

# Zein based magnesium oxide nanoparticles: Assessment of antimicrobial activity for dental implications

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**Abstract:** MgO nanoparticles have been recently discovered as an antibacterial, however, they limited by property degradation due to agglomeration. The addition of a coating agent, such as a zein polymer, is effective in preventing agglomeration without affecting nanosized properties. The aim of this study was to assess the antimicrobial property of MgO nanoparticles when coated with a zein polymer against several oral bacteria and fungi. This was done by utilizing various assessment techniques. The ultimate aim is to use these nanoparticles in dental preparations. The antimicrobial activity of zein-coated MgO nanoparticles at different concentrations of 0.5, 1 and 2% were tested against four different microorganisms: *Staphylococcus aureus*, *Streptococcus mutans* and *Enterococcus faecalis* (gram positive bacteria), and *Candida albicans* (as oral fungus). Two different techniques were utilized: the Kirby-Bauer test, and a modified direct contact test. The results indicated that the antibacterial effect of 1% or 2% zein-coated MgO nanowires were statistically significant ( $p < 0.05$ ) against the four organisms studied: *S. mutans*, *S. aureus*, *E. faecalis* and *C. albicans*. Zein-coated MgO nanoparticles are a new human friendly and potent antimicrobial agent that can be incorporated in the formulation of a variety of new dental materials and products that should provide improvements in dental care and oral health.

**Keywords:** *Enterococcus faecalis*, dental preparation, *Candida albicans*, magnesium oxide, nanoparticles, *Streptococcus mutans*, *Staphylococcus aureus*, zein coated.

## INTRODUCTION

The increase in antibiotic resistance of many bacterial strains has accelerated the development of antibacterial nanomaterials, as they seldom cause the development of resistance (Hamouda, 2012). Nanotechnology involving incorporated nanowires of metal oxides are very attractive for different industrial, medical, and dental products. (Gupta *et al.*, 2013) Metal oxides, especially zinc oxide (ZnO), titanium oxide (TiO<sub>2</sub>), copper oxide (CuO) and magnesium oxide (MgO), have received particular interest as an antimicrobial agent due to their safety, stability and ability to bind to and damage bacterial membranes (Dong *et al.*, 2010).

Recently, researchers have shown interest in MgO after discovering that it can be used in multiple industries. (Gordon *et al.*, 2011, Al-Ghamdi *et al.*, 2012) As a metal oxide, MgO has a high melting temperature, and is utilized in different areas, including as a desiccator in the silicon steel industry, in ceramics (Anhorn *et al.*, 2008), and in adhesives and supplements for industrial chemistry (Satheesh and Omathanu, 2012).

Our previously published studies from this project investigated preparation and assessment of different processing factors that affect the character of MgO nanowires. In this part of the project, we assessed the antimicrobial activity for these nanowires for use in dental applications. (Sharma *et al.*, 2011) The variation in size and concentration of MgO affects their antibacterial activity towards a wide range of bacteria. (Sivaramakrishna *et al.*, 2010 and Jin and He, 2011) These nanowires are newly found antibacterial agents that do not harm mammalian cells or the environment (Huang *et al.*, 2005; Makhluaf *et al.*, 2005) synthesized nanocrystalline particles of MgO in a solution of ethylene glycol in a microwave, and demonstrated its antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* bacteria. Shi *et al.* (2010) investigated the antimicrobial property of inorganic nanometal oxides for pathogen control. They showed that MgO in solution has a bactericidal activity against certain bacteria, whereas ZnO behaves as a growth inhibitor and is more effective against Gram positive organisms. Vicentini *et al.* (2010) studied the antimicrobial effect of nano MgO against food bacteria. They reported that MgO nanowires have strong bactericidal activity against pathogens that is reciprocal to

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the concentration of MgO. Karimi *et al.* (2011) studied the synthesis and characterization of nanowires and nanocomposites of zinc and magnesium oxide for incorporation in a polycarboxylate cement preparation. These nano-metal oxides have good mechanical properties and can be added to dental cements to provide antimicrobial activity.

Unfortunately, nano-metal oxides have the disadvantage of being relatively insoluble, and tend to agglomerate in solution, which degrades their properties. Therefore, the addition of a coating agent or surfactant helps in preventing agglomeration. Zein polymer is a low-cost, alcohol-soluble protein found in corn that has the capability to make nanospheres and film coatings. This makes it of particular interest for particulate- and film-mediated delivery. (Beck *et al.*, 1996) Some investigators believe that zein might serve as an affordable and functional film coating alternative. (Al-Hazmi *et al.*, 2012) Hence, the purpose of this study was to test the efficacy of mixing MgO nanowires with zein polymer in order to preserve nanowire antimicrobial properties.

