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Biomedical Applications of Zinc Oxide Nanomaterials in Cancer Treatment: A review

Satinder Pal Kaur Malhotra and T. K. Mandal

Faculty of Science and Technology

ICFAI University

Rajawala Road, Selaqui

Dehradun-248197, India

E Mail: dr.mandal@iudehradun.edu.in

Abstract

Zinc oxide (ZnO) is a bio-safe material that possesses photo-oxidizing and photocatalysis impacts on chemical and biological species. ZnO holds a unique optical, chemical sensing, semiconducting, electric conductivity and piezoelectric properties. One of the most important features of ZnO nanomaterials is low toxicity and biodegradability. ZnO nanoparticles (NPs) is a low-cost, low-toxic, and versatile material, have shown to have a promising future in biological applications. Specific properties and characteristics of ZnO NPs, such as their inherent toxicity against cancerous cells, at least for cells of lymphocytic origin, their ability to induce intracellular reactive oxygen species (ROS) generation leading to death via an apoptotic mechanism, and their physiochemical properties leading to cellular uptake and ease of functionalization make them an appealing candidate for biomedical applications. In this review, the current status of the use of ZnO nanomaterials for biomedical applications, such as biomedical imaging, drug delivery, gene delivery and cancer therapy has been addressed.

Keywords: Zinc oxide nanomaterial, cancer treatment, biomedical imaging, drug delivery, gene delivery, biomarker mapping.

Introduction

The development of biocompatible, biodegradable, and functionalized nanomaterials for biomedical applications has been an extremely vibrant research area. Zinc oxide (ZnO) possesses unique semiconducting, optical, and piezoelectric properties [1,2]. Therefore, ZnO-based nanomaterials have been studied for a wide variety of applications such as nano-electronic/nano-optical devices, energy storage, cosmetic products, nanosensors, etc [3-8]. ZnO is a wide band gap semiconductor (3.37 eV) with high exciton binding energy (60 meV), which leads to efficient excitonic blue and near-UV emission [9]. ZnO nanomaterials have been used as semiconductors in microelectronic devices and for accelerating degradation of water pollutants via photocatalytic activity. Due to its inherent ability to absorb UV irradiation and optical transparency, ZnO nanoparticles are used in the cosmetic industry, typically in sunscreens and facial creams [10,11]. The use of ZnO in sunscreens has been approved by the food and drug administration (FDA) due to its stability and inherent capability to absorb UV irradiation. Their recognized antibacterial properties are also encouraging a variety of antimicrobial applications. ZnO nanoparticles have gained interest in other biomedical applications based on their high stability, inherent photoluminescence properties which can be useful in biosensing applications. The use of ZnO in sunscreens has been approved by the FDA due to its stability and inherent capability to absorb UV irradiation.

One of the most important features of ZnO nanomaterials is low toxicity and biodegradability. Zn^{2+} is an indispensable trace element for adults and it is involved in various aspects of metabolism. Chemically, the surface of ZnO is rich in -OH groups, which can be readily functionalized by various surface decorating molecules [12,13]. ZnO can slowly dissolve in both acidic (e.g. in the tumor cells and tumor microenvironment) and strong basic conditions if the surface is in direct contact with the solution [14]. Based on these desirable properties, ZnO nanomaterials have gained enormous interest in biomedical applications. In this review, we will summarize the current status of the use of ZnO nanomaterials for biomedical applications, such as biomedical imaging, drug delivery, gene delivery and cancer therapy. The potential use of ZnO nanoparticles in biomedical and cancer applications is gaining

interest in the scientific and medical communities, largely due to the physical and chemical properties of this nanomaterial, and is the focus of this article.

ZnO NANOPARTICLE PROPERTIES: USEFUL FOR BIOMEDICAL AND CANCER APPLICATIONS

Although ZnO nanoparticles have been used in the cosmetic industry for many years, they have only recently been explored for use in cancer applications or as active drugs themselves. ZnO nanoparticles are now being widely researched for their anticancerous properties. Some of the characteristic features of ZnO nanoparticles behind their surge in anticancer therapy are described below.

Biocompatibility

ZnO nanoparticles show relatively high biocompatibility. Their bulkier form is generally recognized as safe (GRAS) by the FDA making them reasonable choices for drug delivery. Zinc is an important co-factor in various cellular mechanisms and plays an important role in maintaining cellular homeostasis; hence ZnO shows biocompatibility. The administered ZnO can be easily biodegraded or can take part in the active nutritional cycle of the body [15].

Easy synthesis

The synthesis process of ZnO nanoparticles is relatively easy, with a wide variety of methods. Owing to these different methods of synthesis, their size and size distribution can be easily controlled. Research has shown that the size of nanoparticles is directly proportional to the toxicity they show; in addition, size manipulation is significant for producing greater EPR effect to increase intra-tumour concentration of nanoparticles [16].

Selectivity

One of the primary advantages for considering ZnO nanoparticles for use in cancer is the inherent preferential cytotoxicity against cancer cells in vitro compared with other nanoparticles. [17,18]. It is anticipated that their cancer cell selectivity may be even further improved by engineering design to minimize harmful effects to normal body cells, which has been observed to occur at very high concentrations of ZnO nanoparticles, particularly those in the smaller size range of 4–20 nm [19].

