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MgO NANOPARTICLES AS ANTIBACTERIAL AGENT: PREPARATION AND ACTIVITY

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Abstract - Bacterial pollution is a great risk for human health. Nanotechnology offers a way to develop new inorganic antibacterial agents. Nano-inorganic metal oxide has a potential to reduce bacterial contamination. MgO is an important inorganic oxide and has been widely used in many fields. Many studies have shown that MgO nanoparticles have good antibacterial activity. Therefore, in this paper, the main synthesis methods, antibacterial activity and antibacterial mechanisms of MgO nanoparticles are reviewed.
Keywords: MgO nanoparticles; Synthesis; Antibacterial activity; Antibacterial Mechanism.

INTRODUCTION

Bacterial contamination continues to draw public attention. It is estimated that approximately 48 million cases of pathogenic diseases occur in the United States (Morris 2011; Jin and He, 2011). Therefore, in order to solve this problem, it is highly necessary to develop effective antimicrobial agents to control the bacterial population (Kumar *et al*., 2008; Li *et al*., 2006). Generally, antibacterial agents can be categorized as organic or inorganic antibacterial agents. Organic antibacterial agents such as organic acids, essential oils, bacteriocins and enzymes have been widely studied. However, they have some shortcomings, such as low resistance to processing conditions, which limit their applications. As a result, inorganic antibacterial agents have attracted much interest for bacterial control (Fang *et al*., 2006; Jung *et al*., 2008). The main advantages of inorganic antibacterial agents, compared to organic antibacterial agents, are the improved stability under harsh processing conditions (Hewitt *et al*., 2001; Makhluf *et al*., 2005). Presently, some of the inorganic antibacterial materials, in particular inorganic metal oxides such as TiO_2, ZnO, MgO and CaO, have been studied (Huang *et al*., 2000; Sawai *et al*., 1995, 1998, 1999, 2000; Sawai, 2003). Among the studied inorganic metal oxides, ZnO, MgO and CaO are of particular interest because they are not only stable under harsh process conditions, but also generally regarded as safe materials to human beings (Stiomenov *et al*., 2002; Sundrarajan *et al*., 2012). Additionally, they have antimicrobial activity without photo-activation, compared to TiO_2 that requires photo-activation (Stiomenov *et al*., 2002; Fang *et al*., 2006; Jones *et al*., 2008; Roselli *et al*., 2003; Manna, 2012).

Recently, nanosciences and nanotechnology has been leading to a technological revolution in the world, which is concerned with materials with significantly novel and improved physical, chemical and biological properties (Wani and Shah, 2012; Sundrarajan *et al*., 2012). In this regard, nanoparticles are recognized as antibacterial agents due to their size, structure, and surface properties (Raghupathi *et al*., 2011). Thus, nanotechnology offers a way to improve the activity of inorganic antibacterial agents. Metal oxide nano-

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particles such as ZnO, MgO and CaO have been investigated as inorganic antibacterial agents (Roselli *et al*., 2003; Stoimenov *et al*., 2002; Shi *et al*., 2012; Tang *et al*., 2012).

MgO is an important inorganic material with a wide band-gap (Al-Gaashani *et al*., 2012). It has been used in many applications such as catalysis, catalyst supports, toxic waste remediation, refractory materials and adsorbents, additive in heavy fuel oils, reflecting and anti-reflecting coatings, superconducting and ferroelectric thin films as the substrate, superconductors and lithium ion batteries, etc (Ouraipryvan *et al*., 2009; Mirzaei and Davoodnia, 2012). In medicine, MgO is used for the relief of heartburn, sore stomach, and for bone regeneration (Bertinetti *et al*., 2009; Boubeta *et al*., 2010). Recently, MgO nanoparticles have shown promise for application in tumor treatment (Di *et al*., 2012). MgO nanoparticles also have considerable potential as an antibacterial agent. Therefore, in this review, the main synthesis methods, antibacterial activity and antibacterial mechanisms of MgO nanoparticles are discussed.