## **MATERIALS AND METHODS**

### ***Synthesis of MgO nanowires***

MgO nanowires were synthesized by directly reacting magnesium acetate and urea using a microwave hydrothermal technique (Maizura *et al.*, 2007) as follows: a solution of magnesium acetate in distilled water (6.44 g/75ml) was stirred for 30 minutes. Next a solution of urea (1.2g/25ml of water) was added by a dropper for 5 minutes. The mix was poured in a Teflon-lined autoclave in a microwave furnace (1000 power) for 15 minutes at a temperature of 180°C. After cooling of the autoclave to room temperature, the white material was collected by centrifugation and rinsed with water and then with ethanol to reduce clumping. This was left to dry for 24 hours at 60°C temperature, and then calcined for one hour at a temperature of 600°C

### ***Coating of MgO nanowires***

#### ***Preparation of MgO-zein nanowires using pH-controlled nanoprecipitation***

Briefly, 0.02g of zein was dissolved in a mixture of ethanol and 0.1 NaOH solution (93.7% (v/v)). A zein solution was added drop wise under ultrasonic shear (750 W and 20 kHz frequency) into 15 ml of aqueous phase containing 0.02g Of MgO and polyvinyl alcohol (0.9% (w/v)). The temperature of the aqueous phase was maintained at 10°C using an ice bath. Subsequently, the zein suspension was placed on a magnetic stirrer at 500 rpm at room temperature to evaporate the ethanol. After complete evaporation of the ethanol, the nanowires were purified to remove excess PVA. The aqueous suspension of zein nanowires were purified by two cycles of differential centrifugation (3,000 rpm for 45 min). After centrifugation, the supernatant was discarded and the

pellet was redispersed in 5ml of buffer. (Satheesh, 2012). Subsequently the suspension was lyophilized (Virtis™, Bench Top model, USA) after adding 2% (w/v) trehalose. (Anhorn, 2008) The zein polymer was then mixed with polyvinyl alcohol (PVA) at a weight ratio of 2:1, and MgO nanowires were added at a weight ratio of 4:1. The solution of MgOzein and PVA was mixed on a magnetic stirrer for a period of 30 minutes.

After separation of the MgO-zein nanowires, the mix was left to evaporate the PVA, then centrifuged and freeze-dried.

### ***Antimicrobial assay***

The antimicrobial activity of the MgOzein nanowires was tested by the Kirby-Bauer agar diffusion test and modified direct contact test. (Zhang *et al.*)

### ***Preparation of materials and bacterial cultures***

An antimicrobial task was determined by the diameter of their inhibition zones against bacteria and fungi on blood and sabouraud agar plates. The following bacterial strains were obtained from the American Type Culture Collection (ATCC) and used to test the antibacterial activity of MgOzein nanowires in the study: *Streptococcus mutans* (ATCC 25175; gram-positive coccus), *Staphylococcus aureus* (ATCC 6538; gram-positive coccus), *Enterococcus faecalis* (ATCC 29212 gram positive coccus) and *Candida albicans* (ATCC10231 fungus).

### ***Determination of antimicrobial activity of metal oxide nanowires***

#### ***By Kirby-Bauer test***

Sterile agar plates were cultured with freshly prepared bacterial inoculum. Sterile paper discs of 6mm in diameter were filled with 100µL of test solutions. These treated filter paper discs were then placed on the plates. The plates were placed in the incubator for one day at a temperature of 37°C. Inhibition zones were measured around each disc. Each plate had 4 discs and every experiment was performed three times. Results represent the average reading +/- SD.

### ***Direct kill test***

#### ***Nanowires preparation***

MgO nanowires solutions and MgOzein coated nanowires solutions in distilled water were prepared at concentrations of 0.5%, 1% and 2%. Samples were sonicated with probe sonicator for 3 minutes each.