High surface area to volume ratio

The electrostatic characteristics of ZnO nanoparticles are another useful feature for biomedical applications. Zinc oxide nanoparticles typically have neutral hydroxyl groups attached to their surface, which plays a key role in their surface charge behavior [19,20]. In aqueous medium and at high pH, the chemisorbed protons (H^+) move out from the particle surface leaving a negatively charged surface with partially bonded oxygen atoms (ZnO^-). At lower pH, protons from the environment are likely transferred to the particle surface, leading to a positive charge from surface $ZnOH_2^+$ groups. The isoelectric point of 9–10 [21] indicates that ZnO nanoparticles will have a strong positive surface charge under physiological conditions. Given that cancer cells frequently contain a high concentration of anionic phospholipids on their outer membrane and large membrane potentials [22,23] interactions with positively charged ZnO nanoparticles are expected to be driven by electrostatic interactions, thereby promoting cellular uptake, phagocytosis and ultimate cytotoxicity. In this regard, the surface chemistry of ZnO nanoparticles readily lends them to functionalization with targeting proteins or chemical groups, and may be a key to rendering them benign to normal cells while still retaining their cancer targeting and killing properties.

Key properties of nanoparticles

Size

The size of nanoparticles, which is comparable to naturally occurring biological molecules, is another feature that makes them well suited for biological applications. Their nanoscale size allows their internalization into cells, and allows them to interact with biomolecules within or on the cell surface, enabling them to potentially affect cellular responses in a dynamic and selective manner. Nanoparticles can interact with biological molecules and manipulate various cellular cycles, disrupting cellular homeostasis and inducing apoptosis, due to their small size, which cannot be checked by the plasma membrane. The size of nanoparticles can facilitate their entry into tumor tissues, and their subsequent retention, by a process recognized as the enhanced permeation and retention (EPR) effect. Studies demonstrate that the cytotoxic properties of ZnO nanoparticles against cancerous cells is directly related to size, with smaller nanoparticles exhibiting greater toxicity [24,25,26].Tissue resident macrophage in the liver and spleen rapidly clears most particles entering into blood vessels. A blood protein called ‘opsonins’ is adsorbed in any foreign particles entering blood and macrophage

targets these adsorbed opsonins. Research has suggested that size is related to blood circulation time of particles. The smaller the size, the more the blood circulation time. Another important consideration is that hydrophilic nanoparticles of 100 nm size or less tend to remain in circulation considerably longer and are more likely to avoid clearance by macrophages and rapid serum clearance by the reticuloendothelial system [27].

Shape and morphology

The overall shape and morphology of the nanomaterial is another important consideration for biomedical applications. ZnO nanowires have been shown to be biodegradable and to eventually dissolve into ions that can be adsorbed by the body and become part of the nutritional cycle, and thereby proposed for in vivo biosensing and biodetection applications [28]. The ability to synthesize ZnO into hollow nanotube-type structures [29,30] also makes them reasonable choices for drug delivery, particularly slow drug release applications.

Dissolution

Studies have recorded some degree of toxicity from ZnO nanoparticles in a wide array of organisms including bacteria, macroalgae, yeast, protozoa, zebrafish, and mice [31-34]. Some of this toxicity has been attributed to the potential dissolvability of ZnO nanoparticles into free Zn^{2+} ions [35,36,37]. Under normal conditions, the cell has a relatively high concentration of zinc bound to various proteins, while the level of free Zn^{2+} ions remain very low and tightly regulated by homeostatic mechanisms [38,39]. Excess zinc can be harmful, however, with intracellular zinc accumulation implicated in neuronal toxicity and brain injury [40]. Excess zinc consumption or inhalation has also been shown to cause ataxia and metal fume fever, respectively [41]. For instances where appreciable nanoparticle dissolution can occur, such as in acidic environments including intracellular lysosomal compartments, hydrated zinc ions in conjunction with intact ZnO nanoparticles, are suggested to lead to mitochondrial damage and disruption of cellular zinc homeostasis leading to cell death.

Enhanced cytotoxicity

While extracellular ZnO shows biocompatibility, elevated levels of administered intracellular ZnO show enhanced cytotoxicity through zinc-mediated protein activity disequilibrium and oxidative stress [42]. ZnO nanoparticles have the unique ability to induce oxidative stress in cancer cells, which has been found to be one of the mechanisms of cytotoxicity of ZnO nanoparticles towards cancer cells. This property is due to the semiconductor nature of ZnO.

ZnO induces ROS generation, leading to oxidative stress and eventually cell death when the anti-oxidative capacity of the cell is exceeded [43].

