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Synthesis, antibacterial activity, antibacterial mechanism and food applications of ZnO nanoparticles: a review

Lu-E Shi^a, Zhen-Hua Li^a, Wei Zheng^a, Yi-Fan Zhao^a, Yong-Fang Jin^a and Zhen-Xing Tang^{a,b,c*}

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Bacterial contamination reduces the shelf-life of foods and presents serious risks to human health. Nanotechnology provides the opportunity for the development of new antibacterial agents. Nano-inorganic metal oxides have shown the potential to reduce bacterial contamination of foods. When the particle size of materials decreases from the micrometre to the nanometre range, nano-functional properties such as diffusivity, mechanical strength, chemical reactivity and biological properties are improved. Significantly, ZnO has been used in many applications with particular success. Many studies have shown that ZnO nanoparticles have enhanced antibacterial activity. This review discusses the main synthetic methods, antibacterial activity, antibacterial mechanisms and food applications of ZnO nanoparticles.

Keywords: ZnO nanoparticles; synthesis; antibacterial activity; antibacterial mechanism; food applications

Introduction

Pathogens present a global public health issue, which leads to increased medical expenses, and affects human health and the economy. Some pathogens are difficult to treat due to the formation of biofilms (Gilbert et al. 1990; Donlan & Costerton 2002). Various pathogens also can cause animal diseases (Desselberger 2000; Donlan & Costerton 2002; Costello et al. 2009; Manna 2012). Therefore, it is necessary to find a new technology that can control and reduce the contamination with pathogens.

Some organic antibacterial agents, such as organic acids, essential oils, bacteriocins and lysozyme, have been widely investigated (Appendini & Hotchkiss 1997; Tripathi & Dubey 2004; Han 2005; Gálvez et al. 2007; Schirmer et al. 2009). However, organic antibacterial agents are sensitive to processing conditions such as high temperature and pressure. Inorganic materials, in particular metal oxides, have strong antibacterial activity at low concentrations (Rai et al. 2009). Compared with organic antibacterial agents, the main advantages of inorganic antibacterial agents are their good stability at high temperatures and pressures, and long shelf-life (Stoimenov et al. 2002; Sawai 2003; Wang 2004). The antibacterial activity of many inorganic or bulk oxide powders such as TiO₂, ZnO, MgO, CaO, CuO, Al₂O₃ and Ag₂O have been studied (Wei et al. 1994; Bellantone et al. 2002; Stoimenov et al. 2002; Liu & Yang 2003; Sawai 2003; Sawai & Yoshikawa 2004; Cioffi et al. 2005; Fu et al. 2005; Brayner et al. 2006; Fang et al. 2006; Shi et al.

2012; Tang, Fang, Zhang, Pan, et al. 2012). Among the studied metal oxides, ZnO, MgO and CaO are regarded as safe materials for human beings, and are part of minerals essential for human health (Stoimenov et al. 2002; Roselli et al. 2003; Cioffi et al. 2005; Chaudhry et al. 2008; Bradley et al. 2011). Furthermore, they have antibacterial activity without photo-activation compared with TiO₂ that requires photo-activation (Yamamoto 2001; Stoimenov et al. 2002; Roselli et al. 2003; Fang et al. 2006; Jones et al. 2008; Manna 2012).

ZnO is a semiconductor inorganic material with three different crystal structures: wurtzite, zinc blende and rock-salt. The structure of wurtzite is thermodynamically stable at ambient conditions, in which every zinc atom is tetrahedrally coordinated with four oxygen atoms (Kulkarni et al. 2011). ZnO has direct wide band gap of about 3.3 eV (Schmidt-Mende & MacManus-Driscoll 2007; Espitia et al. 2012). It has potential applications in many fields, such as ultraviolet light (UV)-shielding materials, gas sensors, biosensors, fillings in medical materials, semiconductors, piezoelectric devices, cosmetics, photocatalytic degradation of pollutants, drug carriers and antibacterial agents (Stoimenov et al. 2002; Roselli et al. 2003; Wang 2004; Kulkarni et al. 2011). The study of ZnO as an antibacterial agent started in the early 1950s. However, the real progressive investigation of ZnO as an antibacterial agent began in 1995. Sawai and colleagues reported that MgO, CaO and ZnO powders had antibacterial activities against some bacterial strains (Sawai et al. 1997, 1998; Sawai 2003).

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In recent years, the development of nanotechnology has promoted the development of new antibacterial agents. When the size of materials decreases from the micrometre to the nanometre range, compared with conventional materials, nanomaterials show better performance such as enhanced diffusivity, increased mechanical strength and chemical reactivity, and enhanced biological properties (Espitia et al. 2012). Research on nano-inorganic materials has been a hot topic. Many studies have indicated that nano-inorganic materials show enhanced antibacterial activity compared with conventional inorganic materials (Yamamoto 2001; Padmavathy & Vijayaraghavan 2008; Raghupati et al. 2011). Presently, the main food applications of ZnO nanoparticles are as an antibacterial agent in packaging materials. In this way, the incorporation of ZnO nanoparticles into packaging materials can decrease the amount of antimicrobials directly in the food products. Furthermore, ZnO nanoparticles also can play an important role in reducing pathogen contamination and extending the shelf-life of food products (Espitia et al. 2012). Thus, in this review the main synthetic methods, antibacterial activity and antibacterial mechanism of ZnO nanoparticles are discussed. Food applications of ZnO nanoparticles, particularly in food packaging, are also mentioned.

Preparation of ZnO nanoparticles

Several methods including the sol-gel method, hydrothermal synthesis, mechano-chemical method and vapour phase method etc., have been adopted for the preparation of ZnO nanoparticles (Liu & Zeng 2004; Xu et al. 2007; Manna 2012). ZnO nanoparticles can be obtained by these methods through adjusting parameters such as temperature, pressure, the hydrolysis ratio and precursors (Meulenkamp 1998; Kim & Sigmund 2004; Liu & Zeng 2004; Chen et al. 2006; Xu et al. 2007; Manna 2012). In this section, the main methods such as the sol-gel method, hydrothermal synthesis, mechano-chemical method and vapour phase method were chosen to discuss the preparation of ZnO nanoparticles. All synthesis methods of ZnO nanoparticles mentioned in this paper can also be conducted on a laboratory scale.

