Chapter 2

Literature Review

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2.1 INTRODUCTION

- The studies on detrimental effects of microorganisms and their remedies are necessary for improving the quality of human life. The variety and variability of microbial flora and fauna coexists in a natural equilibrium within the human body and the living environments; disruption in the natural equilibrium can lead to some serious problems and which is of utmost concern [1–7]. Anti-microbial agents are used to prevent these problems. In recent years, ZnO being a wide band-gap semiconductor (3.36 eV), has turned out to be an efficient antimicrobial agent over the recent years [8]. There are some reports [9] on the considerable antibacterial activity of CaO, MgO and ZnO, which is attributed to the generation of reactive oxygen species on the surface of these oxides, which has been studied by a conductometric method. Inorganic nanocrystalline metal oxides are particularly interesting because they can be prepared with extremely high surface areas, and are more suitable for biological applications. Actually the main advantages of using these inorganic oxides over organic antimicrobial agents are their stability, robustness, and long shelf life and at the same time they contain mineral elements essential for humans and exhibit strong activity even when administered in small amounts. The antibacterial activity of ZnO has been studied largely with different pathogenic and nonpathogenic bacteria such as *S. aureus* and *E. coli* [10]. From the comparative study it had been concluded that, among six metal oxide nanoparticles, ZnO nanoparticles significantly inhibited growth of a wide range of pathogenic bacteria under the normal visible lighting conditions.
ZnO nanoparticles are believed to be nontoxic, biosafe, and biocompatible and have been used as drug carriers, cosmetics, and fillings in medical materials [11-15]. Several reports have addressed the harmful impact of nanomaterials on living cells, but relatively low concentrations of ZnO are nontoxic to eukaryotic cells [16]. The ZnO nanostructures had been selected due to the presence of these beneficial sides.

2.2 ANTIMICROBIAL ACTION OF ZnO

- Sawai et al [17] had examined the minimal inhibitory concentration (MIC) obtained by indirect conductometric assay to estimate the antibacterial activity of insoluble ceramic powders compared with several types of antibiotics. According to their investigation the antibacterial activity was affected by particle size as well as controlled by processing parameters. Many researchers have attempted to correlate the biological activity of inorganic antibacterial agents with the size of the particles [18, 19]. Although many studies on the biological activity of ZnO had been carried out, most of these concerned with the antimicrobial effect of bulk ZnO with a large particle size.

- Yamamoto et al. [20] had studied the antibacterial activity of ZnO with various particle sizes in the range of 0.1–1μm. According to the change in electrical conductivity, the results had clearly demonstrated that nanosized ZnO was a more effective antimicrobial agent than bulk ZnO and this was the first systematic study in which nano and bulk ZnO were compared based on either the inactivation or
the destruction of bacteria using a standard microbial method. Application of inorganic nano-particles and their nanocomposites would be a good alternative and at the same time it can open up a new opportunity for anti-microbial application.

- Jones et al. [21] had studied the antibacterial activity of ZnO nanoparticle suspension on a broad spectrum of microorganisms. They have concluded that among the six tested nanoparticles, four nanoparticles MgO, TiO$_2$, CuO and CeO$_2$, did not show any significant growth inhibition up to 10 mM colloidal suspension, whereas Al$_2$O$_3$ and ZnO had showed significant growth inhibition. No significant growth inhibition was detected for Staphylococcus aureus. At the concentrations of both the colloidal suspension 2mM the ZnO ultrafine powder which was relatively larger in size, had reduced the growth rates to 50% whereas, the smaller ZnO nanoparticles were able to reduce the growth rate to the extend of 99%. The data for both sizes of ZnO nanoparticles had clearly suggested that the antibacterial activity of ZnO nanoparticles in the dark condition was less than that in light conditions. Finally it had been concluded that the light conditions were sufficient for the optimal biocidal activity of the ZnO nanoparticles, which was probably dependent on the size of the nanoparticles.

- Zhang et al. [22] also performed a similar study using ZnO nanofluids. The results had showed that the ZnO nanofluids have bacteriostatic activity against E. coli. The antibacterial activity had increased both with increasing nanoparticle concentration & decreasing particle size but the nanoparticle concentration was observed to be more important than particle size under the conditions of his work.
Electrochemical measurements using a model DOPC (Dioleyl phosphatidylecholine) monolayer had suggested some direct interaction between ZnO nanoparticles and the bacterial membrane at high ZnO concentration. Sawai et al as well as Gomes et al [23] used micron sized ZnO particles for studying the antimicrobial effect of yeast but they were unable to demonstrate the substantial killing of yeast.

