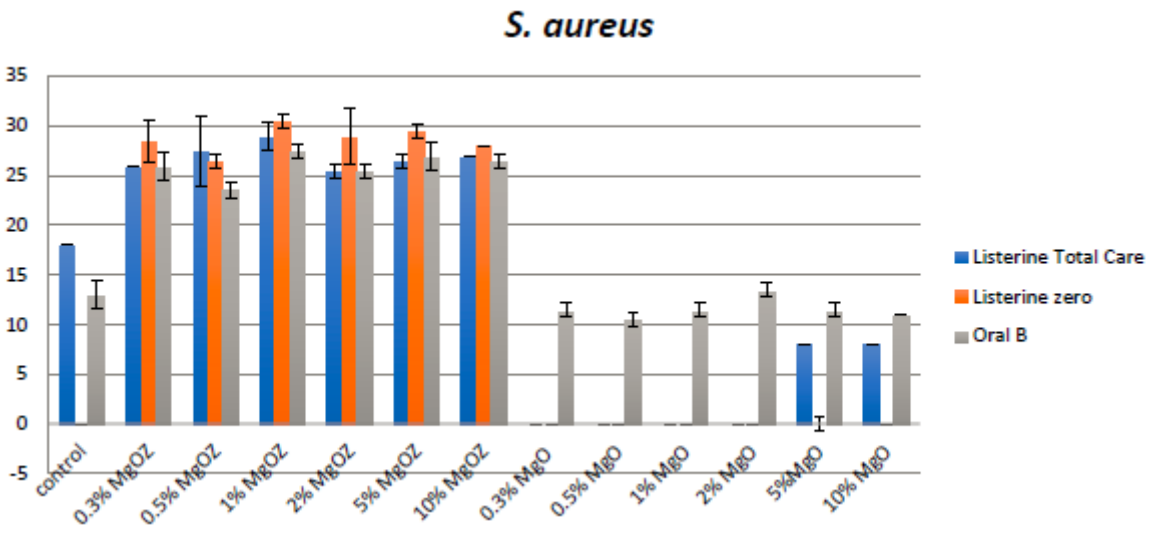

Figure 3

Zones of inhibition zones (in mm) for Oral B, Listerine Zero and Listerine Total Control with and without zein coated MgO nanoparticles with S. aureus.

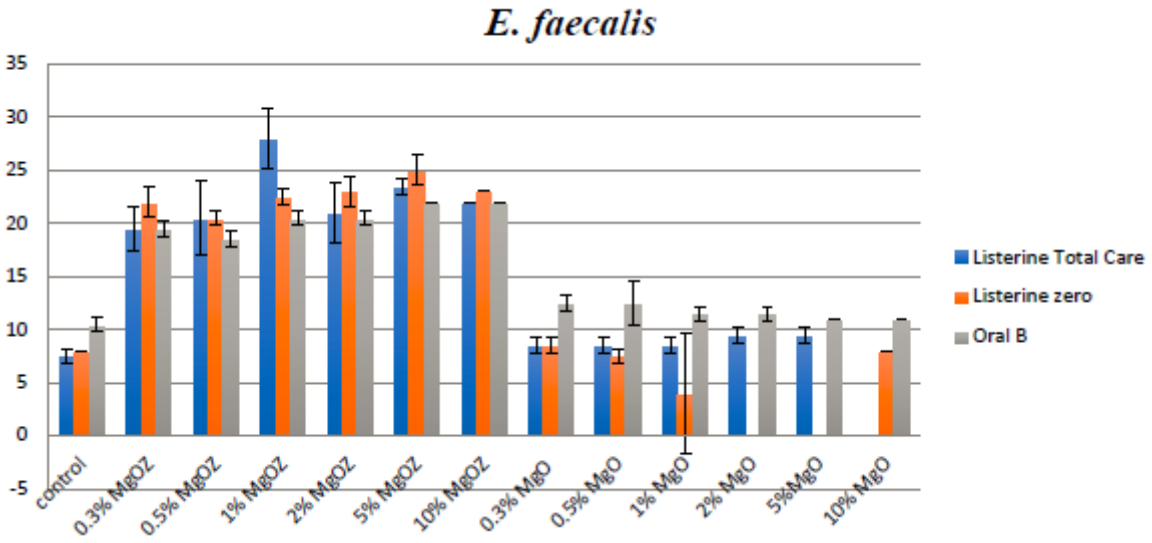

Figure 4

Zones of inhibition zones (in mm) for Oral B, Listerine Zero and Listerine Total Control with and without zein coated MgO nanoparticles with E. faecalis.

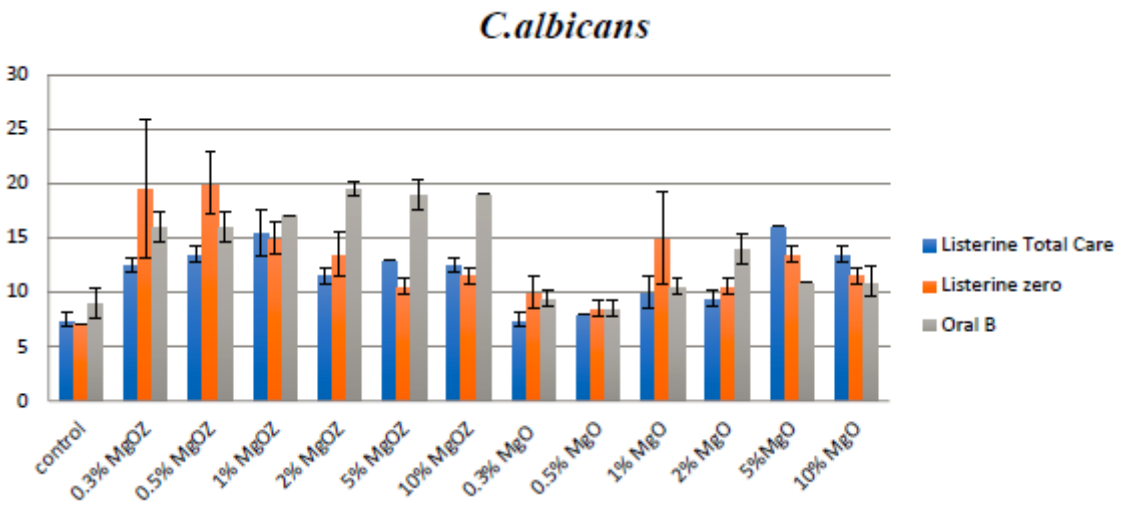

Figure 5

Zones of inhibition zones (in mm) for Oral B, Listerine Zero and Listerine Total Control with and without zein coated MgO nanoparticles with C. albicans.