Sol-gel method

Preparation of metal oxide nanoparticles by the sol-gel method has been widely studied (Meulenkamp 1998). For the process of the sol-gel method, a “sol” suspension is prepared and then subsequently converted to viscous gels and solid materials through hydrolysis, condensation and polymerisation reactions. Several factors, such as the nature of the alkyl group, the concentration of each precursor, temperature, water-to-alkoxide molar ratio and the presence of acid or base catalysts, can affect the size of

ZnO nanoparticles (Manna 2012). The main advantages of the sol-gel method are faster nucleation and growth, and high purity of the final nanoparticles. The disadvantage of the method is the high cost for the precursors. Some examples of the sol-gel method are shown in Table 1.

Tokumoto and Briois (2003) studied the structure of zinc acetate-derived precursor during the preparation process of ZnO nanoparticles in the sol-gel method. The results showed that $Zn_4O(Ac)_6$ was already formed after the dissolution of $Zn(Ac)_2$ in ethanol. The reflux time was one key factor for the preparation of monodispersed ZnO nanoparticles. Sun et al. (2007) studied the purification and stabilisation of ZnO nanoparticles prepared by the sol-gel method using zinc acetate and potassium hydroxide as precursors. The results showed that the purified ZnO nanoparticles could be fully redispersed in methanol and stabilised by adding hexane. Recently, Dejene et al. (2011) synthesised ZnO nanoparticles using the sol-gel method by varying the sodium hydroxide-to-zinc acetate molar ratio. It was observed that the structural properties and particle sizes of ZnO nanoparticles were influenced by the molar ratio of the precursors. The choice of an appropriate molar ratio of the precursors is important in controlling the characteristics of ZnO nanoparticles.

Hydrothermal method

The hydrothermal method may be the simplest route to synthesise ZnO nanoparticles (Meulenkamp 1998; Liu & Zeng 2004; Li et al. 2006; Raghupati et al. 2011; Manna 2012). In a typical hydrothermal method, the precursor zinc salt solution, such as zinc nitrate, zinc acetate or zinc sulfate etc., and an aqueous base solution such as NaOH, KOH, trimethyl (or ethyl) ammonium hydroxide or NH_4OH etc., are prepared in water. The zinc salt solution is then mixed with the base solution by varying the molar ratio of Zn^{2+}/OH^- . Finally, the precipitate is washed and dried in an oven (Manna 2012; Shi et al. 2012). The size and morphologies of ZnO nanoparticles can be controlled by changing the precursors, solvents, molar ratio, temperature and reaction time. The hydrothermal method is attractive due to the simplicity and availability of low-cost precursors. Examples of the hydrothermal method are shown in Table 2.

Table 1. Examples of the sol-gel method.

Precursor	Size (nm)	Reference
Zinc acetate, triethylamine	24	Khan et al. (2013)
Zinc acetate	58	Karunakaran et al. (2011a)
Zinc acetate	20	Armelaio et al. (2011)
Zinc acetate, aniline	20–30	Wahab et al. (2010)
Zinc acetate, NaOH	5–20	Dejene et al. (2011)

Table 2. Examples of the hydrothermal method.

Precursor	Size (nm)	Reference
ZnCl ₂ , NaOH	9–20	Becheri et al. (2008)
Zn(NO ₃) ₂ , (NH ₄) ₂ CO ₃	20–40	Gondal et al. (2011)
ZnCl ₂ , NaOH	8.2	Negi et al. (2012)
ZnCl ₂ , NaOH	2.6–3.3	Ashtaputre et al. (2005)
ZnCl ₂ , NaOH	4	Dutta et al. (2012)
Zn(NO ₃) ₂ , NaOH	15–20	Wahab et al. (2012)

Jia et al. (2009) prepared ZnO nanoparticles with different morphology by controlling the reaction parameters. The results showed that factors such as the solvent, pH and reaction time could significantly affect the morphology of ZnO nanoparticles. Ohira et al. (2008) prepared ZnO nanoparticles by a hydrothermal method in supercritical water. In this work, zinc nitrate aqueous solution was pressurised to 30 MPa at RT and rapidly heated to 400°C by mixing with supercritical water, and then fed into a tubular reactor. ZnO nanoparticles with uniform particle size distribution were obtained.

At present, in order to reduce the reaction time and cost, many new processes have been developed for producing nanoparticles. Power ultrasonic waves can stimulate chemical processes such as nucleation, growth and collapse of cavitation bubbles formed in liquid at extremely high temperature and pressure (Tang & Shi 2008; Shi et al. 2012; Tang, Fang, Zhang, Zhou, et al. 2012). In our group, ZnO nanoparticles were obtained by a sonication-assisted hydrothermal method (Figures 1 and 2). By controlling the calcination conditions and reaction parameters, ZnO nanoparticles with different sizes could be obtained (Shi et al. 2012).

The synthesis of ZnO nanoparticles by the microwave-assistant method has been reported. Jalal et al. (2010) synthesised ZnO nanoparticles by the microwave-assisted method using zinc acetate as a precursor in an ionic liquid,

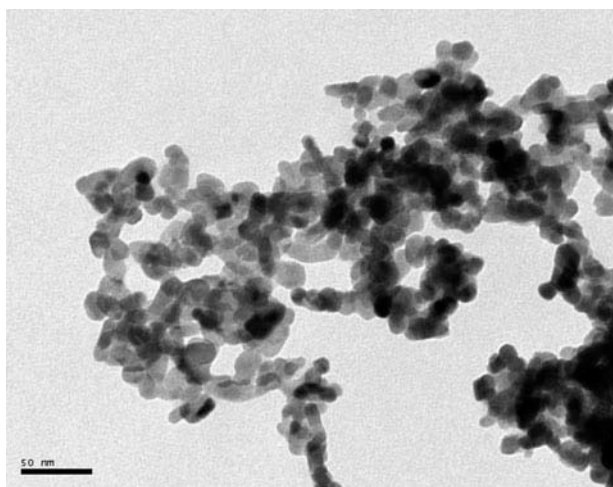


Figure 1. TEM of ZnO nanoparticles (sourced from Shi et al. 2012).

