Bactericidal Activity of Zinc Oxide Nanoparticles

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Abstract. The present study was aimed to evaluate the antibacterial activity of zinc oxide nanoparticles against the pathogen isolated from diabetic foot ulcer. The isolate was identified by MALDI-TOF. The susceptibility of the isolate to antibiotic was evaluated by disc diffusion assay. Chemically synthesized zinc oxide nanoparticle was characterized for its size distribution by dynamic light scattering, morphology by FESEM and elemental composition by energy dispersive X-rayspectroscopy. The wound isolate was identified as E. coli. The strain was resistant to β lactam and aminoglycoside antibiotics. The size of zinc oxide nanoparticles was recorded as 55nm. FESEM revealed the spherical shape of zinc oxide nanoparticles. UV visible spectrophotometry confirms the presence of zinc oxide nanoparticles with an absorbance maximum at 372 nm. Zinc oxide nanoparticles inhibited E. coli with an inhibition zone of 25 mm. Thus, the study proved the bacterial activity of zinc oxide nanoparticles against multidrug resistant E. coli.

1. Introduction
Zinc oxide is a II–VI semiconductor [1]. It is widely used in diverse applications due to its broad band gap (3.3 eV) in the UV region with a binding energy of 60 meV [1, 2]. The photocatalytic activity of zinc oxide is used in waste water treatment plants to oxidize pollutants. It is unique in absorbing light at UVA and UVB region. This UV absorption property is exploited in the preparation of UV shield materials [3, 4]. Advanced nanotechnology tools enable the engineering of materials at the nanoscale. Chemical, mechanical, electrical, and optical properties of materials are unique at the nanoscale [1,2].
Zinc oxide is a potent antimicrobial agent. The hall mark of post antibiotic era. Antibiotics lose their lethal effect against multidrug resistant and extensive drug resistant pathogens. There are only few new antibiotics under clinical trial. There is an urgent need to use alternative materials for augmenting the efficiency of antibiotics. This motivated the scientists to exploit nanomaterials for bactericidal applications. Metal nanoparticles are well studied for their antibacterial activity [3,4]. Among the metal nanoparticles, zinc oxide is biologically compatible and it possesses antioxidant property [9]. Zinc oxide nanoparticles (ZnO NPs) offer highly reactive surface and large surface area for interaction with pathogens. The permeability of bacterial membrane is more for nanomaterials due to its high aspect ratio [6–8]. In addition to its antibacterial activity zinc oxide stimulates angiogenesis and enhances new blood vessel formation which indirectly helps in wound healing [9]. ZnO NPs are used in food application for protection against bacteria [10]. The advantage with ZnO NPs is its biocompatibility and safety to use for human applications [11].
India is the capital of diabetes. Approximately 1.5 million of diabetic patients suffer from chronic wound [12,13]. Majority of the diabetic wound healing is delayed due to infection. Infection turns the wound into chronic and it does not heal in a normal pattern. So, the present study is aimed to evaluate the bactericidal property of zinc oxide nanoparticles against chronic wound isolate.

2. Materials and Method
2.1 Isolation and Identification of pathogens from diabetic wound
The chemicals and media were purchased from Himedia, Mumbai, India. The bacteriafor the evaluation of antibacterial activity of zinc oxide nanoparticles was isolated from diabetic wound. It
was identified by MALDI-TOF[14]. Swab of the wound was collected and suspended in phosphate buffered saline. The sample (100μL) was spread onto blood agar medium. Single colony was selected and processed further for identification by MALDI-TOF. The colony was suspended in 300 μL of sterile water. Cellular constituents were extracted by the addition of 900 μL of absolute ethanol. And mixed well in the cyclomixer for 10 minutes. The sample was centrifuged at 13,000 × g for 2 min. The pellet was dissolved in 50 μL of acetonitrile and mixed with 750 μL of formic acid (70 %).The solution was centrifuged at 13,000 × g for 2 min. The supernatant (1 μL) was placed on MALDI-TOF target plate. 1 μL of matrix solution was overlaid onto the sample and introduced into the MALDI-TOF mass spectrometer (BrukerDaltonik GmbH, Bremen, Germany). Mass spectra were recorded from 2000 to 20,000 Da at a frequency of 20Hz.

2.2 Synthesis and characterization of ZnO NPs
ZnO NPs were synthesized using the chemical method[15]. Zinc nitrate (10 mM) was prepared in pre-chilled nano pure water. Tween 20 (0.1 v/v%) was added to zinc nitrate solution and incubated for 30 minutes at 4°C. Sodium hydroxide (0.1N) was added slowly to zinc nitrate under vigorous stirring till a milky white precipitate was obtained. The addition of sodium hydroxide was carried in a magnetic stirrer at 500rpm. The solution was centrifuged at 5000 rpm for 5 minutes and the pellet was collected and washed thrice using nano pure water. The pellet was dissolved in absolute ethanol and evaporated to dryness. The dried powder was calcinated at 400°C for 3 hrs and stored. The UV-Vis absorption spectrum of the ZnO NPs was analysed by UV Spectrophotometer. The distribution of ZnO NPs was recorded in Particle size Analyser (PSA). The surface characteristics of ZnO NPs were recorded in FESEM.