PREPARATION OF MgO NANOPARTICLES

Many methods, including sol-gel method, hydrothermal method, mechanochemical method, vapor phase method, microemulsion method etc., have been used for the preparation of MgO nanoparticles. The morphology and sizes of MgO nanoparticles can be controlled by adjusting the processing conditions (Kumar and Kumar, 2008; Selvam *et al*., 2011). In this section, three methods, including the sol-gel method, hydrothermal method and microemulsion method, are mainly discussed. Some examples of the preparation of MgO nanoparticles are shown in Table 1.

Sol-Gel Method

For the sol-gel process method, a magnesium alkoxide $Mg(OR)_2$ is hydrolyzed in an alcohol solvent to yield the hydroxide, which is followed by hydrolysis, condensation, polymerization reactions and thermal dehydration (Lopez *et al*., 1998; Stark *et al*., 1996; Znaidi *et al*., 1996; Koper *et al*., 1997). The use of magnesium alkoxides such as magnesium methoxide and magnesium ethoxide has been discussed in a few reports (Bokhimi, 1995; Portillo *et al*., 1996; Jung *et al*., 2003 a, b; Stengl *et al*., 2003). Many factors such as temperature, time, pH, catalytic agent for gel formation, and the environmental conditions can significantly affect the characteristics of the nanoparticles (Klabunde *et al*., 1996; Bokhimi *et al*., 1995). The advantages of the sol–gel method are simplicity, cost effectiveness, high yield of nanoparticles, and low reaction temperature (Jiu *et al*., 2003; Bokhimi *et al*., 1999; Subramania *et al*., 2007).

Stengl *et al*. (2004) described the preparation of magnesium hydroxide aerogels on the basis of the hydrolysis and condensation reactions of the alkoxide. Magnesium oxide aerogels with surface areas of ~ 537 m^2/g were obtained. Kim *et al*. (2005) studied the effect of acetic acid on the stability of the precursor magnesium methoxide and crystallization behavior of sol-gel-derived MgO nanoparticles. Kumar and Kumar (2008) synthesized MgO nanoparticles using magnesium nitrate and oxalic acid as precursors. This process involved gel formation, dehydration of magnesium oxalate, and decomposition of magnesium oxalate at different temperatures (500-1000 °C). MgO nanoparticles with average size 6.5-73.5 nm were obtained.

However, the sol-gel method can usually cause the agglomeration of MgO nanoparticles, which hinders its wide application (Zhou *et al*., 2011; Ouraipryvan *et al*., 2009). Therefore, it is required to develop a surfactant-mediated synthesis method to overcome this limitation. Many polymeric surfactants have been used in the sol-gel method (Esmaeili *et al*., 2009; Zhou *et al*., 2011; Meshkani and Rezaei, 2009, 2010; Jin *et al*., 2009). Mastuli *et al*. (2012) prepared MgO nanoparticles using the sol-gel route assisted with cetyltrimethylammonium bromide (CTAB) as a surfactant to reduce the agglomeration of the particles. The results showed that the use of CTAB in the sol-gel method gave MgO nanoparticles with less agglomeration. CTAB could provide a good control of the morphology and size of MgO nanoparticles.

Table 1: Examples of the preparation of MgO nanoparticles.

Preparation method	Precursors	Particle Size/nm	References
Hydrothermal	$NH_3.H_2O$, $Mg(NO_3)_2$	50-100	Jiu *et al*. (2001)
Hydrothermal	Na_2CO_3, $Mg(NO_3)_2$	30	Zhang (1999)
Hydrothermal	$NH_3.H_2O$, $MgCl_2$	62	Zhu *et al*. (2001)
Hydrothermal	$MgCl_2$, NaOH	15	Suzuki *et al*. (1992)
Hydrothermal	Urea, $MgCl_2$	15-20	Chen *et al*. (2002)
Sol-gel	$Mg(OC_2H_6)_2$, H_2O	30	Alvarado *et al*. (2000)
Sol-gel	$Mg(NO_3)_2$, stearic acid	20-50	Xu (2006)

Hydrothermal Method

Generally, the hydrothermal method may be one of the simplest methods to prepare MgO nanoparticles. Typically, the magnesium salts and base solution are prepared in water. Afterwards, the magnesium salt solution is mixed with base solution by varying the molecular ratio of Mg^{2+}/OH^-. Finally, the precipitate is washed and calcined in an oven. Factors such as magnesium precursors, reactant solvents and reaction conditions play an important role in controlling the morphology and size of MgO nanoparticles (Sutradhar *et al*., 2011; Mel'gunov *et al*., 2003; Reddy *et al*., 2010; Fedorov *et al*., 2007).