ZnO NANOTHERAPEUTICS DRUG DELIVERY

ZnO quantum dots: A smart drug delivery nanocarrier

In many cases, the malignancy of tumors is detected only at advanced stages when chemotherapeutic drugs become increasingly toxic to healthy cells. To improve this condition, both targeted drug delivery [44] and early detection of cancer cells need to be extensively investigated [45**].** Tumor targeting drug delivery systems generally combine a tumor recognition moiety such as folate receptors that are overexpressed on tumor cells with a drug loaded vesicle [46-49**]**. However, current anticancer chemotherapies often show toxic adverse effects and low efficacy due to the failure to differentiate between cancerous and normal cells by the drug itself, as well as the development of drug resistance. Drug delivery systems (DDSs) based on nanotechnology exhibit great potential in anticancer treatment and have been employed to deliver anticancer drugs to the target tissues. Considering the extracellular mildly acidic environment in the solid tumor tissues and the intracellular compartments such as endosomes and lysosomes [50,51] NPs were designed to enter cells through the cellular endocytic pathway and fuse with lysosomes [52] so that a pH-responsive DDS will be an ideal choice for cancer therapy. Fortunately, ZnO NPs can exhibit great stability in physiological condition (pH 7.4), but rapidly dissolve at pH 5~6. ZnO NPs were used as cappers to cover the pores of mesoporous silica NPs (MSNs), and when these DDS met with acids, the ZnO NPs decomposed to release doxorubicin (DOX) molecules from MSNs [53]. However, this type of DDS has difficulty in degradation so that it cannot completely release the drugs [54,55]. Another strategy is based on loading drugs onto the simple ZnO NPs directly, and thus, when the composites meet acid the ZnO NPs decompose completely to release all the drug molecules [56]. The corresponding drug delivery mechanisms is shown in. Figure. 1. Although the stabilized ZnO NPs revealed good biocompability and low toxicity, after ZnO decomposition, Zn^{2+} ions are cytotoxic [57], so they were used directly for cancer treatment [58] or enhancing the DDS toxicity.

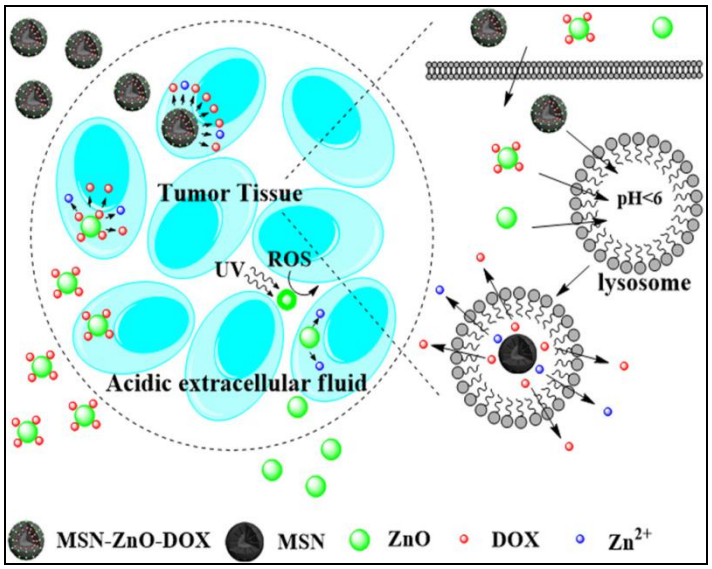

Figure. 1. Schematic illustration of the DOX delivery from the ZnO NPs.

In recent years, ZnO quantum dots have been investigated as multifunctional smart drug delivery nanocarriers due to their low toxicity. The "ZnO-chitosan-folate" system can be used as a nanocarrier for delivery of doxorubicin (DOX), an antineoplastic agent used in tumor treatments, through physical and chemical interactions [58]. The rate of drug release from the nanocarrier depends on several factors, including pH, particle size, surface properties, degradation rate, interaction force of drug binding to the surface and rates of hydration and dehydration of the polymers [59,60]. Conventionally, drugs are loaded into the nanoparticle via weak interactions, e.g. physical adsorption, electrostatic interaction, and p–p stacking and, as a result, the release of drug is achieved by breaking of these interactions. The presence of folic acid in the ZnO-QD-chitosan-folate carrier weakens the electrostatic interaction between DOX and ZnO QDs accounting for the release of DOX.

In another work, ZnO QDs have also been evaluated as a platform for targeted and pH responsive intracellular delivery of DOX [61]. Herein, acidic conditions cause rapid dissolution of ZnO QDs by disfavoring the reaction between Zn^{2+} and DOX, resulting in the release of DOX molecules to the cytosols, killing the cancer cells. Hence, this approach provides a valuable ZnO QDs-based nanovector that can simultaneously realize targeting, diagnosis, and therapy of cancer cells. Recent work [61,62] points towards the application of water dispersed ZnO quantum dots with long term fluorescence stability in the design of new drug release carriers.

Biological Imaging by ZnO NPs: Gd doped ZnO quantum dots: Excellent magnetic resonance and fluorescence imaging (MRI-FI) nanoprobes

Magnetic resonance imaging (MRI), one of the most important noninvasive imaging techniques, has been widely used in radiology to visualize detailed internal structures. This technique has certain advantages, such as deep penetration into tissue, providing anatomical details and high quality three dimensional images of soft tissue in a non-invasive monitoring manner, [63,64] but lower sensitivity and its inability to resolve objects larger than a few micrometers in size makes this technique less beneficial for bio-medical application. On the contrary, the fluorescence imaging (FI) technique promises higher sensitivity and the potential for real-time imaging, but the major drawback of this technique is the limited spatial resolution, making it difficult to translate two-dimensional information to the three-dimensional surgical field [65,66]. Therefore, it was suggested that the limitations associated with both the techniques can be effectively overcome by integrating magnetic resonance and optical imaging functionalities into a single nanostructure [67]. Several different strategies have been directed to developing MRI-FI nanoprobes due to their prominent advantages for medical diagnosis, such as paramagnetic ions doped quantum dots [68,69,70] and silica encapsulated quantum dots [71]. However, encapsulation by silica shell may lead to difficulty in single probe detection, as this strategy increases the particle size, which is not suitable for labeling functional subcellular proteins [72,73]. A simple and versatile method was used to develop excellent as dual modal fluorescence and magnetic resonance imaging (MRI-FI) nanoprobes by doping Gd ion in ZnO QDs [74]. Gd-doped ZnO QDs (with sizes of < 6 nm) were developed for both optical and MRI. These nanoprobes are T_1 positive contrast agents that provide better reliability for clinical diagnosis byfulfilling modern medical criteria such as (a) high relaxivity and quantum yield, (b) small size, (c) simple cost-effectiveness, (d) low toxicity, and (e) chemical stability in air for optimal therapy. The nanoprobes, with exceptionally small size and enhanced fluorescence resulting from Gd doping, are ideally suitable for biological and medical fields Figure. 2. It was found that the emission intensity of the Gd-doped ZnO QDs increased with increasing concentration of Gd^{3+}, with maximum emission intensity at 550 nm. Upon surface coating with N-(2-aminoethyl) aminopropyltrimethoxysilane (AEAPS), the resulting Gd-doped ZnO QDs exhibited low toxicity to HeLa cells and could be imaged with both confocal microscopy and MRI in vitro. They can successfully label HeLa cells in a short time and show no evidence of toxicity on cell growth, even at concentrations up to 1 mM, especially in comparison with the traditional