Recently, Lipovsky et al [24] had reported on the entitled paper “Antifungal activity of ZnO nanoparticles—the role of ROS mediated cell injury” that, nanoparticles of ZnO were much more effective agents in controlling the growth of various microorganisms; furthermore, the smaller particle size had showed the greater efficacy in inhibiting bacterial growth. The results of his study had suggested that ZnO nanoparticles have a marked biocidal activity against Candida albicans and also have a cytotoxic effect which is both concentration-and size dependent. The minimal fungicidal concentration of ZnO was found to be 0.1 mg/ml and this concentration caused an inhibition of over 95% in the growth of C. albicans.

Yuvaraj et al. [25] had reported in their work that antibacterial activity of the plain cotton fabrics and cotton fabrics coated with ZnO nanostructures were tested using Staphylococcus aureus bacterium (Gram positive). The development of antimicrobial cotton fabrics using ZnO nanoparticles had been investigated by other researchers also. R.Rajendran et al. [26] had prepared ZnO nanoparticles by wet chemical method and had directly applied it on to the 100% cotton woven fabric using pad-dry-cure method. The SEM analysis had revealed the presence of
ZnO nanoparticles in treated fabrics and the wash durability study of the treated fabric was also carried out and found to withstand up to 25 wash cycles. The antibacterial activity of the finished fabrics was assessed both by qualitatively & quantitatively where the initial one had been done by agar diffusion and parallel streak method and the later one by percentage reduction test. The topographical analysis of the treated fabric and untreated fabric were studied and compared with each other, which had been clearly demonstrated that the ZnO nanoparticle treated fabrics showed increased antibacterial effect than the untreated fabric. The results had also demonstrated that higher antibacterial activity was observed for *S.aureus* than *E.coli* both in qualitative and quantitative tests but it was in controversial because some other researchers had found that the ZnO nanoparticles coated paper possesses antibacterial activity against *E.coli*. The antibacterial activity was carried out both in the presence of light & dark condition. The best antibacterial activity could be obtained on illumination with 543 nm, 1000 Lux light, i.e. household fluorescent tube light, for 24 h whereas the antibacterial activity was also noted in absence of light, but on treatment for 24 h.

- Regarding the antibacterial fabric material, The ZnO nanoparticles, can be efficiently transferred on the surface of both woven and knitted cotton and polyester/cotton blended fabrics. The woven fabrics were more receptive to antibacterial finishing than the knitted fabrics. Similarly, 100% cotton fabrics were better textile substrates for antibacterial finishing than the polyester/cotton blended fabrics. The antibacterial test had indicated a significant and considerable increment of the antibacterial activity in the ZnO-treated fabrics. Such result can
be exploited for the commercialization in the case of consumer textiles [27].

Beside the cotton & fabric material antibacterial wallpaper had been produced through nanotechnology using ZnO. A simple approach of ultrasound-assisted coating of paper with ZnO nanoparticles of ~20nm without the help of binder was reported. The ZnO coated paper, giving it an antibacterial surface suitable for use as wallpaper in hospitals. The paper could be used on hospital walls, in particular operating theatres, as well as residential complexes, which would reduce the possibility of infections. The ZnO nanoparticles coated paper had antibacterial properties against *E. coli*. Non-impact spray-based coating techniques were generally preferred than ultrasound assisted approach as these avoid web breaks and streak defects and had certain advantages in terms of durability and surface quality.

- According to the above survey it had been demonstrated that many researchers have successfully introduced ZnO nanoparticles on the cotton fabric, wallpaper for producing the antibacterial efficacy. Their main investigation had focused upon the application purpose rather than its mechanism.