1-butyl-3-methylimidazolium *bis*(trifluoromethylsulfonyl) imide, [bmim][NTf₂]. Ma et al. (2012) also synthesised ZnO nanoparticles by the microwave-assisted hydrothermal method using zinc nitrate and triethanolamine as precursors. The results revealed that the as-prepared ZnO nanoparticles had an average diameter of about 150 nm. Mitra et al. (2012) synthesised ZnO nanoparticles by the microwave-assisted route in an aqueous buffer solution (*tris*, *tris*-(hydroxymethyl) aminomethane). Tris buffer solution allowed the reaction to work at mild experimental conditions (Polleux et al. 2005; Mitra et al. 2012). Synthesis of ZnO nanoparticles also can be carried out in an autoclave at different temperatures from 80 to 180°C. Sharma et al. (2010) synthesised ZnO nanoparticles under autoclave conditions (15 min, 5 atm). The size of the as-prepared ZnO nanoparticles was about 2.3 nm.

Mechano-chemical method

The mechano-chemical method has been widely applied to the synthesis of many varieties of nanoparticles including ZnS, CdS, ZnO, SiO₂ and CeO₂ (Aghababazadeh et al. 2006; Espitia et al. 2012). Generally in this process the precursors zinc salts such as ZnCl₂, Zn(NO₃)₂, ZnSO₄ and carbonate salts such as Na₂CO₃, (NH₄)₂CO₃, are simultaneously milled to produce zinc carbonate (ZnCO₃) through a chemical exchange reaction. The reaction results from local heat and pressure at contact surfaces (Zhang et al. 2000; Wang et al. 2002; Zhang et al. 2005; Casey 2006). Afterwards, ZnO nanoparticles can be obtained by calcinations. The mechano-chemical method is suitable for large-scale production of ZnO nanoparticles due to its simplicity and low cost. Moreover, this process is free of any organic solvents (Lu et al. 2008; Espitia et al. 2012). One disadvantage of the method is the agglomeration of particles during milling.

Aghababazadeh et al. (2006) prepared ZnO nanoparticles by this method with an average particle size ranging from 20 to 30 nm. The milling time is an important parameter for controlling the size of ZnO nanoparticles. Ao et al. (2006) showed that the increase of the milling time could effectively reduce the size of ZnO nanoparticles. According to Shen et al. (2006), the size of ZnO nanoparticles was reduced from about 40 to 24 nm when the milling time was increased from 5 to 40 min. However, the size of ZnO nanoparticles slowly increased to 27 nm when the milling time reached 70 min. The heating temperature is another important factor used to control the size of ZnO nanoparticles. Ao et al. (2006) reported the crystal size of ZnO nanoparticles was increased from about 18 nm at 400°C to 36 nm at 800°C.

Vapour phase method (VPM)

Generally, for the process of VPM, the metal bulk of zinc is placed in the vacuum chamber and then it melts and

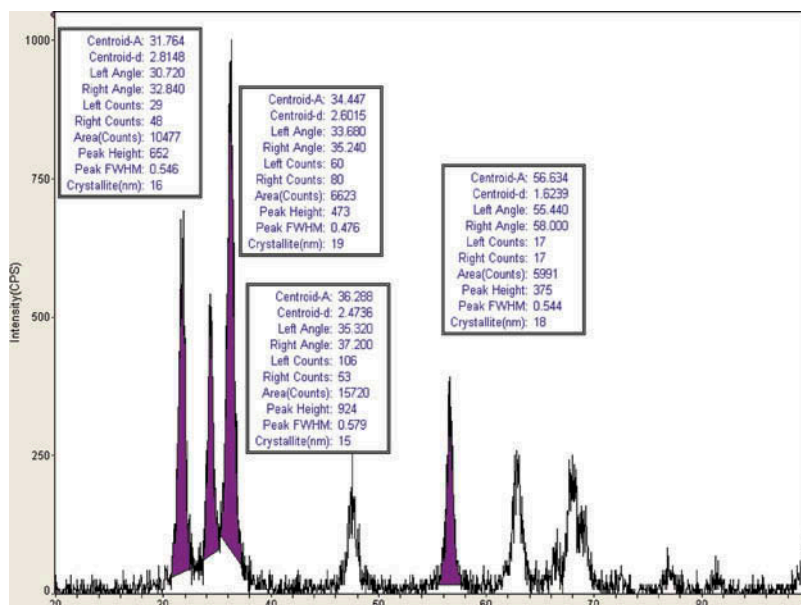


Figure 2. X-ray diffraction (XRD) of ZnO nanoparticles (sourced from Shi et al. 2012).

vaporises into gas under vacuum pressure and vaporised temperature. Afterwards, vaporised zinc forms particles when it is cooled by mixing with cool gas (Swihart 2003; Espitia et al. 2012). Normally, the synthesis of ZnO nanoparticles needs high temperatures from 500 to 1500°C. Different sources such as evaporation, sputtering and laser have been used to evaporate precursors (Espitia et al. 2012). Nanoparticles generated by this method typically have discrete, high crystallinity and average sizes from 8 to 75 nm (Casey 2006). Kim and Sigmund (2004) synthesised various types of ZnO nanoparticles by VPM at a temperature ranging from 800°C to 850°C. Ali et al. (2012) studied the continuous production process for functionalised ZnO nanoparticles by VPM. ZnO nanoparticles were synthesised at 800°C with a size of 13 nm.