2.3 Susceptibility of isolates to antibiotics and ZnO NPs
Agar well diffusion method was used to evaluate the antibacterial activity of all test compounds against the wound isolate[16]. The organism was grown overnight at 37°C in a shaker at 150 rpm. 100μl of culture (10⁸ CFU/mL) was swabbed onto Mueller and Hinton agar plate. Wells of 9 mm size was punctured onto the Mueller and Hinton agar and ZnO NPs (500 μg/ml) was loaded onto the well. The plate was incubated for 24 hours at 28°C. The inhibition zone was measured. Antibiotic discs were used to test the susceptibility of the wound isolate. Antibiotics from various classes like aminoglycoside (amikacin), amoxyclav, ampicillin, ampicillin/sulbactam, aziocillin (ampicillin) were used in the study. The organism was designated as resistant, susceptible and intermediately susceptible to antibiotics based on the CLSI standards[17].

3. Results and Discussion
3.1 Isolation and identification of wound pathogens
In the present study, the bactericidal activity of ZnO NPs was evaluated against the pathogen isolated from burn patient suffering from diabetic foot ulcer. Many studies evaluated the efficacy of antibacterial agents against quality control strains purchased from American Type Culture Collection or Microbial Type Culture Collection. Studies focused on the assessment of antimicrobial agent against clinical isolates represent the original status of resistance in clinical units. Among the clinical isolates, pathogens from diabetic ulcers are highly resistant to drugs. Diabetic conditions provide nutrients for the growth of pathogens. Higher microbial infection requires prolong antibiotic therapy. Inappropriate use of antibiotics or sub therapeutic dose of antibiotics leads to the development of resistance. Identification of pathogen is important to decide the choice and the duration of antibiotic therapy. The technique used for pathogen identification has to be provide accurate results in short time. In the current study, pathogen isolated from diabetic wound was identified by MALDI-TOF. The isolate was identified as E. coli. Mass Spectrum of the isolate is presented in Figure 1. One of the predominant pathogen present in wound is E. coli[18].
MADI-TOF has an advantage of clearly discriminating the closely related species [19]. MALDI-TOF is relatively economical and provides 99.1 to 99.4% accuracy [20].

3.2. Synthesis and characterization of ZnO NPs
Size distribution of ZnO NPs is represented in Figure 2. It revealed the homogenous distribution of ZnO NPs. Optical property of ZnO NPs in UV-VIS region is presented in Figure 3. It revealed the maximum absorbance at 372nm. Similar observations are reported in literature [21–23]. The morphology of the ZnO NPs is depicted in Figure 4. It showed spherical shape, within range of 40-70nm. The lower size range is due to low concentration of zinc nitrate used in the synthesis of ZnO NPs and slow growth of nanoparticles. Energy Dispersive X-Ray Analysis (EDX) spectrum presented in Figure 5 shows the purity of the ZnO NPs.

Figure 1 Mass Spectrum of the isolate

Figure 2 Size of distribution of ZnO NPs

Figure 3 UV-VIS absorption spectrum of ZnO NPs
3.3. Susceptibility of wound isolates to antibiotics and ZnO NPs

The susceptibility of E. coli to various antibiotics and ZnO NPs is shown in Figure 6. The inhibition zone was 14 mm with ampicillin/sulbactam, 12 mm with ampicillin, 0 mm with aziocillin, 16 mm with amikacin and 0 mm with amoxiclav. From the results, it was confirmed the multi-drug resistance of E. coli. Generally, resistance to antibiotics are developed by preventing the entry of drugs into the cell by decreasing the permeability, expelling the drug out of the cell by efflux pump, inactivating the antibiotic or by the modification of target[24]. Most of the Gram-negative organisms develop resistance to β-lactam antibiotic by the synthesis of β-lactamase. As the pathogens are rapidly developing resistance to more than one class of antibiotic, it is inevitable to use other materials to control infection. There is another area of research where the potency of the antibiotics is increased by using with other compounds which works in synergy. ZnO NPs is one among those which can increase the efficiency of antibiotics[25,26].
Figure 6 Susceptibility of wound isolates to antibiotics and ZnO NPs

Inhibition zone exhibited by the ZnO NPs was 25±2 mm. ZnO NPs are well known for their antibacterial activity \([27-30]\). It is proposed that ZnO NPs produce reactive oxygen species and damages the integrity of the bacterial cell membrane. Loss of membrane integrity causes cell lysis and leakage of cellular constituents. Surface interaction of ZnO NPs leads to the production of hydrogen peroxide which is lethal to the bacteria \([8,31]\).

Figure 7: Susceptibility of \(E. \text{coli}\) to antibiotics

Figure 8: Antibacterial activity of ZnO NPs to \(E. \text{coli}\)

From the above observations it is concluded that the ZnO NPs was potent in controlling the multi drug resistant pathogens.

4. Conclusion
The clinical isolate \(E. \text{coli}\) was resistant to multiple class of antibiotics. The multi drug resistant \(E. \text{coli}\) was susceptible to ZnO NPs. Thus, the study demonstrated the potent bactericidal activity of ZnO NPs against clinical multi drug resistant pathogen.

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