### ***Bacteria preparation***

Solutions of 0.5 Mc Farland in broth were prepared from the following bacteria: *Streptococcus mutans* ATCC 25175; *Staphylococcus aureus* ATCC25923; *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231. Equal amount of nanowires solution was added to bacteria solution at a ratio of 1:1 and incubated at 37°C at the following times: 2 hrs, 24 hrs, 2 days, 3 days, 4 days, 5

days, 6 days and 7 days. After each period of incubation, each concentration of the MgO nanowires alone and the MgO nanowires coated with zein were tested for bacteria/fungi growth by streaking agar plates and incubating them at 37°C for 24 hours. Bacterial growth was determined by counting the bacterial colonies (cfu). The results representing the number of bacterial colonies in each plate were graphed according to each type of bacteria.

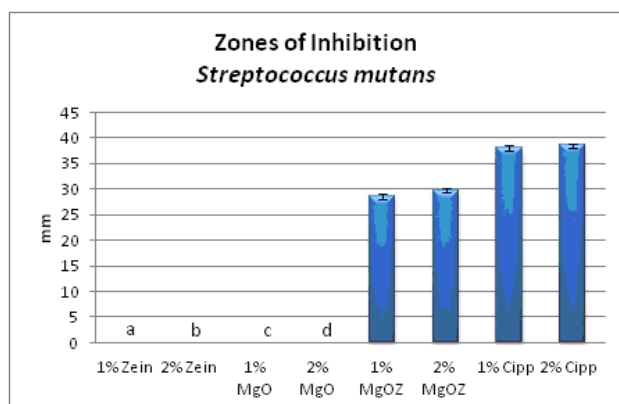
## RESULTS

### Antibacterial activity of MgO nanowires

#### Antibacterial activity on *Streptococcus mutans*

##### Kirby-Bauer test

Zein coated MgO nanowires at both 1 and 2% concentrations showed zones of inhibition against *S. mutans* (28.5, 29.75 mm, respectively). Non-coated MgO nanowires and zein polymer did not show any zones of inhibition (0 mm). The antibacterial effect of the zein coated MgO nanowires was statistically significant and different than the antibiotic at both concentrations of 1 and 2% ( $p < 0.05$ ). There was no statistically significant difference between 1 and 2% zein-coated MgO nanowires ( $p < 0.05$ ). (fig. 1).

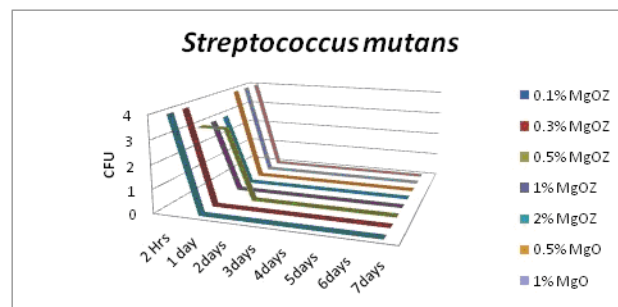


**Fig. 1:** Bar graphs showing the differences in the zones of inhibition in produced by 1 or 2% zein alone, 1 or 2% MgO nanowires alone, 1 or 2% zein-coated MgO (MgOZ) or by 1 or 2% Ciprofloxazine HCl (Cipp) against *Streptococcus mutans*. Height of bars indicates the mean values of zones of inhibition (mm), Brackets indicate +/- S.D. Groups identified by different lower case letters superscripts are significantly different at  $p < 0.05$

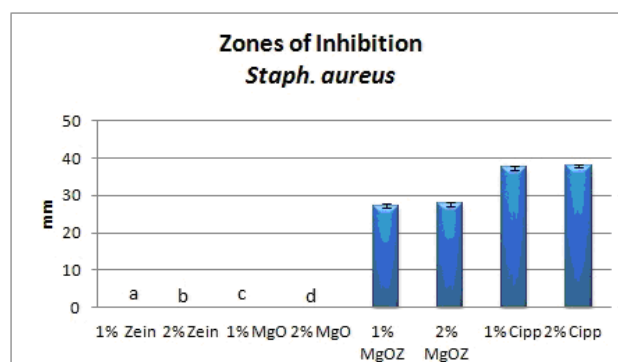
##### Direct kill test

All concentrations of zein coated MgO nanowires and of MgO alone showed growth of *S. mutans* bacteria after 2 hours of incubation at 37°C ( $10^4$  cfu). After 24 hours, all the concentrations showed no growth of bacteria except with 0.5% zein coated MgO nanowires, where the growth was light ( $10^3$  cfu). On day three, all concentrations showed no growth including the solution of 0.5% zein coated MgO nanowires. These results were further