- Zinc oxide nanoparticles blended at 10% (w/w) fraction into dental composites had displayed antimicrobial activity and reduced the growth of bacterial biofilms by roughly 80% for a single-species model dental biofilm. The antibacterial effectiveness of ZnO nanoparticles were assessed against *Streptococcus sobrinus* ATCC 27352 grown both planktonically and as biofilms on composites. Direct contact inhibition was observed by scanning electron microscopy and confocal laser scanning microscopy while biofilm formation was quantified by viable
counts. An 80% reduction in bacterial counts was observed with 10% ZnO nanoparticle containing composites compared with their unmodified counterpart, indicating a statistically significant suppression of biofilm growth. Although, 20% of the bacterial population survived and could form a biofilm layer again, 10% ZnO-NP-containing composites retained at least some inhibitory activity even after the third generation of biofilm growth. Microscopy study had demonstrated continuous biofilm formation for unmodified composites after 1-day growth, but only sparsely distributed biofilms formed on 10% ZnO nanoparticle containing composites. The minimum inhibitory concentration (MIC) of ZnO nanoparticle suspended in *S. sobrinus* planktonic culture was 50 μg mL⁻¹. ZnO nanoparticle containing composites (10%) had qualitatively showed less biofilm after 1-day anaerobic growth of a three-species initial colonizer biofilm after being compared with unmodified composites, but did not significantly reduce growth after 3 days [28].

- The antibacterial activity of four root canal filling materials - Zinc oxide and eugenol cement (ZOE), Calen paste thickened with zinc oxide (Calen/ZO), Sealapex sealer and EndoREZ sealer - against 5 bacterial strains commonly found in endodontic infections (*Kocuria rhizophila*, *Enterococcus faecalis*, *Streptococcus mutans*, *Escherichia coli* and *Staphylococcus aureus*) were studied for primary teeth using the agar diffusion test. It had concluded that *K.rhizophila* was inhibited more effectively by ZOE whereas *E.faecalis* had showed higher inhibition zone due to Calen/ZO. *S.mutans* was inhibited in same intensity separately by Calen/ZO, Sealapex and ZOE whereas *E.coli* had showed the
antibacterial activity in such a manner of ZOE>Calen/ZO>Sealapex and so on.

Among the above mentioned four root canal filling materials, the infections can be presented in a decreasing order of efficacy as follows ZOE>Calen/ZO>Sealapex>Endorez [29].

- Although the antibacterial activity and efficacy of regular zinc oxides have been investigated by Nicole Jones et.al. [30]. The growth analysis data had suggested that nanoparticles of ZnO have significantly higher antibacterial effects on Staphylococcus aureus. The antibacterial activity of ZnO may be dependent on the size and the presence of normal visible light. It had suggested that ZnO nanoparticles have a potential application as a bacteriostatic agent in visible light and may have future applications in the development of derivative agents to control the spread and infection of a variety of bacterial strains.

- In addition, various studies have clearly demonstrated that ZnO nanoparticles have a wide range of antibacterial effects on a number of other microorganisms beside the E.coli & S aureus. Rizwan Wahab et.al. [31] in 2008 had studied the antibacterial study of zinc oxide nanoparticles in the presence of four pathogens such as Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, and Klebsiella pneumonia. The antibacterial activity of ZnO nanoparticles were studied by spectroscopic method taking different concentrations from 5 to 45 μg/ml of nanoparticles. Their investigation had revealed that the lowest concentration of ZnO nanoparticles solution inhibiting the growth of microbial strain was found to be 5 μg/ml for K. pneumoniae, whereas for E. coli, S. aureus, and S. typhimurium, it was calculated to be 15 μg/ml. The particles size of each
nanoparticle was 20 and 30 nm as observed from FESEM and transmission electron microscopy images.