PEGylated CdSe/ZnS or CdSe/CdS QDs, suggesting Gd-doped nanoprobes would find a broad range of applications in the biomedical field by functionalizing these nanoprobes with target ligands.

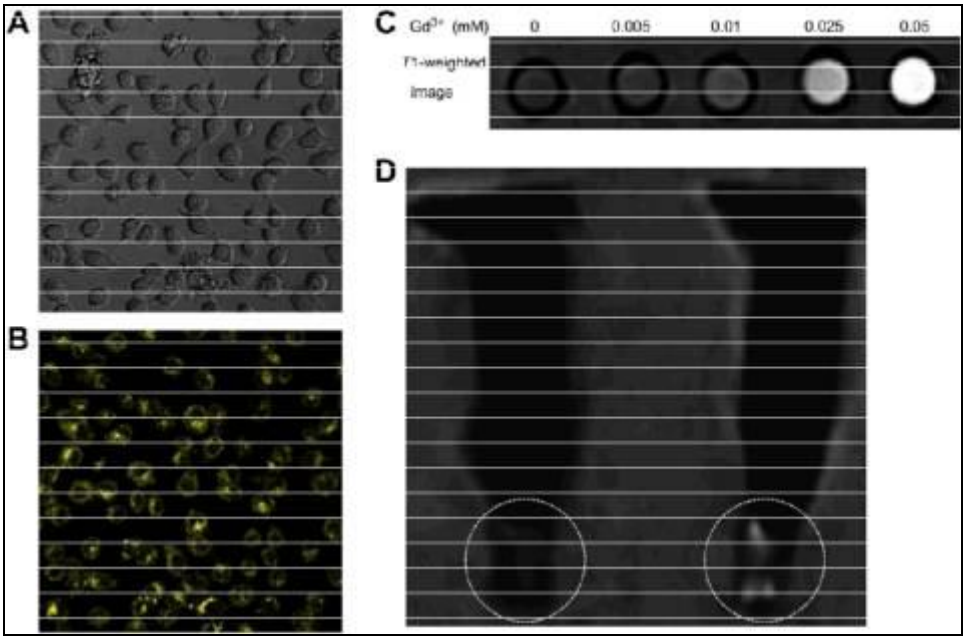

Figure. 2. MRI and optical imaging with Gd-doped ZnO QDs. White field (**A**) and fluorescence (**B**) images of HeLa cells after incubation with Gd-doped ZnO QDs. **C.** A T_1-weighted MRI image of aqueous solutions of Gd-doped ZnO QDs with various Gd^{3+} concentrations, obtained with a 1.5 T clinical MRI system. **D.** A T_1-weighted image of HeLa cells pellet without (left) and with Gd-doped ZnO QDs (right). (Figure is adapted from [75]).

Multifunctional Fe_3O_4-ZnO core-shell magnetic QDs for cancer imaging and therapy

One of the major obstacles in dendritic cell (DC)-based cancer immunotherapy is the development of a delivery system which can efficiently deliver target antigens into DCs [76]. Because of the large surface area, nanomaterials are promising candidates for this application. Multifunctional Fe_3O_4-ZnO core-shell magnetic QDs were also reported for potential cancer imaging and therapy. Recently, Fe_3O_4-ZnO core-shell nanoparticles with an average diameter of 16 nm were prepared to deliver carcinoembryonic antigen into DCs, which could also serve as imaging contrast agents [77], (Figure. 3). Antigen-bound nanoparticles were efficiently taken up by DCs in vitro, where the ZnO shell facilitated cell internalization and significantly reduced the incubation time needed for labeling DCs. No changes in viability or phenotype were observed in the nanoparticle-labeled DCs. More importantly, the uptake of nanoparticle-

labeled DCs in draining lymph nodes of a mouse was successfully detected by MRI, warranting future investigation of these nanoparticles for image-guided antigen delivery and in vivo tracking of the loaded DCs.