Antibacterial activity of ZnO nanoparticles

Many analytical methods have been adopted to evaluate the antibacterial activity of ZnO nanoparticles. One of the most used methods is the broth dilution method, followed by colony count, which plates serial culture broths dilutions containing the bacteria and ZnO nanoparticles incubated at proper conditions, in suitable agar medium. Presently, Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* are mainly chosen as model bacteria to evaluate the antibacterial activity of ZnO nanoparticles. Studies using other bacteria species such as *Streptococcus*, *Enterococcus*, *Bacillus*, *Staphylococcus epidermidis*, *Lactobacillus*, *Salmonella typhimurium*, *Vibrio fischer*, *Pseudomonas*, *Shigella*, *Campylobacter jejuni* and *Proteus vulgaris* to evaluate the antibacterial

activity of ZnO nanoparticles are limited (Liu & Yang 2003; Sawai 2003; Adams et al. 2006; Brayner et al. 2006; Fang et al. 2006; Reddy et al. 2007; Heinlaan et al. 2008; Huang et al. 2008; Jones et al. 2008; Ohira et al. 2008; Liu et al. 2009; Rekha et al. 2010; Gordon et al. 2011; Premanathan et al. 2011; Raghupati et al. 2011; Xie et al. 2011). The antibacterial activity of ZnO nanoparticles depends on the characteristic species tested. Xie et al. (2011) found that the minimal inhibitory concentration of ZnO nanoparticles (about 30 nm) for *C. jejuni* (0.05–0.25 mg ml⁻¹) was eight- to 16-fold lower than *Staphylococcus enteric* and *E. coli* O157:H7 (0.4 mg ml⁻¹) strains. Jones et al. (2008) and Raghupati et al. (2011) also observed there was a difference in the antibacterial activity of ZnO nanoparticles to various clinical isolates of *S. aureus*. Many reports have showed that particle size can affect the antibacterial activity of ZnO nanoparticles (Yamamoto 2001; Jones et al. 2008; Padmavathy & Vijayaraghavan 2008; Raghupati et al. 2011). Yamamoto (2001) investigated the effect of particle size of ZnO nanoparticles over a range of 100–800 nm on antibacterial activity. By measuring the change in electrical conductivity with bacterial growth, it was found that the antibacterial activity of ZnO nanoparticles was increased with the decrease of particle size. Specially, the size-dependent activity was observed in the range 100–800 nm in *S. aureus* and *E. coli*. Generally, with the decrease of particle size, the antibacterial activity of ZnO nanoparticles on *E. coli* and *S. aureus* is increased (Zhang et al. 2007; Jones et al. 2008; Padmavathy & Vijayaraghavan 2008). Some examples of particle size-dependent antibacterial activity of ZnO nanoparticles are

shown in Table 3. Padmavathy and Vijayaraghavan (2008) studied the bactericidal ability against *E. coli* of ZnO suspensions with three different particle sizes (12 nm, 45 nm, 2 μm) in the lowest concentration range (0.01–1 mM) and the highest concentration range (5–100 mM), respectively. The results showed that ZnO suspension with 12 nm particles was more effective than the suspension with large particle sizes. Recently, Raghupati et al. (2011) also found that the antibacterial activity of ZnO nanoparticles was inversely proportional to size. Size-dependent bacterial growth inhibition of *S. aureus* existed in the presence of 6 mM of different sizes of ZnO nanoparticles from 12 to 307 nm. In addition to further verify, the viable *S. aureus* cells were determined by plating cultures from the growing cells in the presence of 6 mM ZnO nanoparticles with sizes from 12 to 307 nm. It was noted that viable cells recovered decreased significantly with decreasing particle size of ZnO nanoparticles. The reason may be ZnO nanoparticles with small size have increased reactivity since the amount of H_2O_2 generated strongly depends on ZnO surface area (Ohira et al. 2008; Padmavathy & Vijayaraghavan 2008).

In addition to particle size-dependent antibacterial activity of ZnO nanoparticles (Table 3), many studies strongly indicate that ZnO nanoparticles have concentration-dependent antibacterial activity. Jalal et al. (2010) indicated that the increase of nanoparticle concentration produced higher antibacterial activity towards *E. coli* due to the increase of amount of H_2O_2 generated from the surface of ZnO. Wahab et al. (2012) investigated the antibacterial activity of ZnO nanoparticles. The results clearly showed that the growth inhibition rate of the test strains increased with the increase of the concentration of ZnO nanoparticles from 5 to 45 $\mu\text{g ml}^{-1}$.

The dimensions and morphology of ZnO nanoparticles also play important roles in determining the antibacterial activity (Yamamoto et al. 2004; Wang et al. 2007; Ohira et al. 2008; Zhou & Keller 2010). However, the relationship between the different forms of ZnO nanoparticles and antibacterial activity is not clear. Many studies on the antibacterial activity of nanostructured ZnO have been reported (Table 4). Wang et al. (2007) studied the

relationship between antibacterial activity and various orientations of ZnO arrays. The results indicated that randomly oriented ZnO nano-arrays showed better antibacterial activity compared with less or well-defined oriented ZnO nano-arrays in *E. coli*. Stankovic et al. (2013) investigated the influence of size scale and morphology on antibacterial properties of ZnO nanoparticles hydrothermally synthesised using different surface stabilising agents such as polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA) and poly(α,γ -L-glutamic acid) (PGA). The morphology and dimensions of ZnO nanoparticles are shown in Figure 3. The differences among the prepared nanoparticles could be attributed to a different nature of the reaction stabilising agents in a synthesis procedure. Significant differences in the antibacterial activity depending on the morphology and size of ZnO nanoparticles were observed. ZnO powder composed of nanospherical particles with an average diameter around 30 nm showed the highest antibacterial activity. Yamamoto et al. (2004) revealed that antibacterial activity of ZnO powder was increased, with the increase of the lattice constant value in the hexagonal structure of ZnO powder. The value in hexagonal structure increased with the increase in the oxidisability of the atmosphere. H_2O_2 generating from all nano-ZnO samples contributed to the occurrence of antibacterial activity, and the generation amount increased with the increase of co value. Ohira and Yamamoto (2012) studied the correlation between antibacterial activity and crystallite size of ZnO. The results showed that the antibacterial activity of ZnO nanoparticles with small crystallite sizes was greater than that of those with large crystallite sizes. More eluted Zn^{2+} ions from ZnO nanoparticles having a small crystallite size contributed to the occurrence of higher antibacterial activity. Therefore, the enhancement of antibacterial activity of ZnO nanoparticles may be probably due to the crystallite size or the lattice strain.