### 2.3 Antimicrobial Action of Capped ZnO

- It had been elucidated from the above review was that ZnO nanomaterials had showed antimicrobial activity for different microorganisms. Here, some reports have been delivered regarding with the capped nanomaterial, which had showed the antimicrobial activity. Zhang et.al [32] had proposed the use of two types of dispersants, Polyethylene Glycol (PEG) & Polyvinylpyrrolidone (PVP), but he mentioned that it had not been affected much the antibacterial activity of ZnO nanofluids but enhanced the stability of the suspensions. Many researchers had been investigated the antimicrobial activity not only by bare ZnO nanomaterial. They have synthesized ZnO in the presence of PVP. Both ZnO nanoparticle & ZnO-PVP had showed significant antimicrobial activities for all three pathogens *Listeria monocytogenes*, *Salmonella Enteritidis*, and *Escherichia coli* O157:H7 in growth media and liquid egg white (LEW). However, the ZnO-PVP coating had less inhibitory effect than the direct addition of ZnO-PVP nanoparticle, while no antimicrobial activities of ZnO-PS film were observed. The ZnO-PVP (3.2 mg ZnO/mL) treatment resulted in 5.3 log reduction of *L. monocytogenes* and 6.0 log reduction of *E. coli* O157:H7 in growth media after 48 hours incubation, as compared to the controls. According to the findings, Listeria cells in the liquid egg white (LEW) control increased from 3.8 to 7.2 log CFU/mL during eight days
incubation, while the cells in the samples treated with 1.12 and 0.28 mg ZnO/mL were reduced to 1.4 and 3.0 log CFU/mL, respectively. The cell populations of Salmonella in liquid egg white (LEW) in the presence of 1.12 and 0.28 mg ZnO/mL were reduced by 6.1 and 4.1 log CFU/mL respectively, in comparison to the reduction levels in the controls. It had been revealed from their study that ZnO nanoparticles possess antimicrobial activities for *L. monocytogenes* and Salmonella in liquid egg white (LEW) and growth media, as well as for *E. coli* O157:H7 in growth media [33]. The enhanced bioactivity of ZnO nanoparticles by studying the antimicrobial activity of suspensions with various particle sizes using a standard microbial method had been analyzed by Nagarajan Padmavathy. The enhanced bioactivity of smaller particles was attributed to the higher surface area to volume ratio. For smaller ZnO nanoparticles, more particles were needed to cover a bacterial colony (2µm). It results in the generation of a larger number of active oxygen species, released from ZnO on the surface of the colony, which had killed the bacteria more effectively. [34].

The antimicrobial activity of ZnO nanoparticles on *Enterobacter aerogenes* was examined. A set of different concentrations of nanoparticles at 50, 75, 100 & 125 µg/ml were tested on gram negative Enterobacter sp. for their concentration dependent antimicrobial effects. A number of other bacteria including *E. coli* & *B. subtilis* were also investigated for zinc oxide nanoparticles suspension to check the bacterial growth. The antimicrobial activity had been examined by drawing growth curves using spectrophotometer assisted absorption observations. The adsorption of ZnO nanoparticles on bacterium surface had visualized by scanning
and transmission electron microscopies. The antimicrobial activity had further confirmed by disc diffusion method. The increased concentrations of zinc oxide nanoparticles had effectively checked the bacteria growth and increased the diameter of inhibition zone in the experiment. In presence of zinc oxide nanoparticles, *Enterobacter sp* growth had significantly reduced while the zinc oxide powder had showed lower levels of antimicrobial activity when compared at same dose levels of 50, 75, 100 & 125µg/ml [35].

- The Polyurethane film coated with ZnO nanoparticles of 27 nm had prepared by solution blending. The ZnO/PU films and coats were generated by a simple method of solution casting and evaporation. Moreover, the antibacterial property test was carried out based on the agar dilution method and the result had indicated that PU films doped with ZnO nanoparticles showed excellent antibacterial activity, especially for *Escherichia coli* [36].

- ZnO nanoparticles loaded cellulose acetate (ZOLCA) films have shown excellent antibacterial action against model bacteria *E.coli* when investigated by both qualitative and quantitative methods and finally the films exhibited great potential applications to protect food stuff against microbial infections [37].

- Zinc oxide nanoparticles in suspension form were used as antibacterial agents for the *E. coli* disease-causing bacterium and this was delivered by L. Zhang et al. [22]. The ZnO nanoparticles had actually damaged the *E.coli* membrane walls, while also being generally regarded as safe for human beings and animals. By studying the effect of varying particle sizes and concentration, the authors found that the bacteriostatic activity was increased with decrease of the particle size of
ZnO nanomaterial. Further advantages were brought by an increased ZnO concentration. Finally, the authors had also noted that dispersing the solution either with PEG (polyethylene glycol) or PVP (polyvinylpyrrolidone) polymers allowed for an improvement in solution stability, while at the same time maintained the antibacterial benefits of ZnO. Such results have great promise for industries such as food, textiles, packaging and healthcare, as nanostructured inorganics' high-temperature, high-pressure stability begins to be fully exploited.

