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REVIEW

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Synthesis, Characterization, and Biomedical Applications of Zinc Oxide Nanoparticles

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Abstract: Nanoparticles of Zinc oxide are biocompatible, have low toxicity, are sustainable, and cost-effective, they have become popular metal oxide nanoparticles and have attracted the attention of researchers worldwide. It becomes apparent that they could be a viable option in the fields of mechanical, electrical, food packaging, and biological applications because of their special optical and chemical qualities. In the long term, biological approaches that use green or natural channels are simpler, less harmful, and more environmental friendly than chemical-based and physical techniques. Not only that, but ZnO NPs can significantly increase pharmacophore bioactivity while being far less toxic and biodegradable. They play a vital role in the apoptosis of cells because they promote the formation of oxygen species that are very reactive and release the ions of zinc, leading to cell death. These ZnO NPs also function effectively in combination with other components that support wound healing and biosensing, which monitors trace levels of biomarkers linked to a range of diseases. Overall, this study covers the production and latest advancements of zinc oxide nanoparticles from green sources such as fruits, vegetables, bacteria, fungi, algae, and proteins. It also sheds light on the potential uses of ZnO NPs in medicine. Lastly, potential uses of synthesized zinc oxide nanoparticles in research as well as biomedical applications are explored.

Keywords: Zinc oxide nanoparticles; Green synthesis; Biomedical applications; Nanotechnology; Photocatalytic activity

1. Introduction

Nanotechnology focuses on particles, specifically those with a size between 1 and 100nm. The word nano first appeared from the Latin term *nanus*. Transdisciplinary scientific areas, active and passive nano assembly, common nanoparticles, and small-size molecular nanosystems are among the four generations of nanomaterials that have evolved[1]. This mesoscopic technology is utilized to create nano-assemblies found in nature, including agricultural goods, nano-medicine, and nanotools for the medical sector's treatment and diagnostic needs[2]. Other potential manifestations include large-surface area nanopores, atomic clusters, nanorods, spots, grains, fibers, and films[3].

The predominant form of zinc oxide is a powder that is white in color and not soluble in water. It is mostly utilized in powder form in industries like paper, paint, rubber, ointments, batteries, glass, and cosmetics. Many metal nanoparticles can be mixed with Zinc oxide to prevent tooth caries[4]. The nanoparticles of these semiconducting metal oxides have received great attention for research as a photocatalyst[5]. Many zinc oxide nanoparticle (ZnO NP) applications require precise control of physical and chemical characteristics, including structure, size distribution, shape, and surface conditions etc[6].

ZnO nanoparticles are robust to high temperatures and do not readily react with other compounds in the photocatalytic process. ZnO is subjected to UV radiation, which causes the electrons to receive light energy and migrate from their valence band region to the conduction band, where they simultaneously form electron-hole pairs. While other carriers travel on the ZnO surface, certain electrons emit heat and light as they migrate back into the valence band. Among these, photo-generated holes and electrons both possess significant oxidizing and reducing capabilities. To accomplish photocatalytic effects, they also react with contaminants[7]. When compared to other semiconductors, ZnO nanostructures are nontoxic and can be produced at a meager cost. They are also extremely photosensitive and photostable[8]. To create nanomaterials without using many dangerous ingredients, a process known as "green synthesis" has gained popularity as awareness of environmental issues rises. Several approaches have been used to create different morphologies of zinc oxide nanostructures, including the hydrothermal process, microwave breakdown, precipitation, and wet chemical approach[9].

1.1 Nanoparticles of Zinc Oxide

ZnO NPs in aqueous solution, which are n-type semiconductors, absorbed UV light and showed phototoxic action, creating H_2O_2 and superoxide ions ($O_2^{\bullet}$), which might impede or eliminate microbes via interacting with proteins, DNA, and active enzymes[10]. While some researchers found that the antibacterial activity was unrelated to Zn^{2+} ion release or levels of reactive oxygen species[11] , others hypothesized that the destruction of cell membranes was caused by electrostatic interactions between negatively and positively charged cell surfaces when zinc oxide nanoparticles were suspended in water.

1.2 Crystal Structures of ZnO

ZnO has three different crystal structures: rock salt, sphalerite, and wurtzite (**Figure 1**). As the pressure reaches 9 GPa at room temperature, the wurtzite-like hexagonal structure of ZnO crystals transforms into a rock salt tetragonal structure, that resembles sodium chloride (NaCl) crystals. The energy values for three distinct ZnO crystal structures are 5.638 eV for the wurtzite structure, 5.586 eV for the sphalerite structure, and 5.478 eV for the rock salt structure.

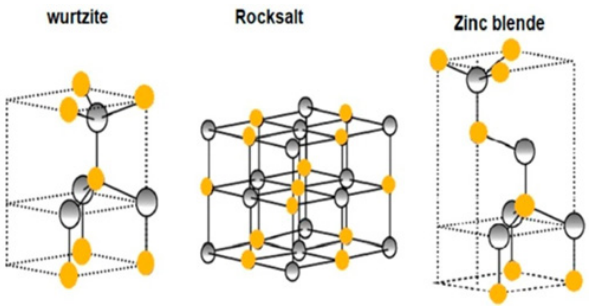


Figure 1. Three structures of ZnO (a)Wurtzite (b) Rock Salt Structure (c) Zinc Blend Reprinted with permission from[12]. Copyright 2024 American Chemical Society.