Huang *et al*. (2005) studied the controllable preparation of MgO nanoparticles by the hydrothermal method. The results showed that the particle size increased with the increase of calcination temperature. Through adjusting the concentration of $Mg(NO_3)_2$, reaction temperature and calcination conditions, MgO nanoparticles with different particle sizes were obtained. Camtakan *et al*. (2012) synthesized MgO nanoparticles by the hydrothermal method using $MgCl_2$ and NaOH as precursors. The results revealed that the as-prepared MgO nanoparticles had an average diameter of about 24 nm. Recently, Sundrarajan *et al*. (2012) prepared MgO nanoparticles by the hydrothermal method using magnesium nitrate and sodium hydroxide as precursors and soluble starch as a stabilizing agent. MgO nanoparticles with different sizes could be obtained by controlling different calcination temperature. Calcination temperature could significantly affect the morphology and size of MgO nanoparticles. Similarly, Krishnamoorthy *et al*. (2012) prepared MgO nanoparticles using magnesium nitrate and sodium hydroxide as precursors and cellulose as a stabilizing agent. The size of the as-prepared MgO nanoparticles was in the range from 10 to 30 nm.

At present, in order to reduce reaction time and cost, many new processes have been developed for preparing MgO nanoparticles. The microwave-assisted hydrothermal method has been attracting significant attention because it has advantages such as the short reaction time, narrow size distribution, high purity of the prepared particles, and high yield rate of nanoparticles (Aslan and Geddes, 2009). Moreover, it is potentially more cost effective compared to conventional synthesis methods (Nishioka *et al*., 2011a,b; Bhatte *et al*., 2012). In the microwave-assisted hydrothermal method, the precursor solution is irradiated by a microwave source. The efficient energy transfer results in a rapid heating process (Parida and Parija, 2006; Moghaddam and Saeisian, 2007). Polyol solvents such as ethylene glycol are extremely suitable for this method because of their relatively high dipole moment and loss factor. Takahashi (2007) reported the preparation of cubeshaped MgO nanoparticles by the microwave-assisted hydrothermal method. Recently, Selvam *et al*. (2011) synthesized MgO nanoparticles from magnesium and urea as precursors by the microwave-assisted route. Compared to nanoparticles prepared by the conventional method, MgO nanoparticles obtained by the microwave-assisted method had higher surface area (63.56 m^2/g).

Currently, the sonochemical method has been extensively used to generate nanoparticles (Gandhi *et al*., 2011). Ultrasonic waves can stimulate certain novel chemical processes such as nucleation, growth, and collapse of cavitation bubbles formed in the liquid through localized hot spots in the liquid of extremely high temperature (~ 2,700 °C) and pressure (~1,000 atm). Gandhi *et al*. (2011) obtained MgO nanoparticles with sizes from 5 to 10 nm through this ultrasound-assisted hydrothermal method. In our group, MgO nanoparticles were obtained by a sonication-assisted hydrothermal method (Tang *et al*., 2012; Tang and Shi, 2008). By controlling calcination conditions and reaction parameters, MgO nanoparticles with different sizes were obtained (Figs. 1 and 2).

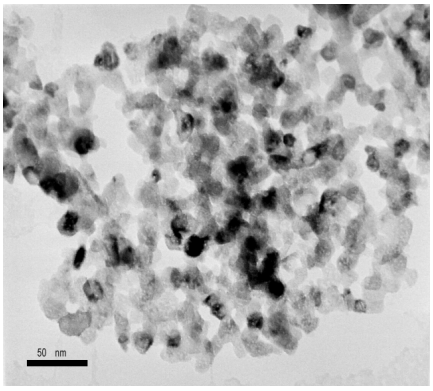

Figure 1: Transmission electron microscopy (TEM) image of MgO nanoparticles (Tang *et al*., 2012)