Many reports have indicated that ZnO nanoparticles have better activity towards Gram-positive bacteria than towards Gram-negative bacteria (Jones et al. 2008; Xie et al. 2011). Tawale et al. (2010) justified the low inhibition rate of Gram-positive bacteria *S. aureus* compared with Gram-negative *E. coli*. Kim and An (2012) evaluated the effect of ZnO nanoparticles on the activity of *E. coli* and *Bacillus subtilis*. The image observation indicated that reflectors assumed to be due to cell necrosis were found in *E. coli* exposed to ZnO nanoparticles. However, *B. subtilis* did not show any reflectors. The reason may be probably due to the difference of cell membrane structure (Figure 4). The cell wall of Gram-positive bacteria is primarily made up of a peptidoglycan layer, teichoic and lipoteichoic acids. The cell wall of Gram-negative bacteria has an outer membrane, which mainly consists of lipopolysaccharide and a thin peptidoglycan layer (Jiang et al. 2004; Epand & Epand 2009; Le et al. 2010; Espitia et al.

Table 3. Examples of particle size-dependent antimicrobial activity of ZnO nanoparticles.

Particle size (nm)	Organism tested	Reference
100, 800	<i>Escherichia coli</i>	Yamamoto (2001)
12, 45, 2000	<i>E. coli</i>	Padmavathy and Vijayaraghavan (2008)
12–307	<i>Staphylococcus aureus</i>	Raghupati et al. (2011)
50–70	<i>S. aureus</i>	Jones et al. (2008)
230, 2417	<i>E. coli</i>	Zhang et al. (2007)

Table 4. Effect of dimension and morphologies of ZnO on antimicrobial activity.

Nanostructured ZnO	Particle size	Reduction rate of the organism tested	Reference
ZnO nanowire	Length > 1 μm , diameter 150 nm	<i>Escherichia coli</i> (83.3%)	Wang et al. (2007)
ZnO nanoparticles	<1 μm	<i>Staphylococcus aureus</i> (50%)	Jones et al. (2008)
Nanorods	Length 800 nm, diameter 55–70 nm	<i>E. coli</i> (100%)	Tam et al. (2008)
Nanospheres	25–40 nm	<i>E. coli</i> (93%), <i>S. aureus</i> (63%)	Banoee et al. (2005)

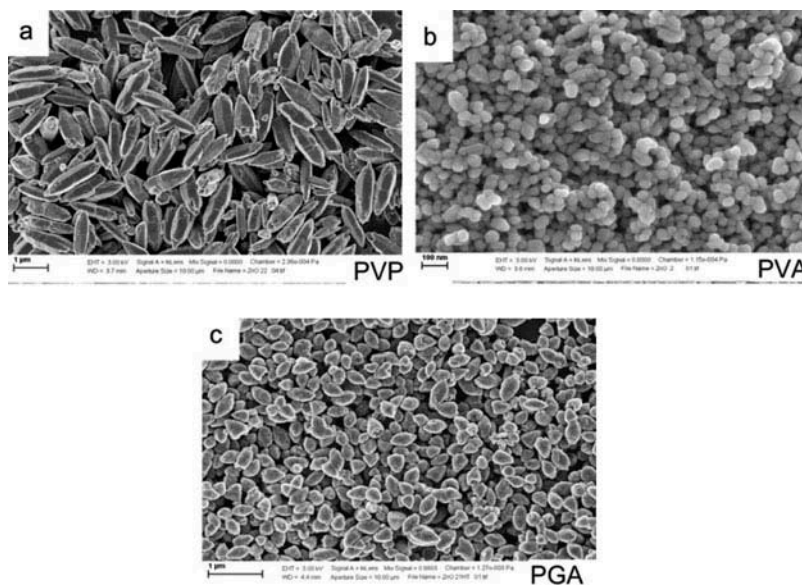


Figure 3. Scanning electron microscopy (SEM) images of ZnO powders (sourced from Stankovic et al. 2013): (a) ZnO/PVP; (b) ZnO/PVA; and (c) ZnO/PGA.

2012). Thus, the outer membrane of Gram-negative bacteria can reduce the damage from ZnO nanoparticles (Russell 2003). Applerot et al. (2009) also found that *E. coli* showed higher susceptibility to ZnO nanoparticles compared with *S. aureus*. However, according to the explanation of Applerot et al. (2009), the reason may be the differences in the intracellular antioxidant content such as carotenoid pigments, as well as the presence of potent detoxification agents such as antioxidant enzymes within the bacteria.

Studies have showed that the antibacterial activity of ZnO nanoparticles combined with other antibacterial agents has better activity compared with that of uncombined ZnO nanoparticles. Bhadra et al. (2011) found chitosan-capped ZnO nanoparticles showed higher antibacterial activity against *E. coli* compared with uncapped ZnO nanoparticles. The researchers also observed that the antibacterial activity of chitosan-capped ZnO nanoparticles was higher than that of the antibiotic amoxicillin. AbdElhady (2012) synthesised chitosan/ZnO

nanoparticles using different concentrations of ZnO at different temperatures. ZnO/chitosan nanoparticles with an average length of 60 nm and an average width of 5–15 nm showed good antibacterial activity. ZnO nanoparticles combined with antibiotics showed the enhanced antibacterial activity compared with uncombined ZnO nanoparticles. Banoee et al. (2005) found that ZnO nanoparticles ranging from 20 to 45 nm combined with ciprofloxacin had enhanced antibacterial activity against *E. coli* and *S. aureus*. The possible reason may be due to the action of nanoparticles on the surface of the bacteria complementing the action of the antibiotic.

Doping is a widely studied method for the modification of nanoparticles (Yamamoto et al. 2000; Lin et al. 2009; Manna 2012). Desselberger (2000) synthesised Mn-doped ZnO nanoparticles and found the doped ZnO nanoparticles had an increased antibacterial activity against both Gram-negative and Gram-positive bacteria than undoped ZnO nanoparticles. Dutta et al. (2010) also reported that Fe- or Co-doped ZnO nanoparticles had