2. Preparation methods of ZnO Nanoparticles-based Material

For ZnO NPs, several synthesis techniques have been devised; the preparation method used will depend on the specificity of the application. Two main approaches were typically employed: the bottom-up and top-down,

which involve chemical-based, physical, and biological preparation of zinc oxide nanoparticles[13]. The synthesis of nanomaterials using a Microfluidic reactor is one of the non-conventional approaches. The bulk materials are physically cut or sliced into nano-sized materials using the top-down method. Conversely, the bottom-up technique fabricates nanomaterials by chemical and biological synthesis using atoms or molecules[14]. Compared to the top-down technique, it is quicker and less expensive. **Table 1** lists several strategies for synthesizing ZnO NPs, the benefits and drawbacks of each synthesis method.

The traditional methods for synthesizing ZnO NPs involve the usage of harmful reducing agents and organic solvents[15]. These substances are known to be extremely reactive and can pose hazards to the environment. However, the adoption of green synthesis approaches offers a more environmentally friendly alternative.

Table 1: Various methods employed in the synthesis of Zinc Oxide Nanoparticles

Preparation Techniques	Advantages	Disadvantages
Physical Methods (Ultrasonic irradiation, Sputtering, Laser ablation, Explosion processes Mechanical milling and Thermal evaporation etc)	Free from catalyst Industrial manufacture on a large scale Uncomplicated	Control of parameters Durable machinery Intensive energy input Impedes crystalline structure High expenses Instability in discharge
Chemical Methods (Solgel processes, Precipitation, Spray Pyrolysis, Solvothermal method, Chemical vapor deposition etc)	Minimal energy input Simple management of chemical reagents Equipment usability Straightforward parameter manipulation Large-scale industrial production	Widespread utilization of Surfactants Costly precursors Toxic nature Limited yield Slow deposition rate Poor penetration
Biological (Plant extracts, algae, fungi, Microorganisms and other bio-molecular extracts)	Promising substitutes for chemical and physical approaches Eco-friendly Utilization of affordable organic solvents Cost-effective Reproducible	Ambiguous mechanism Stability of nanoparticles
Microfluidic Reactor (Co-flow, Segmental flow and Continuous flow)	Highly valuable products Reproducible	Control of parameters

2.1 Chemical Methods for Synthesis of Zinc Oxide Nanoparticles

Chemical processes can be used to create ZnO NPs in a variety of ways, some of which are depicted in **Table 2** such as controlled precipitation, solvothermal and hydro thermal processes, emulsion and microemulsion environments, sol-gel processes, the vapour transport method, and mechanochemical processes.

2.1.1 Sol-gel method

This approach is used for producing ZnO NPs because of its easy process, high product yield, and quick reaction to temperature changes[16]. To address the issue of insufficient catalytic as well as reactive capacity, this method also yields low-cost, narrow-size-distribution ZnO NPs that contain ZnO with a larger surface area; typically, an annealing action at a particular temperature is carried out following nanoparticle formation in sol–gel[17].

Because the oxide reforms at elevated temperatures throughout the annealing process, metal oxide crystal defects may increase. As crystal defects expand, they can create active spots on the surface of solids that act as donation hubs[18]. These locations can alter the overall basicity of surface of the resulting ZnO NPs and enhance the size of crystallites. Large surface areas of ZnO powder have been successfully created using the sol-gel synthesis method, and then calcination and drying were carried out. Variables including pH, a gelling agent, and the rate of temperature at which calcination takes place also affect the dimensions and form of ZnO NPs during manufacture[19].

2.1.2 Hydrothermal method

ZnO NPs were synthesized using a hydrothermal method with hexamethylenetetramine (HMTA) acting as a surfactant. Using various co-surfactants, this hydrothermal approach revealed distinct morphologies,

such as belt sheet structures that resembled flowers or wires, etc[20].The same substance has numerous usages as a catalyst in different organic processes and could also be employed as a photocatalyst, a fuel cell, and a solar cell, or in semiconductors, among other uses, depending on their surface shape[21]. The impact of key variables (i.e., temperature, time, and surfactant concentration) on ZnO particle size as well as form was examined using statistical design. This method yields ZnO particles with a size range of 55–110 nm[22].

2.1.3 Co-precipitation method

This approach is also used to synthesize ZnO NPs. When compared to other traditional ways, the precipitation approach offers an easy way to produce huge quantities of product at low cost without the need for costly raw materials or sophisticated machinery[23]. ZnO NPs produced via co-precipitation with precursors of sodium hydroxide and zinc nitrate. According to the X-ray diffraction analysis, the produced ZnO nanoparticles range in size from 35 to 40 nm and have a wurtzite structure[24]. Research shows that ZnO nanoparticles in co-precipitation have a spherical surface shape. The nanoparticles' UV-visible spectrum exhibits a red shift in comparison to the bulk sample's.