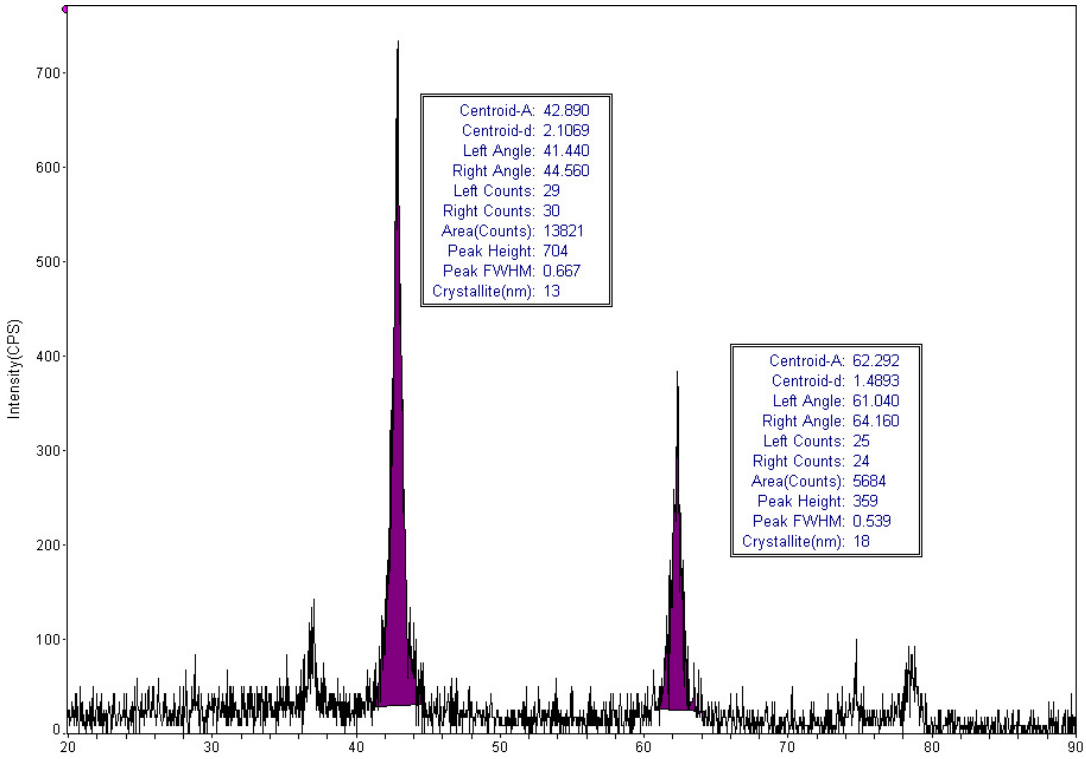

Figure 2: X-Ray diffraction (XRD) of MgO nanoparticles (Tang *et al*., 2012)

Micro-Emulsion Method

There are some reports on the synthesis of MgO nanoparticles using surfactants. Khairallah and Glisenti (2007) reported that non-ionic surfactants like Brij-56 and TX-100 could produce particles with surface areas of 22 m^2/g and 65 m^2/g with crystallite sizes 16 and 18 nm, respectively. However, in these syntheses the precursor is usually heated along with the surfactant to get the oxide, which probably leads to the decrease in surface area. In order to solve this problem, the micro-emulsion method has been used (Ganguli *et al*., 2010; Eastoe *et al*., 2006; Ranjan *et al*., 2009; Vaidya *et al*., 2008). The size of the reverse micelles dispersed homogeneously in the micro-emulsion is in the nano-regime and thus they can be used as nano-reactors to synthesize nanomaterials. The morphology and size of the particles can be adjusted by the proper choice of the surfactant and a number of parameters, which include the concentration of water, surfactant, and nature of non-polar phase. Ganguly *et al*. (2011) synthesized MgO nanoparticles by the micro-emulsion method. The results showed that monodisperse and uniform MgO nanoparticles 8-10 nm in size with a surface area ~ 108 m^2/g were obtained.