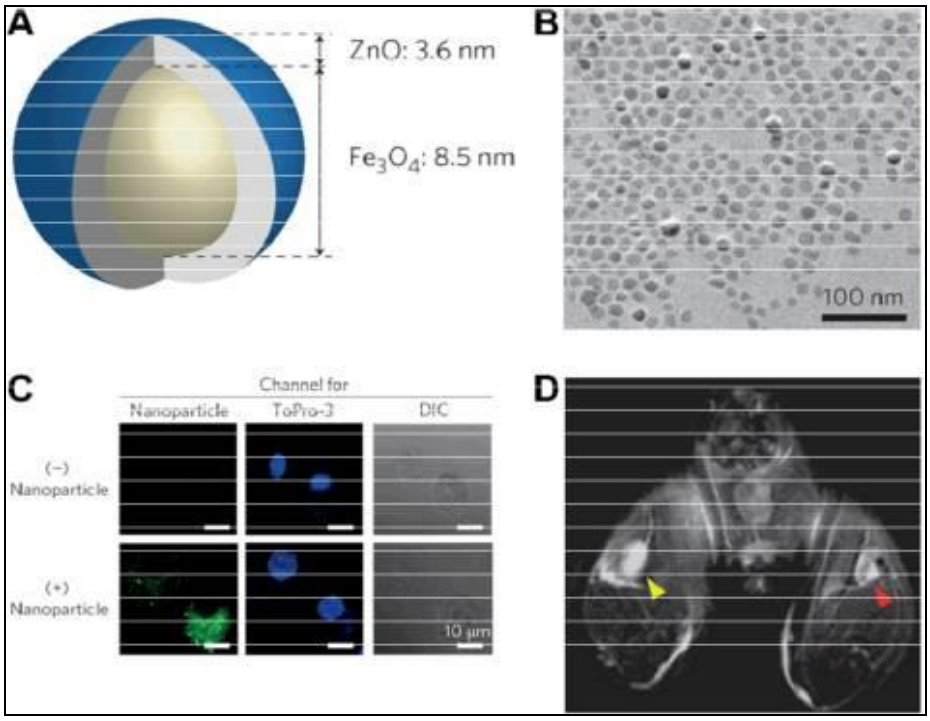

Figure. 3. **A.** A diagram of the core-shell Fe_3O_4-ZnO nanoparticle. **B.** A transmission electron microscopy image of the core-shell Fe_3O_4-ZnO nanoparticles. **C.** Fluorescence images of dendritic cells (DCs) without (top) or with (bottom) the nanoparticles. The fluorescence signal is shown in green and the nuclei (in blue) were stained with ToPro-3. DIC: differential interference contrast. **D.** An in vivo MRI image of draining lymph nodes of a mouse injected with DCs labeled with Fe_3O_4-ZnO (red arrowhead) or ZnO nanoparticles (yellow arrowhead) into the ipsilateral footpads. (Figure is adapted from [77]).

Targeted optical imaging with green fluorescent ZnO nanowires (NWs)

The major hurdles for biomedical applications of ZnO nanomaterials include low-intensity and short-wavelength luminescence of ZnO, limited capability in size control, and sharpness/stiffness of ceramic-based nanostructures (rigid and sharp tips/edges could potentially cause cell/tissue damage). Recently green fluorescent ZnO nanowires (NWs) were synthesized, which could overcome the abovementioned hurdles, and demonstrated that the ZnO NWs can be employed for targeted imaging of cancer cells [78,79]. The c(RGDyK) (abbreviated as RGD) peptide, which is a potent antagonist of integrin $\alpha_v\beta_3$ (a key protein

involved in tumor angiogenesis and metastasis) [80,81] was used as the targeting ligand. After surface functionalization to render the ZnO NWs water solubility, better biocompatibility, and lower cytotoxicity, RGD-conjugated green fluorescent ZnO NWs selectively bound to U87MG human glioblastoma cells (which express a high level of integrin $\alpha_v\beta_3$) and the intrinsic fluorescence signal of ZnO NWs could be detected by a fluorescence microscope (Figure 4A, 4B).

Red Fluorescent Zinc Oxide Nanoparticle: A Novel Platform for Cancer Targeting

Novel red fluorescent ZnO NPs were developed and the successful conjugation of 64Cu ($t_{1/2}$ = 12.7 h) and TRC105, a chimeric monoclonal antibody against CD105, to these ZnO NPs via well-developed surface engineering procedures was done. The produced dual-modality ZnO NPs were readily applicable for positron emission tomography (PET) imaging and fluorescence imaging of the tumor vasculature. ZnO NPs with dual-modality imaging properties can serve as an attractive candidate for future cancer theranostics. In addition, these ZnO NWs were also labeled with a positron emission tomography (PET) isotope, ^{64}Cu ($t_{1/2}$: 12.7 h), to evaluate its biodistribution in normal mice without the use of tumor-targeting ligands (e.g. RGD peptides) (Figure 4C) [82]. PET findings revealed that the ZnO NWs accumulated mainly in the reticuloendothelial system (RES) and they could be degraded and cleared from the mouse body Much further improvement will be needed before these ZnO NWs can be applied for in vivo targeting/imaging of cancer.