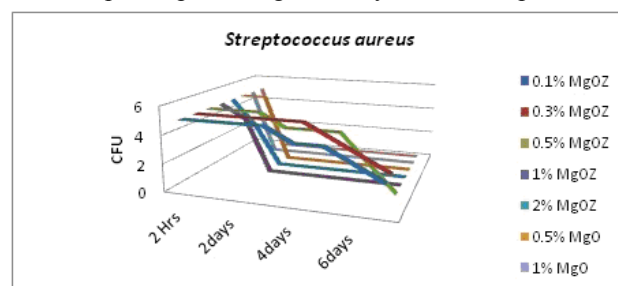
confirmed at day 3, 4, 5, 6 and 7, where there was no *S. mutans* growth. (fig. 2).



**Fig. 2:** Line Graph showing the bacteria count (cfu) of *Streptococcus mutans* after direct contact with different solutions from 2hrs till 7 days.



**Fig. 3:** Bar graphs showing the differences in the zones of inhibition of *Staphylococcus aureus* by 1 or 2% zein alone, 1 or 2% MgO nanowires alone, 1 or 2% zein-coated MgO (MgOZ) or by 1 or 2% Ciprofloxazine HCl (Cipp). Height of bar indicates the mean (mm), Brackets indicate +/- S.D. Groups identified by different lower case letters superscripts are significantly different at  $p < 0.05$ .



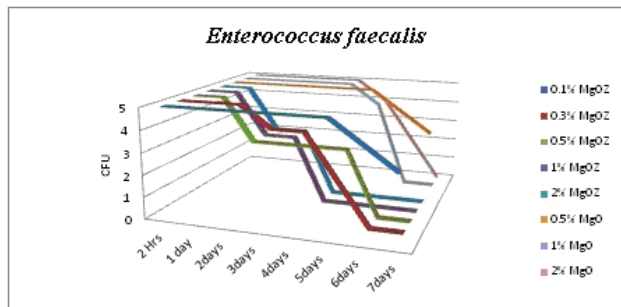
**Fig. 4:** Line Graph showing the bacteria count (cfu) of *Staphylococcus aureus* after contact with different solutions from 2hrs till 7 days.

### Antibacterial activity on *staphylococcus aureus*

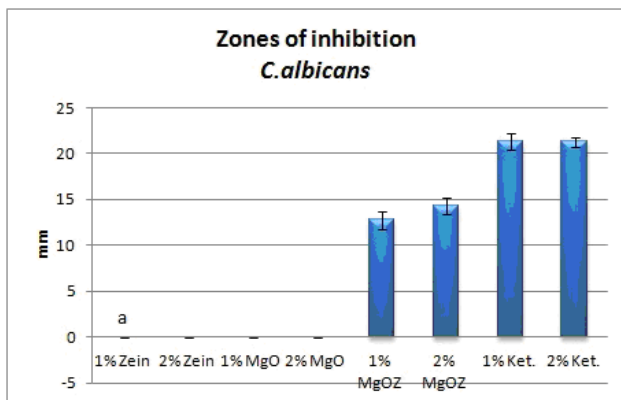
#### Kirby-Bauer test

Zein coated MgO nanowires at 1 and 2% showed zones of inhibition against *S. aureus* (27.25, 28mm, respectively). ZnO nanowires alone and zein polymer showed no zones of inhibition (0mm) against *S. aureus*. The antibacterial effect of the zein coated MgO nanowires was statistically significant and different than the

antibiotic at both concentrations of 1 and 2% ( $p < 0.05$ ). There was no statistically significant difference between 1 and 2% zein coated MgO nanowires ( $p < 0.05$ ) (fig. 3).



**Fig. 5:** Line Graph showing the bacteria count (cfu) of *Enterococcus faecalis* after contact with different solutions from 2hrs till 7 days.