ANTIBACTERIAL ACTIVITY OF MgO NANOPARTICLES

Many analytical methods have been used to evaluate the antibacterial activity of MgO nanoparticles. One of the most used methods is the broth dilution method, followed by colony count, which plates serial culture broth dilutions containing bacteria and MgO nanoparticles incubated at proper conditions in suitable agar medium. Many reports have shown that the antibacterial activity of MgO nanoparticles is size-dependent. Huang *et al*. (2005) reported that antibacterial activity was increased with the decrease of the particle size of MgO. A relationship between the bactericidal efficacy against B. *subtilis* ATCC 9372 and the particle size of nano-MgO was demonstrated. For particles in the size range ~ 45-70 nm, the bactericidal efficacy of nano-MgO increased slowly with decreasing particle size. Below ~ 45 nm however, the bactericidal efficacy showed a much stronger dependence on particle size. Makhluf *et al*. (2005) demonstrated that small MgO nanoparticles had an efficient antibacterial activity towards *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Small, electron-dense black dots could be observed in the cytoplasm of MgO-nanoparticle-treated bacteria (Fig. 3). In the *E. coli* case, a low-density area in the

middle of the cell was observed (Fig. 3d). These MgO particles within the cells were suggested to have been re-formed from individual MgO nanoparticles that penetrated the bacterial cell wall and cell membrane. The results revealed a clear size effect, where the amount of killed bacteria was strongly dependent on particle size. Sundrarajan *et al*. (2012) investigated the effect of MgO nanoparticles size on the antibacterial activity. The results indicated that small-sized MgO nanoparticles had better antibacterial activities towards both gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria. Furthermore, MgO nanoparticles had more activity towards gram positive bacteria compared to gram negative bacteria. Generally, the specific surface area of MgO nanoparticles increases as the size of the nanoparticles decreases. The increase in surface area determines the potential number of reactive groups on the particle surface, which are expected to show high antibacterial activity (Nel *et al*., 2006; Pal *et al*., 2007).

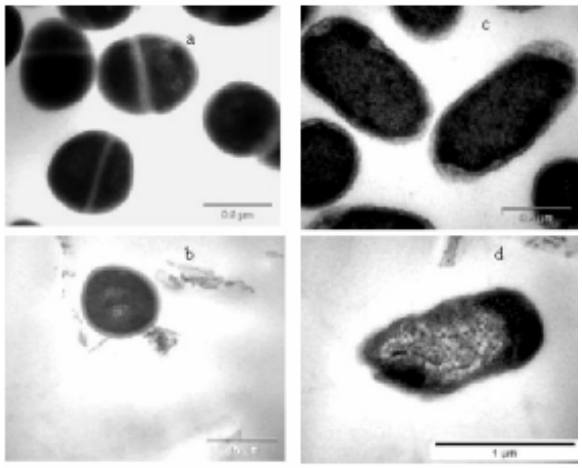

Figure 3: The effect of MgO nanoparticles on the ultra-structure of *S. aureus* and *E. coli* cells (reproduced with permission from Makhluf *et al*., 2005) TEM of *S. aureus* untreated cells (a), and treated cells (b). *E. coli* untreated cells (c) and treated cells (d). Magnification 60 K. Scale bar: 0.5 μm.

In addition to the particle size-dependent antibacterial effect of MgO nanoparticles, some studies have indicated that MgO nanoparticles have dosage-dependent antibacterial activity. Sawai (2003) reported that the activity of MgO nanoparticles against *E. coli* increased with the increase of MgO concentration. Jin and He (2011) found that higher MgO nanoparticle concentrations resulted in greater bacterial inactivation. An approximate seven log unit reduction in *E. coli* O 157: H7 was achieved by an 8 mg/mL MgO nanoparticle treatment at 24 h. At 7 h, the anti *E. coli* O157: H7 activity of MgO nanoparticles was dependent on its concentration, as in the case of low inoculum levels. The treatment with 3 mg/mL or higher MgO nanoparticles significantly reduced cell concentrations to undetectable levels after 24 h at room temperature, indicating 3 mg/mL MgO nanoparticles would be enough to kill all cells. Shrivastava *et al*. (2007) reported the preparation of silver nanoparticles in the range of 10-15 nm with increased stability and enhanced anti-bacterial potency. The antibacterial effect was dose dependent. An *et al*. (2011) and Zhang *et al*. (2011) also found that high MgO nanoparticle concentrations resulted in greater bacterial inactivation. Many reports have indicated that MgO nanoparticles have better activity towards gram-positive bacteria than towards gram-negative bacteria. The reason is probably due to the difference in cell membrane structure (Fig. 4). The cell wall of gram-positive bacteria (*E. coli*) consists primarily of thin layers of lipid A, lipopolysaccharide, and peptidoglycan, but that of gram-negative bacteria *S. aureus* consists of only a peptidoglycan layer. Membrane functions, activity of enzymes associated with the membrane, and maintenance of cell integrity depend on the structure of the cell surface (Espitia *et al*., 2012). Therefore, *E. coli* shows a stronger resistance to MgO nanoparticles, compared to *S. aureus* (Yim *et al*., 2006). The findings are similar to the antibacterial activity of Ag nanoparticles against gram-positive and gram-negative bacteria. Yoon *et al*. (2007) observed that *B. subtilis* was more sensitive than *E. coli* to Ag nanoparticles, meaning that *E. coli* was more resistant to nanoparticles than *B. subtilis* was. The lower sensitivities of *E. coli* as compared to *B. subtilis* was ascribed to the fact that the outer membrane of gram-negative bacteria such as *E. coli* is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provide an effective barrier against Ag nanoparticles.