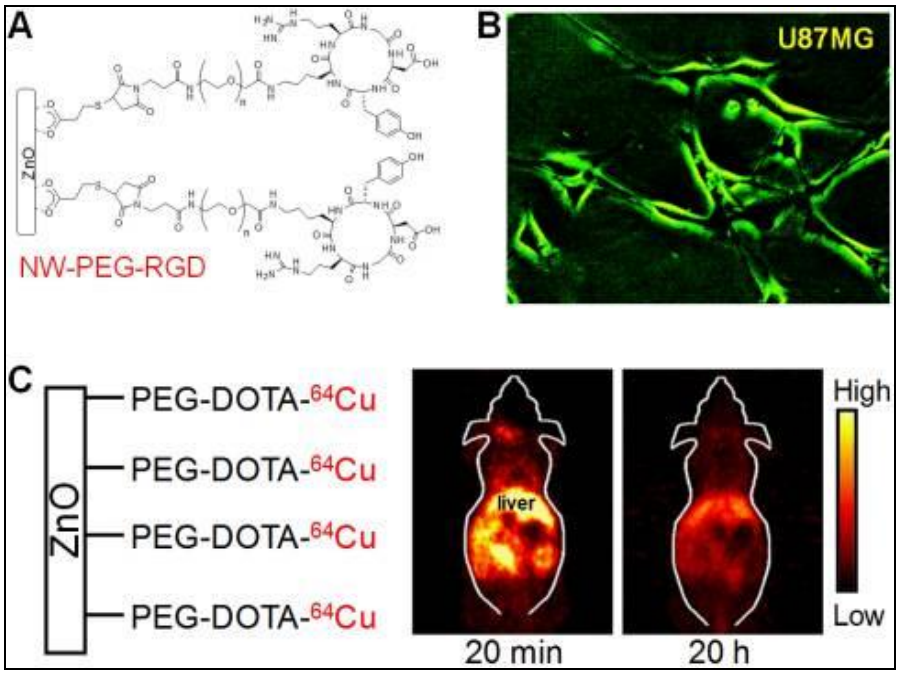

Figure. 4. Targeted optical imaging with green fluorescent ZnO nanowires (NWs). **A.** A schematic structure of RGD peptide conjugated ZnO NWs. PEG denotes polyethylene glycol. **B.** Fluorescence imaging of integrin $\alpha_v\beta_3$ on U87MG human glioblastoma cells with NW-PEG-RGD. Magnification: 200×. **C.** Representatives positron emission tomography images of ^{64}Cu-labeled non-targeted ZnO NWs at 20 min and 20 h postinjection into female Balb/c mice. (Figure is adapted from [82]).

Fluorescence lifetime imaging (FLIM) with ZnO NPs

ZnO NPs have been employed to fluorescence lifetime imaging in human skin [83] as shown in Figure. 5. The skin was treated with ZnO NPs for 4 h, then the skin was visualized with multiphoton imaging at different depths below the skin surface, as was the untreated skin. At 10 μm from the surface of human skin, an additional peak at 270 ps can be observed in the treated sample but cannot be seen in the control Figure. 5.a,b. This peak represents the short lifetime of ZnO NPs as the dark blue region in the false colored image. At 31 μm depth, there is a peak at about 3000 ps apparent in the treated skin but not in the control Figure. 5.c,d. However, no signals from ZnO are found in the treated skin at 45 μm depth. This research confirmed ZnO FLIM for human skin can reach several tens of micrometers in depth.

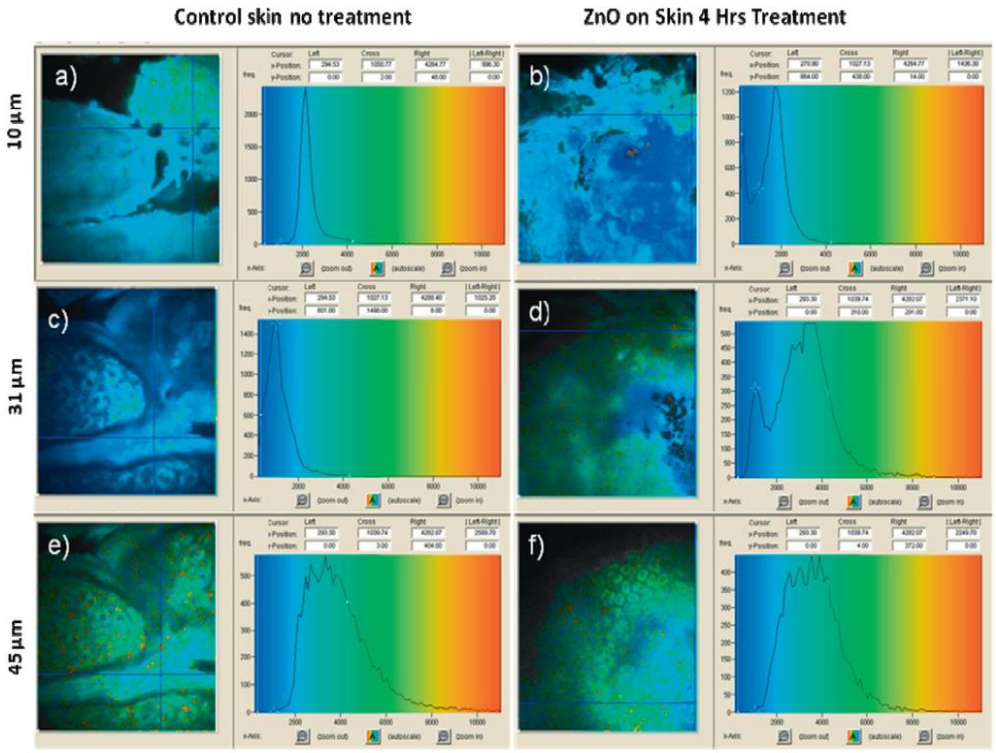

Figure. 5. In vivo control and treated skin after exposure of ZnO for 4 h intensity versus life times for ZnO, stratum corneum, and viable epidermis. 740 nm excitation, channel 1 (350~415 nm), field of view 175:175 μm. (**a**) Stratum corneum control, (**b**) stratum corneum after ZnO treated for 4 h, (**c**) first layer of viable epidermis control, (**d**) first layer of viable epidermis after ZnO treated for 4 h, (**e**) viable epidermis control and (**f**) viable epidermis after ZnO treated for 4 h. (Figure is adapted from [83]).