- Yunhong Jiang had been attempted to develop ZnO coated PVC films for exhibiting its antimicrobial activity, and had assessed its potential use in self-cleaning and self-sterilizing materials such as food packaging and items for use in hospitals. The size of ZnO nanoparticles coated on the films was estimated in the range of 50-100 nm. The antibacterial activities of ZnO coated films for *E.coli* (Gram-negative) were estimated by the growing inhibitory effect in the solutions. The specific surface area was examined by N₂ adsorption using the Brunauer, Emmett and Teller (BET) method. The antibacterial activity had increased with both decreasing particle size and increasing surface area of ZnO. The minimum inhibitory concentration (MIC) of ZnO coated films against *E.coli* was 0.25 g/L.

Xihong Li *et al.* [38] had also investigated the antimicrobial activity of PVC coated ZnO powder against two bacteria *E.coli* & *S.aureus* and two different fungi *P.citrinum* & *A.flavus*. However, antifungal activity of the ZnO-coated films against *Aspergillus flavus* and *Penicillium citrinum* was not observed whereas the film had exhibited good antibacterial effect for *E.coli* & *S.aureus*. Regarding to
the antibacterial activity both Yunhong Jiang & Xihong Li have suggested that ZnO coated PVC film had exhibited good antibacterial activity against *E.coli*.

- According to the recent research publications, nanoparticle metal oxides offer a wide variety of potential applications in medicine due to the unprecedented advances in nanobiotechnology research. The effect of zinc oxide (ZnO) nanoparticles prepared by mechano-chemical method on the antibacterial activity of different antibiotics was evaluated using disk diffusion method against *Staphylococcus aureus* & *E.coli*. In their study they have decorated the ZnO nanomaterial through different antibiotics and finally checked the antimicrobial assay [39].

- From the above mentioned literature reviews, it can be inferred that research regarding mechanism of antimicrobial effect of functionalized ZnO with changing structural configuration had not been well established. The main thrust area of the research documented above dealt with the study of MIC as well as MBC whereas less information regarding attachment & internalization of nanomaterial with microbial cell thereby initiating antimicrobial activity was generated. Many researchers have found that the antimicrobial activity of ZnO nanomaterial had dependent on the particle size & concentration.

- ZnO nanofluids have been prepared in glycerol as a base fluid in the presence of ammonium citrate as a dispersant. The experimental result had revealed that low concentrations of ZnO nanofluids could not inhibit bacterial growth. The antibacterial activity of ZnO had increased with increasing nanofluid concentration and time. In the antibacterial tests, it was found that the ZnO
nanofluids were good bactericidal agents. Ammonium citrate could slightly, but not significantly, reduced the number of colonies on the agar plates. Ammonium citrate could act as a weak antibacterial agent but it had not affected the antibacterial activity of ZnO nanofluid respectively. Survival ratio of bacteria had decreased with increasing the concentrations of ZnO nanofluids and time and it had produced strong antibacterial activity toward *E. coli* [40]. Recently antibacterial activity of ceramic powders had attracted attention. Ceramic powders of zinc oxide (ZnO), calcium oxide (CaO) and magnesium oxide (MgO) were found to show marked antibacterial activity. Nanocomposites containing nano particle ZnO (average size 20 nm) in TiO$_2$sol-gel matrix had been synthesized with various loading weight percentage of nano ZnO powder with respect to TiO$_2$ sol weight. After the nanocomposites were dried into flakes, antibacterial activity of the nanocomposites with various ZnO nano particles weight percentage were investigated for *Staphylococcus aureus* and *Escherichia coli* by conductimetric assay. Nanocomposites with 20 weight percentage and 30 weight percentage of ZnO nanoparticles in TiO$_2$ solgel matrix had inhibited 40 to 95% of both antibacterial proliferations from different series of nanocomposite products. Both nanocomposites selectively inhibited towards *E.coli* compare with *S.aureus* [41].