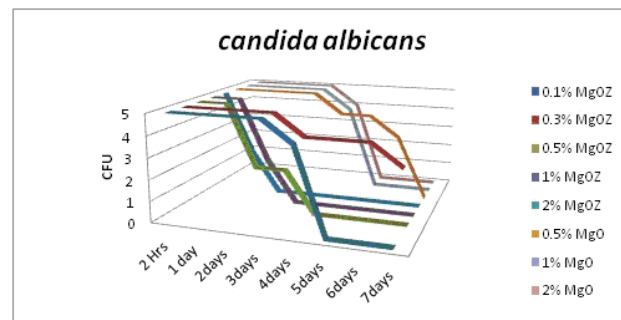


**Fig. 6:** Bar graphs showing the differences in the zones of inhibition of *Candida albicans* by 1 or 2% zein alone, 1 or 2% MgO nanowires alone, 1 or 2% zein-coated MgO (MgOZ) vs positive control antifungal agent, 1 or 2% Ketoconazole (Ket). Height of bar indicates the mean (mm), Brackets indicate  $\pm$  S.D. Groups identified by different lower case letters superscripts are significantly different at  $p < 0.05$ .

**Direct kill test**

All concentrations of zein coated MgO nanowires and of MgO alone showed growth of *S. aureus* bacteria after 2 hours of incubation at 37°C ( $10^5$  cfu). After 24 hours, there was no growth with 1 and 2% MgO, light growth with solutions of 2% MgOZ ( $10^3$  cfu), and heavy growth ( $10^5$  cfu) in the rest of the solutions. After two days, all solutions of 1, 2% zein coated MgO nanowires (MgOZ) and 0.5, 1 and 2% of MgO nanowires had no growth, while 0.1, 0.3 and 0.5% of zein-coated MgO nanowires had heavy growth ( $10^5$  cfu). The same results appeared on day 3 ( $10^5$  cfu) for the three concentrations. Meanwhile, 1, 2% zein-coated MgO nanowires and 0.5, 1 and 2% of MgO showed no bacterial growth after 3 days. The same pattern was observed on day 4 with a slight decrease in bacterial growth in solutions of 0.1, 0.3 and 0.5% MgOZ ( $10^4$ ,  $10^5$ ,  $10^4$  cfu, respectively). By day 7 0.5% MgOZ

had no growth, while 0 and 0.3% MgOZ had light growth of *S. aureus* ( $10^2$  cfu) (fig. 4).



**Fig. 7:** Line Graph showing the bacteria count (cfu) of *Candida albicans* after contact with different solutions from 2hrs till 7 days.

**Antibacterial activity on enterococcus faecalis**

**Kirby-Bauer test**

There were no zones of inhibition against *E. faecalis*, a gram-positive coccus, for either 1% or 2% zein, or 1% or 2% MgO nanowires alone, or 1% or 2% zein coated-MgO nanowires that exhibited any antibacterial effects.

**Direct kill test**

All concentrations of MgOZ nanowires and of MgO alone showed growth of *E. faecalis* bacteria after 2 hours and 24 hours of incubation at 37°C ( $10^5$  cfu). After two days, 0.5%, 1% and 2% of zein-coated MgO nanowires showed a decrease in bacteria count ( $10^3$  cfu), but there was bacterial growth with all other concentrations ( $10^5$  cfu). By day 4, 1 and 2% zein-coated MgO nanowires showed no growth, while the rest of the concentrations showed the same pattern for day 2. On day 6, all concentrations showed no growth except for the 0.1% zein-coated MgO nanowires and 0.5 and 2% MgO nanowires showed a slight decrease in bacterial concentration ( $10^4$ ,  $10^4$  and  $10^2$  cfu, respectively). By day 7 there was no bacterial growth for all concentrations, except for 0.1% MgOZ and 0.5% MgO nanowires, which showed the same bacterial count ( $10^3$  cfu) (fig. 5).

**Antifungal activity of MgO nanowires**

**Kirby-Bauer test**

Similarly, zein-coated MgO nanowires at 1 and 2% showed zones of inhibition against *C. albicans* (13, 14.25 mm, respectively). ZnO nanowires alone and zein polymer showed no zones of inhibition (0mm) against *C. albicans*. The antibacterial effect of the zein-coated MgO nanowires was statistically significant and different than the antifungal drug at both concentrations of 1 and 2% ( $p < 0.05$ ). There was no statistically significant difference between 1 and 2% zein-coated MgO nanowires ( $p < 0.05$ ) (fig. 6).