Multifunctional Au–ZnO hybrid NPs for targeted induction lysosomal membrane permeabilization (LMP)-dependent apoptosis in cancer cells and real-time imaging.

Heterostructural ZnO/Au nanocomposites, where Au NCs grow at the tip of ZnO nanorods or along the nanorod surfaces, were synthesized and investigated for their optical properties and biocompatibility [84]. When incubated with HeLa cells, these ZnO/Au nanocomposites were found to be internalized into the endosomes and cytosol. In addition, these nanoparticles showed selective cytotoxicity to bacteria, as well as preferential killing of leukemic T cells while sparing normal immune cells, because of the dissolution of surface ZnO layer.

Zinc Oxide Nanoparticles: Cancer Therapy and Photodynamic Therapy

Photodynamic therapy (PDT) is an emerging and promising alternative for non-invasive treatment of cancer [85]. Upon uptake of photosensitizers into cancer cells, irradiation with light of suitable wavelength and dosage can generate reactive oxygen species (ROS) which can induce cell death and/or necrosis [86]. ZnO nanoparticles can induce ROS such as hydroxyl radical, hydrogen peroxide, and superoxide in aqueous solutions upon absorption of UV illumination, making them good candidates for PDT (Figure 6) [87].

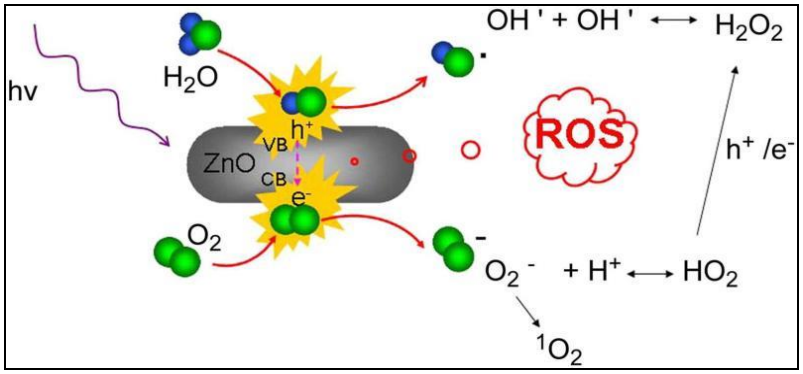

Figure. 6. Possible mechanism of ROS production by ZnO nanorods under UV irradiation. (Figure is adapted from [87]).

ZnO nanoparticles combined with graphene can be used for targeting photodynamic therapy (PDT) under visible light irradiation. Folic acid (FA), a targeting agent toward tumor cells, was conjugated onto graphene oxide (GO) via imide linkage [88]. The combination of ZnO with GO–FA induced a remarkable improvement in tumor targeting, which has been demonstrated by the cellular uptake assay. Due to the high electrical conductivity of graphene, the interaction between graphene and ZnO, and the inhibition of aggregation, the hybrid of GO–FA and ZnO significantly enhances the photodynamic activity. It was noted that the photodynamic activity of the non-cytotoxic GO–FA–ZnO is mediated by reactive oxygen species (ROS) generation under visible light irradiation. Following the ROS generation, GO–FA–ZnO caused a significant decrease in cell viability, mitochondrial membrane potential, superoxide dismutase activity, catalase and glutathione peroxidase, as well as an increase in malonodialdehyde production. Moreover, GO–FA–ZnO induced apoptotic death by elevating the caspase-3 activity. Thus, GO–FA–ZnO can act as a novel tumor targeting photosensitizer and a promising strategy in PDT for cancer treatment [Figure 7].

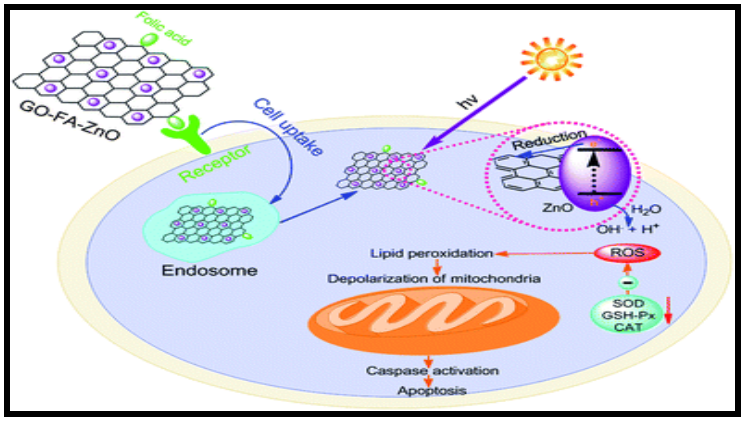

Figure. 7. Folic acid-conjugated graphene–ZnO nanohybrid for targeting photodynamic therapy under visible light irradiation [88].