There were relatively small numbers of publications in the literature focused on the antibacterial activity study of functionalized ZnO nanostructures for *E.coli* and *S.aureus* bacteria. Beside this, there were lacks of insufficient reviews regarding to antibacterial activity study by capped ZnO nanomaterial. Functionalization of ZnO nanomaterial through 3- Mercaptobenzoic acid & 3-
Aminopropyltriethoxysilane and its antibacterial efficacy had not been analyzed. Several studies have proposed the antibacterial activity of bare ZnO nanostructures. Due to the encapsulation different functional groups can be attached and through this attachment the surface chemistry of bare ZnO may be engineered. ZnO nanostructures had been shown good antibacterial activity but the functionalized material is still not so popular. It would be interesting to determine if any modification of ZnO nanoparticles with chemical groups or bioagents are more effective in eliminating various microorganisms. Some reports have suggested that modification of nanoparticle surfaces can efficiently target and kill both Gram-positive and Gram-negative bacteria [34].

The above investigation reports have found various methods for synthesizing surface functionalized ZnO nanostructures. Initially the bare ZnO nanomaterials were synthesized & then functionalized it with different capping agents by post capping. Some of them directly bought ZnO nanostructures from market and then functionalized it based on the same post capping. In all cases the structure of the ZnO nanomaterial was predefined due to the post capping. In situ synthesis of functionalized ZnO nanomaterial may introduce new nanostructures as well as it can reduce the preparation process timing.

2.4 MECHANISM OF ANTIBACTERIAL ACTION OF ZnO

Generally, the antimicrobial mechanism of chemical agents can be understood by studying the specific binding of the chemical agent with a microorganism and the
consequent metabolism of the agents inside the microorganism. ZnO nanoparticles in aqueous suspensions had been shown to produce various active oxygen species. In light of these results it had been concluded that the cyto-toxic effect of ZnO on Candida may be mediated through ROS (reactive oxygen species). Perelshtein et al.[42] had reported that the antibacterial activity of ZnO nanostructures was due to the formation of the oxygen radicals, which had effectively prevented the growth of *Staphylococcus aureus* bacterium on cotton cloth. Antibacterial activities of ZnO nanoparticles and their mode of action had studied against an important foodborne pathogen, *Escherichia coli* O157:H7. Both ZnO nanoparticle with sizes of 70 nm and concentrations of 0, 3, 6 and 12 mmol l(-1) and nanoparticle free solutions were used in antimicrobial tests for *E. coli* O157:H7. ZnO nanoparticle had showed increasing inhibitory effects on the growth of *E. coli* O157:H7 as the concentration increased. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Raman spectroscopy were used to characterize the changes of morphology and cellular compositions of bacterial cells treated with ZnO nanoparticle and had studied the mode of action of ZnO nanoparticle for *E. coli* O157:H7. The intensity of lipid and protein bands in the Raman spectra of bacterial cells had increased after exposure to ZnO nanoparticle, while no significant changes in nucleic acid bands had been observed. Finally it had concluded the fact that ZnO nanoparticle were found to have antibacterial activity for *E. coli* O157:H7. The inhibitory effect had increased as the concentration of ZnO nanoparticle increased. Results had indicated that ZnO nanoparticle might be distorted and damaged the bacterial cell.
membrane, resulting in a leakage of intracellular contents and the death of bacterial cells. The result had suggested that ZnO nanoparticle could potentially be used as an effective antibacterial agent to protect agricultural product and food safety.

- It had supported the fact that antibacterial activity was induced by the hydrogen peroxide (H$_2$O$_2$) generated from ZnO. It was well known that H$_2$O$_2$ had showed harmful effect to the living cells and was the major contributor of antibacterial activity. Perelshtein et al had mentioned, the natural tendency of cellulose fibers was to absorb the moisture and the ZnO nanoparticles coated on the fibre had generated H$_2$O$_2$ from the absorbed moisture. The freshly produced H$_2$O$_2$ can be taken to the advantage of forming antibacterial wall papers.

- The synthesis and bioactivity of nano particles and their antimicrobial properties individually and combination with different antibiotics had not been demonstrated. Recently the effect of zinc nano particles along with different antibiotics on the antibacterial activity had evaluated against *Staphylococcus aureus*. Disk diffusion method was used to determine the antibacterial activity of various classes of antibiotics in the absence and presence of sub inhibitory concentration of Zinc nano particles. *S. aureus* was used as the test strain. In the presence of sub-inhibitory concentration of Zinc nano particles i.e. 100µg/disc, the antibacterial activities of all antibiotics have increased from 2 mm to 10mm. The highest increase was observed for Penicillin G and Amikacin. These results had signified that the Zinc nano particles revealed the antimicrobial action of beta
lactums, cephalosporins, amino glycosides, glycopeptides, macrolids and lincosamides, tetracycline for *S. aureus* bacteria [43].