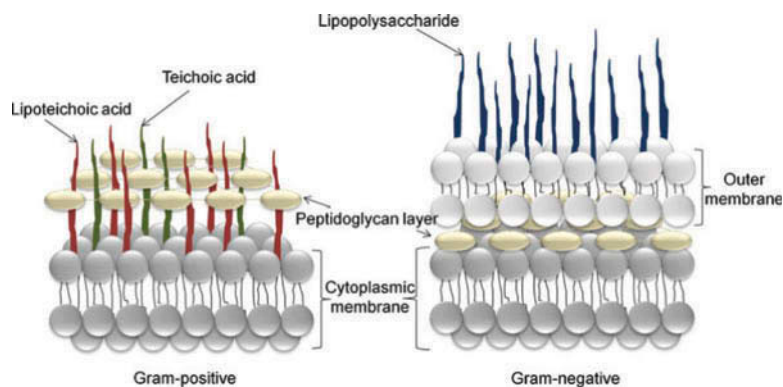


Figure 4. Membrane structure of Gram-positive and Gram-negative bacteria (sourced from Espitia et al. 2012).

enhanced antibacterial activity against *E. coli*. Karunakaran et al. (2010, 2011a, 2011b) synthesised Ag-ZnO nanoparticles using microwave, sono-chemical and sol-gel methods. The results showed that Ag-doped ZnO nanoparticles had better antibacterial activity compared with ZnO nanoparticles. Gordon et al. (2011) combined ZnO with iron oxide to produce magnetic nanoparticles with antibacterial activity. The results showed that the antibacterial activity of the combined nanoparticles was dependent on the weight ratio [Zn]/[Fe], i.e., the higher the ratio, the higher the antibacterial activity.

Antibacterial mechanism of ZnO nanoparticles

The exact antibacterial mechanism of ZnO nanoparticles is still unknown. However, many antibacterial mechanisms of ZnO nanoparticles such as the formation of reactive oxygen species (ROS), the interaction of nanoparticles with bacteria, subsequently damaging the bacterial cell, and the release of Zn^{2+} have been proposed (Table 5) (Sawai et al. 1998; Stoimenov et al. 2002; Brayner et al. 2006; Nel et al. 2006; Xia et al. 2008; Zhang et al. 2008; Ma et al. 2009; Jalal et al. 2010; Espitia et al. 2012; Manna 2012).

Many studies have indicated that the formation of ROS is the main antibacterial mechanism of ZnO nanoparticles (Sawai et al. 1998; Yamamoto 2001; Padmavathy & Vijayaraghavan 2008; Zhang et al. 2008; Jalal et al. 2010; Gordon et al. 2011; Espitia et al. 2012). Many studies have clearly indicated that ZnO nanoparticles or

powders in aqueous solution can produce various ROS such as hydroxyl radicals ($\bullet OH$), singlet oxygen or superoxide anion ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2). The formation of hydroxyl radicals and singlet oxygen species in ZnO suspension can be determined by electron spin resonance (Lipovsky et al. 2009; Jalal et al. 2010; Gondal et al. 2011; Manna 2012), while the formation of hydrogen peroxide can be measured by direct quantification (Sawai et al. 1998; Sawai 2003; Sawai & Yoshikawa 2004; Manna 2012). Hydroxyl radicals and singlet oxygen species are negatively charged species that cannot penetrate the cell membrane, whereas hydrogen peroxide can easily penetrate the cell (Padmavathy & Vijayaraghavan 2008). The generation process of H_2O_2 is shown in Figure 5. The generation of ROS depends on the surface area of ZnO nanoparticles, i.e. the higher the surface area, the higher the ROS production (Jones et al. 2008; Padmavathy & Vijayaraghavan 2008). The generation of ROS and the disruption of cell membranes caused by ZnO nanoparticles are actually bacteriocidal. Lipovsky et al. (2009) reported that ROS from ZnO suspension could be produced even under ordinary room light with a light intensity of 10 mW cm^{-2} . Furthermore, the amount of ROS can be increased significantly when ZnO suspension is irradiated with visible light in the range of 400–500 nm or with UV light. Subsequently, the antibacterial activity of ZnO is increased further (Applerot et al. 2009; Lipovsky et al. 2009; Ma et al. 2009; Raghupati et al. 2011; Manna 2012). Dutta et al. (2012) confirmed that the production of ROS during the interaction of ZnO

Table 5. Main antimicrobial mechanisms of ZnO nanoparticles.

Antimicrobial mechanisms of ZnO nanoparticles	Reference
Formation of reactive oxygen species (ROS)	Sawai et al. (1998), Jones et al. (2008), Jalal et al. (2010)
Interaction of nanoparticles with bacteria, damaging the bacterial cell	Brayner et al. (2006), Zhang et al. (2007, 2008), Xie et al. (2011)
Release of Zn^{2+} from nanoparticles	Reddy et al. (2007), Padmavathy and Vijayaraghavan (2008)

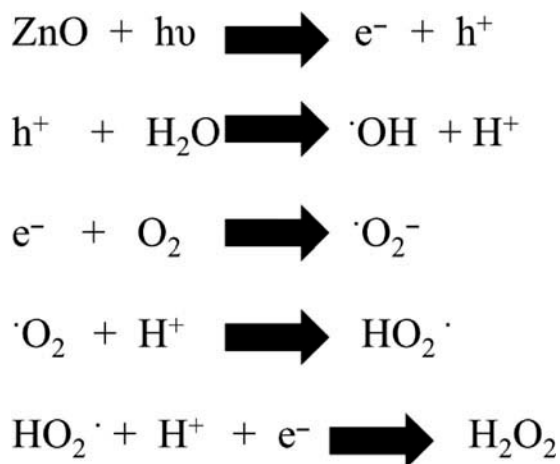


Figure 5. Generation process of H₂O₂ from ZnO nanoparticles.

nanoparticles with *E. coli* was a key factor for antibacterial activity. The generated ROS could cause the oxidation of lipid membrane in the cell wall of *E. coli*. Recently, Stankovic et al. (2013) studied the effect of particle size of ZnO powder on antibacterial activity and found ZnO powder with the largest specific surface area and the smallest particle size had the highest antibacterial activity. It has been supposed that H₂O₂ generated from the ZnO surface contributed to antibacterial activity. However, some studies have indicated the generation of ROS from ZnO nanoparticles is not a unique mechanism for the antibacterial activity of ZnO nanoparticles (Adams et al. 2006; Zhang et al. 2007; Hirota et al. 2010; Espitia et al. 2012). Adams et al. (2006) found the antibacterial activity of ZnO nanoparticles could occur in the dark. Hirota et al. (2010) also indicated that ZnO nanoparticles (about 30 nm) had sustainable antibacterial activity against *E. coli* in the absence of light. These results indicated that there were probably additional mechanisms for the production of ROS in the absence of light.

