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Toxicity mechanism of titanium dioxide and zinc oxide nanoparticles against food pathogens

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ABSTRACT

Food preservation is an important field of research. It extends the shelf life of major food products. Our current study is based on food preservation through TiO_2 and ZnO nanoparticles. TiO_2 and ZnO are biocompatible nanomaterial. The biocompatibility of the materials were established through toxicity studies on cell lines. Titanium dioxide and Zinc Oxide nanoparticle were synthesized by wet chemical process. They are characterized by X-Ray diffraction and TEM. The antibacterial activities of both the materials were analysed to ensure their effectiveness as food preservative against *Salmonella typhi*, *Klebsiella pneumoniae* and *Shigella flexneri*. The results indicates that TiO_2 and ZnO nanoparticle inhibits *Salmonella*, *Klebsiella* and *Shigella*. The mode of action is by the generation of ROS in cases of *Salmonella*, *Klebsiella*. Mode of action in *Shigella* is still unclear. It was also proved that TiO_2 and ZnO nanoparticle are biocompatible materials.

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1. Introduction

Preservation of food has been a great challenge to mankind. People tried to preserve food by inactivation of the spoiling microorganisms. The food preservation techniques includes drying, salting, heating or fermentation [1]. Prolonged use of antibiotics had let to the emergence of resistant microorganisms. So the antibiotics are banned as a food preservation agent [2]. So we need to develop an antimicrobial agent which is effective and less harmful to human beings with less or no side effects. Nanoparticles have shown more positive results towards food preservation.

Nanomaterials are an increasingly important product of nanotechnologies. Nanoparticles are normally defined as being smaller than 100 nanometres in at least one dimension. Nanomaterials are coming into use in healthcare, electronics, cosmetics, textiles, information technology and environmental protection. Chemical and physical properties of the food materials can be changed and improved by nanomaterials. It is been incorporated in food processing, food packaging and in the production of functional foods etc [3]. Nanomaterials had been used in food preservation for a long time. The mechanical and heat resistant properties of the food packing materials can be altered with titanium dioxide, silver, silicone dioxide. Nanomaterial coating will form an antimicrobial surface. This increases the shelf life of the food products [4].

Various nanoparticles like Copper, Aluminum, Gold, Silver and metal oxides like Zinc Oxide, magnesium oxide titanium dioxide exhibits good antibacterial activities [5]. Nanoparticles are more effective than antibiotics. They kill more microorganisms than antibiotics [6]. Nanomaterials will overcome the problem of antibiotics resistance, which is usually produced by excessive usage of antibiotics. They target the bacteria at different stages of metabolism and hence it becomes difficult for bacteria to develop resistance [7].

Metal oxides nanoparticles such as titanium oxide, magnesium oxide, zinc oxide and copper oxide are cheaper than nanosilver. ZnO nanoparticles are more efficient as antibacterial agent than bulk powder. ZnO nanoparticles also have antibacterial activity against spores that are high temperature and high-pressure resistant. Metal oxides such as TiO_2, ZnO and MgO are used for the preparation of antimicrobial packaging films due to their strong antimicrobial activity. ZnO nanoparticles exhibited antibacterial activity against Gram positive and Gram negative bacteria. TiO_2 is inert non-toxic,

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cheap, eco-friendly. It has antibacterial activity against a wide variety of bacteria [8,9].

Titanium dioxide has been used to disinfect medical devices. It is used in pharmaceutical preparations. It is also used in paints. It is used for drug delivery and targeted drug delivery applications [10,11]. Titanium dioxide is a biocompatible material [12]. It is used to purify water [13]. Titanium dioxide has good antibacterial activity. Titanium dioxide nanoparticles will produce free radicals like superoxide, singlet oxygen which interacts with bacteria and kills them. The production of free radicals is the main mechanism behind its antibacterial activity [6]. Titanium dioxide though inactivates microorganism, it is found to be more effective only on irradiation [14]. Zinc Oxide been given GRAS (Generally Regarded as Safe) status by the U.S. Food and Drug Administration (21CFR182.8991) for being used as a food additive [15]. Zinc oxide has gained more importance because it is stable under harsh processing conditions [16]. The antibacterial mechanism of zinc oxide may be due to release of free radicals, cell membrane disruption or protein degradation etc. To make the best use of the nanoparticle, the underlying mechanism of its activity has to be well established [17].

In nanomaterials there is a change in surface chemistry and increase in chemical reactivity. Interaction mechanisms between nanoparticles and living systems are complex. Nanomaterials bind and interact with biological matter and change their surface characteristics. Nanoparticles, have the ability to enter, translocate within, and damage living organisms. The toxicity of these materials depends greatly upon the arrangement of its atoms. Some nanoparticles can produce irreversible damage to cells. After inhalation and through oral exposure, NPs are distributed to the liver, heart, spleen, and brain in addition to lungs and gastrointestinal tract. During metabolism, some of the NPs are congregated in the liver tissues. NPs are more toxic to human health in comparison to large sized particles of the same chemical substance, and it is usually suggested that toxicities are inversely proportional to the size of the NPs [18–20].