- The specific role of size scale, surface capping, and aspect ratio of ZnO particles on toxicity toward prokaryotic and eukaryotic cells were investigated. ZnO nano and microparticles of controlled size and morphology were synthesized by wet chemical methods. Cytotoxicity test of the mammalian cell was studied using a human osteoblast cancer cell line and antibacterial activity using both *Escherichia coli* & *Staphylococcus aureus* [44]. Scanning electron microscopy (SEM) was conducted to characterize the visual features of the antibacterial action of ZnO. They observed that antibacterial activity had increased with decrease in particle size. Aqueous suspensions containing $4.45 \times 10^{-5} \text{ - } 1.25 \times 10^{-3} \text{ M}$ ZnO particles had exhibited a strong antibacterial activity against *E. coli* under the dark conditions. The dominant mechanisms of such antibacterial behaviour were found to be either or both of chemical interactions between hydrogen peroxide and membrane proteins, and chemical interactions between other unknown chemical species had generated due to the presence of ZnO particles with the lipid bilayer. The effect of direct physical interactions between nanoparticles and biological cells were found to play a relatively small role under the conditions of this study.

- The antibacterial behaviour of ZnO nanopowder and their composite coatings were investigated for *E. coli*. ZnO nanopowder and Aluminum based ZnO composite powders were synthesized using in-house powder processing techniques. Bacteria culture results had showed that ZnO nanopowder and their composite powders had displayed excellent bacteriostatic activity for *E. coli*. The
antibacterial activity had increased with increasing concentration of ZnO nanoparticle in their composite powders as well as increasing surface area of compacted pellets. These nanocomposite powders were subsequently used to generate antibacterial coatings using cold spray technology. The ratio of weight percentage of Al to ZnO in their composite powders was 80:20, 50:50 and 20:80. The results had indicated that the antibacterial activity increased with increasing ZnO nanopowder concentration in the composite powder feedstock and cold sprayed coating [45]. The experimental results had suggested three possible antimicrobial mechanisms of suspensions of ZnO particles:

- Physical interaction by attaching onto the bacterial cell wall directly
- Biological interaction with the cell membrane components and
- Chemical interaction by producing reactive oxygen species.

Most significantly, the results had indicated that ZnO suspensions containing different sized particles inhibited the growth of *E. coli* bacterial cells through different mechanisms. Micron-sized ZnO particles had killed the *E. coli* through producing H₂O₂, whereas little H₂O₂ was found in nano-sized ZnO suspensions.

The aim of the study was to determine whether metal oxide nanoparticles had damaged the bacterial cell membranes. Bacterial cell membrane integrity was determined by using fluorescence microscopy, after 1h treatment of TiO₂ and ZnO nanoparticles (0, 10, 50, 100, and 500mg/L) in ultrapure water and in the presence of natural organic matter. Results had revealed a measurable impact on the cell membrane integrity of *Escherichia coli* bacteria at 100 and 500mg/L TiO₂ even in the presence of natural organic matter. Whereas, no statistically significant
change in cell membrane integrity was observed with ZnO that aggregated and precipitated at concentrations at or above 100 mg/L. In addition, *Enterococcus faecium* were not impacted in the presence of ZnO or TiO$_2$. Transmission electron microscopy images had showed an interaction between TiO$_2$ nanoparticles and *E. coli* cells. This interaction was not observed for ZnO nanoparticles or *Enterococcus faecium* cells. The cell membrane integrity assay had indicated nanoparticle impact on bacterial cells that were not detected by plating techniques. [46].

- There were few publications in the literature regarding the interaction between ZnO nanoparticles and bacterial cells. According to Sawai *et al.* [8, 9], Yamamoto *et al.* [20, 47, 48], Stoiimenov *et al.* [19], Makhluf *et al.* [49], Brayner *et al.* [18], Jeng and Swanson *et al.* [50], Adams *et al.* [51] and Zhang *et al.* [22], the ZnO nanoparticles were mostly effective for inhibiting both Gram-positive and Gram-negative bacteria, and even spores that were high-temperature and high-pressure resistant. A higher concentration of smaller particles with a higher surface area had exhibited a better antibacterial behavior but the crystalline structure and shape had a small effect.

