

Functional Zinc Oxide Nanocrystals for Photodynamic Therapy: A Brief Review

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Abstract

The emergence of cancer nanomedicine is the result of fruitful advances in the fields of nanotechnology, bioimaging, formulation development, and molecular biotechnology. Zinc oxide nanoparticles (ZnO NPs) are able to accumulate specifically within a tumor region thanks to the enhanced permeation and retention effects. However, the photodynamic therapy (PDT) shows limited tissue penetration owing to the small penetration depth of ultraviolet light used to excite the NPs. Thus, PDT can be efficiently exploited for superficial tumors or with optical waveguide irradiation in the case of deeper but accessible cancer tissues. The role of ZnO NPs in these multifunction nanoplateforms and biomedical applications as well as the recently emerged PDT are discussed.

Keywords: Zinc oxide nanoparticles; Cytotoxicity; Photodynamic therapy

The n-type semiconductivity of ZnO is due to the deviation from stoichiometry and the presence of intrinsic defects such as oxygen vacancies (V_O), zinc interstitials (Zn_i), and zinc vacancies (V_{Zn}). Interestingly, the doping of ZnO provides a method for controlling its structure, and consequently electrical, magnetic, and optical properties [1, 2], which leads to a ranges of changes, including band gap value, transparency, room-temperature ferromagnetism, piezoelectricity and magneto-optical properties [3]. Transition metal doping in ZnO crystal lattice is one of the well-known strategies for tuning the band gap of ZnO to enhance its range of absorption from UV to visible light [4, 5], and can modify the morphology, particle and crystallite size of intrinsic ZnO [2]. The introduction of Ag in ZnO nanostructures have received increasing attentions for productions of UV-visible light absorption materials. When Ag is doped in the ZnO crystal lattice, Ag ions acts as an electron acceptor inside energy level of band gap in ZnO material [6]. Therefore, it can be used to generate p-type conductivity in pristine. Moreover, introducing Ag creates acceptor

levels inside the ZnO energy band, and as a result, decreases the E_g value. However, according to results in some cases, Ag-doped ZnO shows n-type conductivity behavior. In addition, they considered 2.08 at.% Ag dopant concentration, which contained one Ag substitution at an interstitial sites (Ag_i), one Ag substitution at an O sites (Ag_O) and one Ag substitution at a Zn site (Ag_{Zn}). As the result, the Ag dopant prefers to substitute on the Zn site under both O-rich and Zn-rich condition. Thus, high concentrations of the Ag_{Zn} and Ag_O would be effective in making ZnO a visible-light active photocatalysts. Due to a larger radius of Ag^+ than Zn^{2+} , Zn ions may be hardly substituted by Ag ions. Therefore, more incorporation of Ag in ZnO than a critical value leads to the aggregation of Ag atoms at the grain boundaries and formation of separated particles and clusters [7-9]. Nanocrystalline Ag-doped ZnO powders was synthesized via a precipitation method at low temperature with various concentrations of Ag precursors [7]. It was found that when the Ag loading is less than or equal to 0.5 mol%, Ag ions introduce in ZnO lattice by substitution on Zn^{2+} sites. But, if the Ag doping increases more than this critical values, from 0.6-1.0 mol%, Ag atoms bond together and form Ag clusters at the ZnO grain boundaries. Shinde *et al.* [10] approved that when Ag doping exceeds the optimum value, electron mobility of ZnO films decreases due to enhancement in the film disorder. Therefore, at a high doping concentration, the Ag-substituted Zn^{2+} ions act as electron acceptor levels, which compensate the donors and decrease the conductivity and photocatalytic of ZnO films.

For biomedical applications of ZnO, Methicillin-Resistant Staphylococcus Aureus (MRSA) commonly linked to both hospital-associated infections and new community-acquired strains, which is brought by a variety of disease-causing bacteria, such as *Enterococcus*, *Staphylococcus*, and *Streptococcus*, have been thought to be a serious threat to public health worldwide [11]. Therefore, new strategies need to identify and develop next generation of drugs or agents to control bacterial infections, bacteriostatic, antimicrobial, or biocidal action [12]. For example, Hirota *et al.* [13] have fabricated ZnO ceramics starting from fine ZnO powders which were hydrothermally treated. Sustainability in antibacterial activity was evaluated using a colony count method with *E. coli* bacteria on nutrient agar medium in a Na-P-buffer solution. Antibacterial activity (f) was defined as the following equation: $f = -\log(N/N_0)$, where N_0 (10^7) the number of colony before the addition of ZnO. f was found to be equal to seven indicating all the colonies were perfectly disappeared after the antibacterial activity test. Thus, ZnO exhibits an antibacterial activity even under the dark condition [14]. Premanathan *et al.* [15] investigated the toxicity of ZnO NPs toward prokaryotic and eukaryotic cells. Antibacterial activity of ZnO was tested against the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, and the gram positive bacteria *Staphylococcus aureus*. The effect was more pronounced with the gram-positive than the gram-negative bacteria. Furthermore, cytotoxicity of ZnO to mammalian cells was also studied by using human myeloblastic leukemia cells (HL60) and normal peripheral blood mononuclear cells (PBMCs). ZnO NPs