Interaction of ZnO nanoparticles with bacteria, subsequently damaging the bacterial surface, has been proposed to explain the antibacterial activity of ZnO nanoparticles. Zhang et al. (2007) clearly showed that the presence of ZnO nanoparticles led to the damage of the cell membrane of *E. coli*. Electrochemical measurements indicated that such damage might be due to direct interactions between ZnO nanoparticles and the membrane at high ZnO nanoparticles concentrations. Zhang et al. (2008) also indicated a strong bind between nanoparticles and the bacteria surface due to electrostatic forces, consequently producing cell membrane damage. The zeta potential of ZnO nanoparticles was around +24 mV at pH 7.0. The *E. coli* surface was negatively charged at pH 7.0 due to the polysaccharides of lipopolysaccharide. Thus, the surface interaction of ZnO nanoparticles with *E. coli* was favoured mainly by the electrostatic forces. Similarly, Xie et al.

(2011) observed the interaction between ZnO nanoparticles and *C. jejuni* induced morphological changes, the leakage of the intracellular content and substantial increases in oxidative stress gene expression. The damage to cell membranes by the interaction of ZnO nanoparticles and bacteria that led to the leakage of the cell membrane was also observed by typical bio-TEM technology in the study of Wahab et al. (2012). Through bio-TEM images, a possible mechanism by which ZnO nanoparticles attached initially to the outer membrane of the cell resulting in formation of pits in the cell wall followed by breakdown of the outer membrane of the cell was proposed. Additionally, ZnO nanoparticles also can either be transported into the cytoplasm or penetrate the cell wall or membrane (Brayner et al. 2006; Applerot et al. 2009; Raghupati et al. 2011; Manna 2012). Thus, according to these studies, direct interaction between ZnO nanoparticles and the bacterial surface, along with the produced ROS, leads to damage of the cell membrane or wall. Therefore, the mechanism of antibacterial activity of ZnO nanoparticles has not been fully understood.

It is well known that zinc ions can inhibit multiple activities in bacteria, such as glycolysis, transmembrane proton translocation and acid tolerance. The presence of zinc ions can prolong the lag phase of bacteria, and thus contribute to the antibacterial activity (Applerot et al. 2009). However, low concentrations of soluble Zn²⁺ can promote the growth of bacteria. Reddy et al. (2007) found complete inhibition of *E. coli* growth at a concentration of ZnO nanoparticles of ≥0.1 mM, while growth of *S. aureus* was completely inhibited at a concentration of ZnO nanoparticles of ≥0 mM. Moreover, it also showed that *E. coli* treated with 1 mM of ZnO nanoparticles could increase in the number of colony forming units (CFU) compared with the control due to low concentrations of Zn²⁺ in the growth medium. Padmavathy and Vijayaraghavan (2008) also indicated that ZnO nanoparticle suspensions in lower concentrations (0.01–1 mM) had less antibacterial activity against *E. coli* due to the presence of soluble Zn²⁺ ions.

Safety issues of ZnO nanoparticles

The application of nanotechnology in the food industry and the medical field requires an evaluation of its health risks (Mu & Sprando 2010). During the last few years, research on the toxicological characteristics of nanoparticles has increased significantly. Due to their small size and corresponding large surface area, it is believed that different degrees of biological effects are related to their special characteristics (Oberdorster et al. 2005; Adisehah et al. 2009).

Several studies on the potential toxicity of ZnO nanoparticles or powders in various animal systems, such as rats, guinea pigs, mice, human skin, zebrafish, *Daphnia* etc., have been reported (Lam et al. 1988; Hirano et al.

1989; Wesselkamper et al. 2001, 2005; Ma-Hock & Burkhardt 2008; Wang et al. 2008; Zhu et al. 2008, 2009; Zvyagin et al. 2008; Ma et al. 2009; Bai et al. 2010). Ma-Hock and Burkhardt (2008) found that ZnO nanoparticles could induce inflammatory reactions or oxidative stress responses in the respiratory tracts and lungs after inhalation of 0.5, 2.5 and 12.5 mg m⁻³. Wang et al. (2008) investigated oral toxicity of ZnO nanoparticles with two different sizes (20 and 120 nm) in mice. The target organs for ZnO nanoparticles' acute oral administration are liver, heart, spleen, pancreas and bone. The mice treated with 120 nm ZnO nanoparticles were found to have dose-effect pathological damage in gastric, liver, heart and spleen; however, the mice treated with 20 nm ZnO nanoparticles presented lessened liver, spleen and pancreas damage with the increase of the treated dose. Zvyagin et al. (2008) found that ZnO nanoparticles stayed in the stratum corneum and accumulated into skin folds and/or hair follicle roots after exposure to human skin with 20–30 nm ZnO nanoparticles. Thus, it indicated that the studied ZnO nanoparticles could result in safety issues.

The cytotoxicity of both bulk and nanoparticles of ZnO in several cell cultures including mouse neural stem cells, mouse embryo fibroblast cells, epithelial, NIH3T3 fibroblast, endothelial cells, human lung epithelium cells, human liver cells, human bronchial epithelium cells, human cardiac microvascular endothelial cells and human kidney cells (Lee et al. 2008; Deng et al. 2009; Yang et al. 2009; Heng et al. 2010; Huang et al. 2010; Hsiao & Huang 2011; Pujalté et al. 2011; Sun et al. 2011; Sharma et al. 2012) has also been studied. There is no doubt that ZnO nanoparticles have cytotoxicity against different culture cells mostly due to the induction of oxidative and inflammatory responses. Deng et al. (2009) found that toxic effect of ZnO nanoparticles (10, 30, 60 and 200 nm) in mouse neural cells were dose dependent rather than size dependent. Zn ions from ZnO nanoparticles at 12 ppm or higher in the culture after 24 h of treatment could induce cell damage. The cytotoxicity of ZnO nanoparticles depended on the availability or concentration of zinc ions. Heng et al. (2011) evaluated the cellular association, cytotoxic and inflammatory potential of spherical and sheet-shaped ZnO nanoparticles on mouse and human cell lines as well as with primary cultures of mouse bone marrow-derived dendritic cells. The results also demonstrated dose-dependent effects on the cytotoxicity of spherical and sheet-shaped ZnO nanoparticles on human cell lines.