In the present study, the Zinc oxide and Titanium dioxide nanoparticles were synthesized and characterized. Their antibacterial activity, mechanism of inhibition against food pathogens and cytotoxic effect are analysed.

2. Materials and methods

2.1. Synthesis of titanium dioxide nanoparticles

A 7.4 ml of Titanium tetraisopropoxide was added, drop by drop, to 30 ml of 1 M HNO_3 aqueous solution, and then agitated for 2 h to give a transparent sol, in which 2.0 g TiO_2 are contained. The pH of the colloidal solution was adjusted to pH 3, with the addition of 1 M NaOH solution after dilution of the colloid with 100 ml water, resulting in a turbid TiO_2 colloid. The suspension was agitated at room temperature, centrifuged and then washed with distilled water. The isolated TiO_2 was dried for 1 h at 100° C in air. The resulting powder was then calcinated at 450° C for 3 h [21].

2.2. Synthesis of zinc oxide nanoparticles

$ZnAC_2 \cdot 2H_2O$ and $(NH4)_2CO_3$ were dissolved in high-purity water to form solutions with certain concentrations, respectively. The two solutions were slowly dropped into the vigorously stirred polyethylene glycol (PEG) solution (5% (w/w), water solution). The precipitate was collected by filtration and rinsed three times with high-purity water and ethanol, respectively. Then the precipitate was dried at 100 °C under vacuum for 12 h to have the precursor ready. Afterwards the precursor was calcinated at 450 °C for 3 h to obtain the ZnO [22].

The phase purity of the synthesized NPs was recorded using powder X-ray diffractometer (Seifert, JSO-DE BYEFLEX 2002, Germany).The average size and size distribution of the synthesized NPs were determined by Transmission Electron Microscopy (TEM). The sample was scanned at 200 kV (JEOL 2000Fx-II, Tokyo, Japan) high Resolution, analytical TEM with a W-source and a point–point resolution of 2 Å. The functional groups were analysed by Fourier transform infrared spectroscopy using (Perkin Elmer Spectrum One Spectroscopy, Branford, CT, USA).

2.3. Antibacterial assay

The antibacterial activity of TiO_2 and ZnO-NPs was tested against four implant associated pathogens including *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Salmonella typhi* by micro-broth dilution assay, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) using Muller-Hinton broth (HiMedia, Mumbai, India). TiO_2 and ZnO-NPs were serially diluted to obtain the final concentration ranges from 500 to 39 μg/ml in a sterile titer plate. 10 μl suspension of inoculum whose density was adjusted to that of 0.5 McFarland standards (approximately 1 to 2×10^8 CFU/ml) were added and incubated at 37 °C for 24 h. The MIC values were read at 630 nm in an ELISA Reader (Robonik Readwell plate ELISA Analyzer, India) and MIC_{50} values were calculated.

2.4. Growth curve profiling

To examine the bacterial growth curve in presence of TiO_2 and ZnO-NPs, the test organisms were grown in Luria-Bertani (LB) media supplemented with MIC_{50} concentration of NPs. Subsequently, 2×108 CFU/ml of test organisms were added to the above broth as inoculum and all the flasks were put on shaker incubator (180 rpm) and incubated at 37 °C. The control group was maintained without NPs. The bacterial growth was indexed by measuring optical density at every 2 h (up to 20 h) at 600 nm using spectrophotometer (Shimadzu UV-Spectrophotometer UV-1601).

2.5. Determination of Reactive oxygen species (ROS)

Generation of intracellular ROS was measured by the oxidation-sensitive fluorescent probe using DCFH-DA (Sigma-Aldrich). The bacterial cells were incubated for a period of 3 h with the nanoparticles. 1 ml of each culture was pelleted by centrifugation at 5000*g* for 5 min and resuspended in PBS containing 30 μg/ml DCFH-DA at 37 °C for 30 min in the dark. As positive control cells were treated with 10 μM H_2O_2 for 30 min added prior to the DCFH-DA and untreated cells were used as negative control. The cell suspension was transferred to 96- well plate and fluorescence was measured at an excitation wavelength of 480 nm and emission wavelength of 520 nm using a fluorescence multiwell plate reader (Perkin Elmer).

2.6. DNA fragmentation analysis

All the bacterial cultures were incubated for 24 h in Luria-Bertani broth supplemented with the MIC_{50} concentration of the nanoparticles. DNA was extracted by resuspending the cell pellet in 200 μl of TEG buffer, 40 μl of 10% SDS and 5 μl of Proteinase K (20mg\ml). The mixtures were then incubated at 56 °C for an hour. An equal volume of phenol/chloroform/isoamylalcohol (25:24:1,v/v/v) was added to the mixture and centrifuged for 10 min at 13,000g at 4 °C. The aqueous layer was digested with 800 μl of RNase (10 mg/ml) and DNA was precipitated from the aqueous layer by adding two volumes of absolute ethanol and 400 μl of 0.1 M sodium acetate. The resulting pellet was rinsed twice with 70% ethanol, air-dried, and dissolved in 1 ml of TE buffer.

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