Doping is a widely studied method for the modification of nanoparticles (Lin *et al*., 2009; Yamamoto *et al*., 2000; Manna, 2012). Yamamoto *et al*. (2000) studied the change of antibacterial characteristics with the doping amount of ZnO in MgO-ZnO solid solution. The results showed that, with increasing doping amount of ZnO in MgO-ZnO solid solution, a decrease in the antibacterial activity against *E. coli* and *S. aureus* was observed. The pH in physiological saline at the powder concentration of 2.5 mg/mL showed an alkali region above 10.0, and decreased with the increase of ZnO amount in MgO-ZnO solution. The reason for the decrease in antibacterial activity may be the decrease of the stability of O_2^- generated at the surface of the solid-solution and the decrease of pH value in the medium.

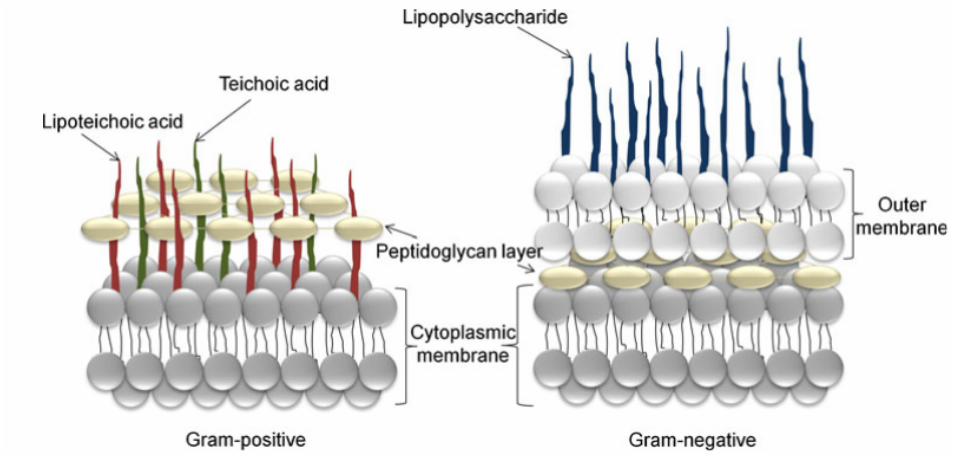

Figure 4: Membrane structure of gram-positive and gram-negative bacteria (reproduced with permission from Espitia *et al*., 2012).

Avanzato *et al*. (2009) investigated the antibacterial activity of magnesium oxide-germanium oxide composite powder. The prepared nano-composite powder showed good bactericidal activity toward both gram-negative (*E. coli*) as well as gram-positive bacteria (*S. aureus*), though they were more efficient against gram-positive bacteria. The results showed that nanocomposite powders were more effective against *S. aureus* than *E. coli* at lower concentrations. At higher concentrations (>5 mg/mL), the growth of bacteria was almost completely inhibited (>95 %) in both cases. The minimal inhibitory concentration (MIC) for *S. aureus* was found to be 0.05 mg/mL whereas that of *E. coli* was found to be 0.25 mg/mL.