- There were several number of mechanisms have been proposed to interpret the antibacterial behavior of metal oxides. Makhluf *et al* in 2005 had attributed the antibacterial activity of MgO whereas Sawai *et al.* in 1996 had measured the active oxygen species generated in ZnO slurries by using chemiluminescence and oxygen electrochemical analyses and found
That H$_2$O$_2$ was produced in ZnO slurries and its concentration was directly proportional to the ZnO particle concentration. *E. coli* cell has a rod-like morphology with a length of approximately 1–3 µm and a diameter of 0.5–1 µm. It consists of an outer membrane as well as an inner membrane.

Given the structure of the *E. coli* cell envelope, the following mechanisms could contributed to the antibacterial behavior of ZnO suspensions:

- There were two types of interaction occurs i.e. chemical and physical interaction.
- Chemical interactions occurs between ZnO and the components of the cell envelope (lipid bilayer, peptidoglycan, membrane proteins, lipopolysaccharides).
  - Chemical reaction between cell envelope components and Zn$^{2+}$ due to the presence of ZnO particles.
  - Chemical reaction between the Zn$^{2+}$ ions and components in the interior of the cell. This involves transportation of Zn$^{2+}$ into the interior of the cell first and then the reaction occurs.
  - Chemical reaction between the cell envelope components and chemical species such as hydrogen peroxide, generated due to the presence of ZnO particles.
  - Chemical reaction between the chemical species generated due to ZnO particles and components in the interior of the cell.
- Physical interactions between ZnO nanoparticles and the cell envelope structure as follows:
• Physical blockage of the transport channels of the cell membranes by ZnO particles
• Physical damage to the membrane envelope components by ZnO particles.
• Penetration of ZnO particles through the cell envelope and interact with the interior of the cell.

Several researchers have proposed several mechanisms to establish the antibacterial activity against *E. coli* and *S. aureus* bacteria.

Several studies have suggested, there may be two possible mechanisms involved in the interaction between nanoparticles and bacteria –

• The production of increased levels of reactive oxygen species (ROS), mostly hydroxyl radicals and singlet oxygen and
• Deposition of the nanoparticles on the surface of bacteria or accumulation of nanoparticles either in the cytoplasm or in the periplasmic region may cause the disruption or disorganization of cellular function. It indicates that both production of the ROS and deposition of ZnO nanoparticles within the cell cytoplasm or on the surface of *S. aureus* leads to either inhibition of bacterial growth or killing the cells.

Although there were numerous studies regarding the antibacterial effect of ZnO. Focus had been drawn on *E. coli* with relatively few reports on *S. aureus*. Not much information could be obtained regarding the actual mechanism on the antimicrobial effects.

There were no thorough investigation had been reported for different structured ZnO nanostructures with that of known antibiotic Amoxicilline.
There is a lack of sufficient reports regarding to surface functionalized nanomaterial and its antibacterial activity along with the cellular internalization.
2.5 REFERENCES


[27] Prof S Kathirvelu, Prof (Dr) L.D. Souza, Prof(Mrs) Dr B Dhurai, Journal of the Textile Institute, 101(2010)6 520-526


[29] Alexandra Mussolino de QUEIROZ¹, Paulo NELSON-FILHO¹, Lea Assed Bezerra da SILVA¹, Sada Assed¹, Raquel Assed Bezerra da SILVA¹, Izabel YOKO ITO², Braz Dent J 20(4) (2009) 290-296


[35] Shilpa Newati, Sarita Sachdeva, Varsha M.Singh and Riaz A.Khan, 10AIChE, Annual Meeting, Salt Lake city, UT, November 7-12, 2010

[36] J.H.Li¹, R.Y.Hong²,*, M.Y.Li³, H.Z.Li³, Y.Zheng⁴, J.Ding⁵, Progress in Organic Coating 64(2009) 504-509

[38] Xihong Li, Yage Xing, Yunhong Jiang, Yulong Ding & Weili Li, International Journal of Food Science and Technology 44 (2009) 2161–2168