Toxicity studies of ZnO nanoparticles are developing rapidly; however, it is still not sure whether ZnO nanoparticles are safe for health and the environment due to the lack of environmentally relevant conditions used in the experiments (Franklin et al. 2007). Generally, ZnO powders or nanoparticles are bio-safe within a certain range, but may become hazardous at higher concentrations.

Reddy et al. (2007) reported that ZnO nanoparticles reduced the viability of human T-cells at a high concentration of 5 mM. Presently, due to a lack of sufficient experimental studies and data, it is difficult to set threshold limits for various forms of ZnO for community members. Beckett et al. (2005) suggested that the threshold limits of ZnO for welders and others in the workplace were at 5 mg m⁻³.

Food applications of ZnO nanoparticles

The use of nanotechnology can extend and improve the functions of packaging materials, leading to a new kind of active food packaging (Chaudhry et al. 2008). Presently, the main food applications of ZnO nanoparticles are as antimicrobial agents in food packaging materials. In addition to antimicrobial activity, ZnO nanoparticles can improve the properties of packaging materials such as mechanical strength, barrier properties and stability. ZnO nanoparticles have been incorporated in different materials including glass, PVC, low-density polyethylene (LDPE), cellulose, polyurethane (PU), cellulose acetate, polypropylene (PP), and chitosan by different incorporation methods (Applerot et al. 2009; Emamifar et al. 2010; Li et al. 2010, 2009; Vicentini et al. 2010; Jin & Gurtler 2011; John et al. 2011; Lepot et al. 2011; Seil & Webster 2011; Anitha et al. 2012). Microorganisms are used to evaluate the antimicrobial activity of ZnO nanoparticles including Gram-negative bacteria such as *E. coli* as well as Gram-positive bacteria such as *B. subtilis*, *S. aureus* and *Lactobacillus plantarum* (Applerot et al. 2009; Emamifar et al. 2010; Jin & Gurtler 2011).

Li et al. (2009) studied the effects of ZnO nanoparticles on the mechanical and antibacterial properties of PU films. It was found that significant improvement of the PU films in Young's modulus and tensile strength was achieved by incorporating ZnO nanoparticles (27 nm) up to 2.0%. Moreover, PU films doped with ZnO nanoparticles had excellent antimicrobial activity against *E. coli* and *B. subtilis*, especially for *E. coli*. Li et al. (2010) investigated the characterisation of chitosan/ZnO nanoparticles composite film. The results showed that ZnO content had an effect on the mechanical properties of chitosan/ZnO nanoparticles composite film, and the antibacterial properties of the composite film for *B. subtilis*, *E. coli* and *S. aureus* were enhanced by the incorporated of ZnO. Further, chitosan/ZnO nanoparticles composite film with 6–10% ZnO nanoparticles showed high antibacterial activities. The researchers suggested that the antibacterial activity of the composite material could be attributed to interactions with the strongly electronegative microbial surface, inducing changes in permeability, metabolic disturbance and ultimately death.

Until now, most studies on the antimicrobial activity of ZnO nanocomposite films have been investigated *in vitro*.

Few studies on the antimicrobial activity of ZnO nanoparticles were performed *in vivo*. Emamifar et al. (2010) tested the antimicrobial activity of LDPE films incorporated with silver (Ag) and ZnO nanoparticles in orange juice. The results showed that LDPE films incorporated with ZnO nanoparticles could prolong the shelf-life of fresh orange juice by up to 28 days without causing negative effects on sensory parameters. Furthermore, Emamifar et al. (2011) also tested the antimicrobial activity of LDPE nanocomposite films containing Ag and ZnO in orange juice inoculated with $8.5 \log \text{CFU ml}^{-1}$ of *L. plantarum*. The results showed that the rate of microbial growth was significantly reduced by the use of ZnO nanoparticles.

Chaudhry et al. (2006) evaluated existing regulatory frameworks relevant to food and food packaging, among a number of other current and projected products and applications of nanotechnology. The main obstacle to the application of nanotechnology in food relative areas is the general lack of knowledge in relation to potential consumer health risks (Chaudhry et al. 2008). In addition to applications in food packaging, ZnO nanoparticles also show potential for application in cosmetic products. One of the primary advantages of using ZnO nanoparticle formulations in cosmetic products is to increase the efficacy and tolerance of UV filters on the skin surface. ZnO nanoparticles have become popular because they can retain their UV filtration and absorption properties while eliminating the white chalky appearance of traditional sunscreens (Mu & Sprando 2010). Furthermore, many modifications to the standard ZnO nanoparticle protection system have been reported to increase the sun protection factor (Villalobos-Hernandez & Muller-Goymann 2006; Mu & Sprando 2010).

Conclusions

ZnO in nanoparticle form is a promising antibacterial agent due to its wide activity against both Gram-positive and Gram-negative bacteria, and high resistance to harsh processing conditions. Many synthetic methods, such as the sol-gel method, hydrothermal method, VPM and mechano-chemical method, have been used to prepare ZnO nanoparticles. The hydrothermal method has received most attention due to its simplicity. The antibacterial activity of ZnO nanoparticles is size and concentration dependent. The antibacterial activity of ZnO nanoparticles can be enhanced by doping ZnO with another metal. Although the exact antibacterial mechanism of ZnO nanoparticles has not been established, three main antibacterial mechanisms such as the formation of ROS, interaction of nanoparticles with bacteria, subsequently damaging the bacterial cell, and the release of Zn^{2+} have been proposed. It is still not clear whether ZnO nanoparticles are safe for human health. Results to date show that

ZnO nanoparticles are safe up to a certain level, but may become toxic at higher concentrations. ZnO nanoparticles used as antimicrobial agents in food packaging materials show good antimicrobial activity. In future, more research should be focused on low-cost preparation methods for ZnO nanoparticles and on studies of their mechanism of antibacterial activity. Furthermore, more studies on the safety evaluation and antimicrobial activity of ZnO nanoparticles *in vivo* need to be undertaken.

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