ANTIBACTERIAL MECHANISM OF MgO NANOPARTICLES

The exact antibacterial mechanism of MgO nanoparticles is still unknown. A number of mechanisms, such as the formation of reactive oxygen species (ROS), the interaction of nanoparticles with bacteria, subsequently damaging the bacterial cell, and an alkaline effect have been proposed to explain the antibacterial mechanism of MgO nanoparticles. Several similar mechanisms have been proposed to explain the inhibitory effect of silver nanoparticles on bacteria. Silver has long been known to cause microbial inhibition. The antibacterial mechanisms of silver include induction of oxidative stress due to generation of ROS, which may cause the degradation of the membrane structure of the cell. Release of ions from the surface of nanoparticles has been reported to cause bacterial death due to binding to cell membrane (Emamifar *et al*., 2011; Kim *et al*., 2007).

Many studies have indicated that the antibacterial mechanism of MgO nanoparticles is due to the formation of ROS such as superoxide anion (O_2^-) (Huang *et al*., 2005 a, b; Lin *et al*., 2005; Yamamoto *et al*., 2010; Yamamoto *et al*., 2001). It has been reported that the increase of the surface area of MgO particles leads to an increase of the O_2^- concentration in solution and thus results in a more effective destruction of the cell wall of the bacteria. However, when the particle size of MgO is below 15 nm, the aggregation effect becomes very significant due to the very high surface energy of the particles. The large size of aggregated MgO inhibits the interaction with bacteria and particles so that the bactericidal efficiency becomes lower (Sawai *et al*., 2000; Yamamoto *et al*., 2000). Hewitt *et al*. (2001) reported that the enhancement of the antibacterial activity of MgO nanopowder against *E. coli* was due to the generation of a large amount of O_2^- by the surface of MgO powder. Recently, Krishnamoorthy *et al*. (2012) evaluated the antibacterial activity of MgO nanoparticles against the gram-negative bacteria *E. coli* and *Pseudomonas aeruginosa* (*P. aeruginosa*), as well as the gram-positive bacterium *S. aureus*. MgO nanoparticles exhibited antibacterial activity with MIC of 500 μg/mL against *E. coli* and 1000 μg/mL for *P. aeruginosa* and *S. aureus*. It was suggested that the mechanism of the antibacterial activity of MgO nanoparticles may be lipid peroxidation and ROS due to the presence of defects of oxygen vacancy at the surface of the nanoparticles.

The interaction of MgO nanoparticles with bacteria, subsequently damaging the bacterial surface, has been proposed to explain the antibacterial activity of MgO nanoparticles. Stoimenov *et al*. (2002) suggested that the cell death was caused by the electro-

static interaction between the bacteria surface and MgO nanoparticles. Peter *et al*. (2002) and Makhluf *et al*. (2005) demonstrated that nano-MgO exhibited high activity against bacteria due to the interaction of particles and bacteria. It was found that nano-MgO particles could take up halogen gases due to the defect nature of their surface and its positive charge, which resulted in a strong interaction with bacteria, which are negatively charged (Stoimenov *et al*., 2002).

The alkaline effect has been considered as another primary factor in the antibacterial action of MgO nanoparticles (Sawai *et al*., 2001; Yamamoto *et al*., 2000). Sawai *et al*. (1997) proposed that the possible antibacterial mechanism was the adsorption of water moisture on the MgO nanoparticle surfaces, which could form a thin water layer around the particles. The local pH of this thin water layer formed around the nanoparticles might be much higher than its equilibrium value in solution. When the nanoparticles are in contact with the bacteria, the high pH in this thin surface water layer could damage the membrane, resulting in cells death.

CONCLUSIONS

MgO nanoparticles are a promising antibacterial agent due to their high resistance to harsh processing conditions. Many synthetic methods, such as the sol–gel method, hydrothermal method and micro-emulsion method, have been used to prepare MgO nanoparticles. The hydrothermal method has been given more attention due to the simplicity. The antibacterial activity of MgO nanoparticles is size and concentration dependent. Although the exact antibacterial mechanism of MgO nanoparticles is not clear, three main antibacterial mechanisms have been proposed, such as the formation of ROS, the interaction of nanoparticles with bacteria, subsequently damaging the bacterial cell, and an alkaline effect. In the future, more research should be focused on the preparation of MgO nanoparticles with low cost and studies of the antibacterial mechanism of MgO nanoparticles. Also, more studies should be carried out on the activity of MgO nanoparticles towards other microorganism species.

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