exhibited a preferential ability to kill cancerous HL60 cells as compared with normal peripheral blood mononuclear cells (PBMCs), and also enhanced ultrasound-induced lipid peroxidation in the liposomal membrane.

The numerous reports of the unusually high cytotoxicity of ZnO NPs and their widespread uses in various applications cause significant environmental and health concerns. This calls for a detailed investigation to identify the specific material features and properties that contribute to their strong cytotoxic effects. This information will also help researchers design new synthetic approaches to produce safer ZnO NPs with minimal cytotoxicity. However, only a few studies have attempted to make safer ZnO NPs by design. George *et al.* [16] developed safer ZnO NPs prepared by using a flame spray pyrolysis process that showed significant reduction in their cytotoxicity by doping with Fe ions. The lower of the Fe-doped ZnO NPs was attributed to improve ZnO bond strength and reduced NPs dissolution. Another group recently reported a safer-by design concept that involved hermetic encapsulation of ZnO nanorods in biologically inert, nanothin, amorphous SiO₂ coating using a modified flame spray pyrolysis synthesis [17, 18]. The SiO₂ coating provided a 3-fold reduction in their DNA damage, while preserving their optoelectronic properties. Both approaches involved the introduction of foreign metal or metal oxide components, and these methods were limited to NPs synthesis in gas phase.

Ancona *et al.* [19] proposed the synthesis and characterization of lipid-coated ZnO NPs as new photosensitizer for PDT against cancer. The phospholipid bilayer coating induced the photo-generation of short chain carbon centered free radicals, thus, the NPs surface chemistry plays a crucial role in determining the type of photo-generated free radicals. Moreover, lipid-coated NPs are effectively internalized by HeLa cells through an endosomal-lysosomal pathway which can generate ROS even once internalized and kill cancer cell at non-toxic concentration under UV-stimuli activation. On the other hand, Ahamed *et al.* [20] investigated the anticancer potential of Ag-doped (0.5-5%) anatase TiO₂ NPs by sol-gel process. Biological studies showed that Ag-doped TiO₂ NPs induced cytotoxicity and apoptosis in human liver cancer (HepG2) cells. The toxic intensity of TiO₂ NPs was increased with increasing the amount of Ag-doping. It was further found that the Ag-doped TiO₂ NPs provoke ROS generation and antioxidant depletion. Toxicity induced by Ag-doped TiO₂ NPs in HepG2 cells was efficiently abrogated by antioxidant N-acetylcysteine. Ag-doped TiO₂ NPs also induced cytotoxicity and oxidative stress in human lung (A549) and breast (MCF7) cancer cells. Moreover, these NPs have potential to selectively kill cancer cells while sparing normal cells. In terms of antibacterial activity of Ag-doped TiO₂ and Ag-doped ZnO NPs, Nigussie *et al.* [21] reported that the doping of Ag on ZnO and TiO₂ by sol-gel method play a vital role in the increased antibacterial activity performance. The reduction in the viability of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* bacteria to zero using Ag-doped ZnO NPs occurred at 60 µg/mL of culture, while Ag-doped TiO₂ showed zero viability at 80 µg/mL.

Therefore, several studies have proposed the photoexcitation of nanostructured ZnO to produce intercellular ROS as an effective therapeutic strategy or PDT, causing severe toxicity in different cancer cell line. This therapy promises better selectivity and fewer side-effect compared to most traditional chemo-and radio-therapies. ZnO NPs can indeed accumulated specifically within tumor region thanks to the enhanced permeation and retention effect [22]. However, the PDT shows limited tissue penetration owing to the small penetration depth of UV light (less than 1 mm) used to excite the NPs. Thus, PDT can be efficiently exploited for superficial tumors or with optical waveguide irradiation in the case of deeper but accessible cancer tissues.

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