Biodegradable ZnO@polymer Core–Shell Nanocarriers loaded with doxorubicin (DOX) used for the treatment of human brain cancer cells

Luminescent ZnO@polymer core–shell nanoparticles were synthesized and loaded with doxorubicin (DOX) for the treatment of human brain cancer cells. Analyses by confocal laser scanning microscopy proved that the ZnO@polymer–DOX nanocomposites were

decomposed at lysosomes to release DOX molecules, which penetrated into the nucleus and finally killed the cells Figure 8 [89].

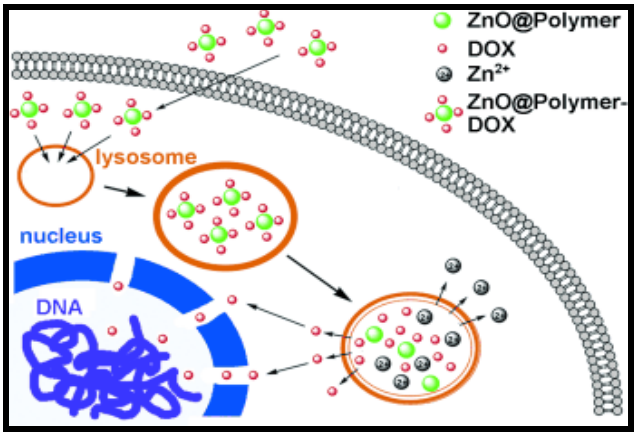

Figure. 8. Luminescent ZnO@polymer core–shell nanoparticles were synthesized and loaded with doxorubicin (DOX) for the treatment of human brain cancer cells.

Lipid-coated ZnO nanoparticles as lymphatic-targeted drug carriers

Lymphatic metastasis plays an important role in tumour recurrence. The applications of nanoparticles in the treatment of lymphatic metastatic tumours have been limited by targeting inefficiency and nonselective toxicity. Hence, lipid-coated ZnO nanoparticles (LZnO NPs) have been developed to solve these issues. Using the microreactor method, ultrasmall (~30 nm) core–shell-structured nanoparticles loaded with 6-mercaptopurine (6-MP) were fabricated. In vivo results show that the lipid shell induces a remarkable improvement in lymphotropism and biocompatibility compared to ZnO nanoparticles [90]. Furthermore, the ZnO core exhibits not only pH-responsive behaviour to guarantee effective drug delivery, but also a strong preferential ability to kill cancerous cells, due to the generation of higher levels of reactive oxygen species in rapidly dividing cells. Furthermore, LZnO NPs enhance the cytotoxicity of 6-MP, resulting from the improved internalization of nanoparticles through endocytosis. These findings indicated that LZnO NPs are a promising candidate for use as lymphatic-targeted drug carriers.

Zinc Oxide Nanoparticles and Biomarker Mapping

Nanoparticles have been used as a tool for the detection of disease biomarkers in both in vivo and ex vivo diagnostic applications, consequently leading to an advancement of proteomics

and genomics technologies [91,92,93]. Recent studies have shown that ZnO nanoparticle cores capped with polymethyl methacrylate are useful in the detection of low abundant biomarkers [94]. These nanobeads work by facilitating surface absorption of peptide/proteins from cell extracts enabling increased sensitivity and accuracy of cancer biomarker detection using mass spectrometry. Using another approach, a ZnO nanorod-based cancer biomarker assay has been developed for high-throughput detection of ultralow levels of the telomerase activity for cancer diagnosis and screening [95].

ZnO Nanoparticles and Targeted Gene Delivery

Gene therapy is defined as delivery of functional genes to target cells for achieving therapeutic effects. The application of gene therapy in the suppression or replacement of malfunctioning genes holds great promise for the cure of a number of diseases at the genetic level. Gene therapy has attracted considerable interest over the last several decades for cancer treatment [96]. One major challenge of gene therapy is the development of safe gene vectors which can protect DNA from degradation and enable cellular uptake of DNA with high efficiency. A wide variety of nanomaterials have been investigated for gene delivery and gene therapy applications, including ZnO nanomaterials. Recently, ZnO tetrapod-like nanostructures have been synthesized as novel carriers for gene delivery. These functionalized tetrapods, consisting of silica-coated amino-modified tetrapod-like ZnO nanostructures, are able to effectively bind plasmid DNA through electrostatic interactions and enhance transfection efficiency of A375 cells [97,98]. Polycation-capped ZnO quantum dots have been recently developed and shown to mediate efficient DNA transfer into COS-7 cells, and at the same time allow for real-time imaging of gene transfer [99]. Thus, with continued research, ZnO and metal oxide nanomaterials may provide an effective means for targeted gene delivery and gene silencing for next-generation cancer applications.

Conclusion

Nanotechnology has provided significant breakthroughs in medicine and cancer applications. The potential benefits of zinc oxide nanomaterials for tumor imaging, controlled drug delivery, and targeted cancer cell killing can be enormous and may offer clinical therapeutic platforms that simply do not exist today. There are multiple characteristics of ZnO that make these

nanomaterials attractive considerations including their versatility, biodegradability, intrinsic fluorescence, relative ease of synthesis, ability to tailor their physiochemical characteristics, ability to functionalize them with chemotherapeutic drugs and cancer targeting molecules and their desirable cancer cell cytotoxicity profile make them appealing candidate for biomedical applications. Due to their low toxicity, low cost and biocompatibility, ZnO NPs are used in vivo bio-imaging, gene/drug delivery and cancer detection. Thus, ZnO NPs can serve as novel platforms for the treatment of cancer.

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