**Direct kill test**

All concentrations of MgOZ nanowires and of MgO alone showed candidal growth after 2 hours of incubation at

37°C ( $10^5$  cfu). By day 2, 2% MgOZ had no growth, whereas 0.5 and 1% MgOZ had scanty growth ( $10^2$  cfu) and 0.5, 1, 2% MgO and 0.1, 0.3% MgOZ had heavy growth ( $10^5$  cfu). On day 3, 1 and 2% MgOZ showed no growth of *C.albicans*, while a 0.5% MgOZ concentration was scanty ( $10^2$  cfu) and the rest still had heavy growth ( $10^5$  cfu). On day 4, 0.5, 1 and 2% MgOZ showed no growth while the rest showed medium growth ( $10^4$  cfu). By day 5, All concentrations had no candidal growth, except for 0.3% MgOZ and 0.5% MgO, which showed growth of *C. albicans* ( $10^4$  cfu). Day 6 had the same pattern as day 5, and day 7 showed no growth with all the concentrations, except for 0.3% MgOZ that still had candida growth ( $10^3$  cfu) (fig. 7).

## DISCUSSION

Previous studies of metal nanowires have shown great antimicrobial activity that made them useful in many industries. Low concentrations of nano-metal oxides killed bacteria, and had no harmful effect on mammalian cells. This was attributed to their large area of contact with bacteria that allows them to react differently with the bacterial surfaces. (Dong *et al.*, 2010) One of the advantages of nano-metal oxides is their high melting point and their ability to raise intracellular oxidative stress after penetrating the bacterial cell and binding to a specific target by generating reactive oxygen species (ROS). (Gordon *et al.*, 2011) This allows them to attack bacteria through different mechanisms. Hence, bacteria fail to acquire resistance against these nanowires as resistance would need a series of mutations.

Human mouth bacteria like *S.mutans*, *S.aureus* and *E.faecalis*, as well as *C.albicans* are responsible for many dental diseases. The use of antimicrobial metal oxides nanowires that can eliminate oral microorganisms without generating resistance is of great promise. In the current study, MgO-zein nanowires showed excellent inhibition of *S.mutans*, *S.aureus* and *C.albicans*, and also against *E.faecalis*, which is resistant to many current antimicrobial agents, which has made it capable of surviving conventional root canal therapy (Maizura *et al.*, 2007).

The use of the Kirby Bauer test provided some hints on the antimicrobial behavior of the MgO nanowires. However, it has some limitations as it does not only depend on the materials toxicity to a given microorganism, but also depends on the diffusion and affinity of the material in bacteria suspension. The direct kill test confirmed the results and provided an opportunity to plate the bacteria suspension from wells, and also the inoculum from controls, directly on agar plates after 2 hours of contact time as well as after 1-7 days contact time. This method provides a direct evaluation of the bactericidal effect of the different concentrations. The results of this study showed that coated and uncoated

MgO nanowires have an antimicrobial effect against *S. mutans*, *S.aureus*, *E. faecalis* and *C. albicans*.

The zein polymer has been used as a surfactant to decrease MgO nanowires agglomeration and this may enhance their antimicrobial activity. The results showed that coating MgO with zein was effective. Zein-coated MgO nanowires in different concentrations showed enhanced and more rapid antimicrobial activity. They showed antimicrobial activity on one day in the diffusion disc method with all microorganisms tested, in the direct kill test it took one day with *S.mutans*, two days with *S. aureus* and *C. albicans*, and four days with the resistant bacteria of *E. faecalis*. This could be attributed to the coating of zein that decreases nanowire agglomeration and helps in their dispersion, which in turn affected their antimicrobial activity. As zein-coated MgO nanowires were faster in producing their action, the use of zein as a coating to nanowires reduced agglomeration and enhanced antimicrobial activity. Hence, the use of zein-coated MgO nanowires can be applied as antimicrobials and can be added to medical and dental products. This is especially useful in dental composites, dental bonding agents and dentures where *C. albicans* rapidly contaminates denture bases and colonizes the subsurface porosities of polymerized polymethyl methacrylates (Kakehashi *et al.*, 1965, Zhang *et al.*, 2009 and Odonkor and Addo, 2011). The optimal use of metallic oxide nanowires as antimicrobial agents in dentistry requires more research, but they hold great promise.

## CONCLUSION

The results indicated that the antibacterial effect of 1% or 2% zein-coated MgO nanowires were statistically significant ( $p < 0.05$ ) against the four organisms *S.mutans*, *S.aureus*, *E.faecalis* and *C.albicans*. Zein-coated MgO nanowires are a new human friendly and potent antimicrobial agent that can be incorporated in the formulation of a variety of new dental materials and products to improve dental care and oral health. Of course, it will not obviate the need for further clinical evaluation for this novel formula, which may inform clinicians of other important data.

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