2.1.4 Microwave assisted method

A variety of nanoparticles, including oxides, hydroxides and sulfides have been produced using microwave-assisted synthesis techniques[25]. The advantages of this method over practical hydrothermal procedures include (a) quick response, (b) simple medium, (c) rapid rise to reaction temperature, and (d) control over particle morphology. Two processes—dipolar rotation and ionic conduction—that convert electromagnetic waves into heat energy constitute the foundation of microwave heating[26]. These processes are closely tied to the reaction mixture's chemical composition. Because various compounds absorb microwaves in various capacities, the reaction mixture's components may be heated selectively due to this property[27].

ZnO nanoparticles were created by combining glycine and Zn $(NO_3)_2 \cdot 6H_2O$ at a molar ratio of 1:2, respectively. Following a thorough mixing and grinding of these two raw ingredients, the resulting white liquid was poured into the porcelain crucible Next, the prepared mixture was heated at 80% power for two minutes in a microwave oven. The mixture ignited and produced a lot of foam as it evaporated, leaving behind a fluffy, grey-white substance in the container[28]. To eliminate unreacted components, the resulting fine solid was finally repeatedly rinsed with deionized water a few times before being treated with ethanol. The ZnO nanoparticles in solid form were dried for three hours at 100°C in an oven.

2.1.5 Solvothermal method

The primary goals of the solvothermal method are to create metal oxide nanoparticles that have a lot of surface area, a lot of crystallinity, and a lot of thermal stability[29]. A solvothermal method employing an ethylene glycol and ethanol solvent system was used to prepare ZnO NPs. By adjusting the liquids' volume composition and the synthesis temperature, ZnO NP's size and form could be adjusted[30]. The liquids' volume ratio was varied to produce spherical and ellipsoidal nanoparticles. At a lower synthesis temperature, a greater volume percentage of ethylene glycol was used to create primarily spherical nanoparticles with an average diameter of 4 nm. For the lowest nanoparticle size, which is attributed to the quantum confined effect, a blueshift during a room temperature photoluminescence observation from the free excited state transitions is detected[31].

The ammonia gas sensing properties of the nanoparticles with the lowest diameter demonstrated superior sensitivity at a comparatively lower temperature in comparison to those of the particles with larger diameters[32].

Table 2. Chemical Processes for Zinc Oxide Nanoparticle Synthesis

Method	Materials and devices	Strengths	Weaknesses	Reference
Direct precipitation	Ammonium carbonate $[(NH_4)_2CO_3]$, ammonium hydroxide $(NH_3 \cdot H_2O)$, etc	Purity is high, the operation is simple and equipment	Limited dispersibility, distribution of the size of particles are wide	[33]
Homogeneous precipitation	Hexamethylenetetramine $[(CH_2)_6N_4]$, urea $[CO(NH_2)_2]$	The sediment particles are compact, consistent, and easily achievable.	The reaction duration is excessively long, and the required temperature is excessively high.	[34]

Continuation Table:

Method	Materials and devices	Strengths	Weaknesses	Reference
Solid phase method	$ZnSO_4 \cdot 7H_2O$ and anhydrous Na_2CO_3	The reaction conditions are moderate, and the efficiency of the output is outstanding.	It is challenging to grind effectively.	[35]
Spray Pyrolysis	The zinc acetate aqueous solution dehydrates	Excellent dispersibility and tiny, very pure particles	Cost and usage of energy are high	[36]
Laser-induced chemical vapour deposition	A laser system operating in a continuous wave mode using carbon monoxide, in combination with zinc salt and an inert gas.	Efficient energy conversion rates and a consistent particle size.	The yield is limited, and the associated costs are high.	[37]
Sol-gel method	$ZnCl_2$, $Zn(NO_3)_2$ and $Zn(CH_3OO)_2$	Required temperature is low, purity of product is high	The processes of washing and filtering are intricate.	[38]
Microemulsion Technique	Zn salt emulsion	Particles exhibit uniformity and controllability, making them straightforward and convenient to manipulate.	Cost of material is high	[39]

2.2 Green Synthesis Method

Nanoparticles with biological uses can be synthesized by a process called biosynthesis, which involves the use of microbes and plants. This method is safe, economical, biocompatible, green, and environmentally beneficial. Green synthesis comprises the synthesis carried out by algae, bacteria, fungi, and plants, among others[40]. They make it possible to produce ZnO NPs on a big scale without adding any more contaminants. Increased catalytic activity and less usage of costly and hazardous chemicals are two benefits of using biomimetic-synthesized nanoparticles[41]. Some naturally occurring strains and extracts from plants release phytochemicals that serve as capping or stabilizing agents as well as reducing agents. For instance, the production of ZnO nanoflowers with uniform size from B. *licheniformis* cell soluble proteins demonstrated enhanced photocatalytic activity and photostability, as evidenced by the 83% degradation of the pollutant dye methylene blue (MB) in the presence of ZnO nanoflowers, taking into account the fact that MB's self-degradation was blank and that after three repeated cycles of the experiment at various time intervals, degradation was found at 74%, clearly demonstrating the photostability of the ZnO nanoflowers produced[42]. **Figure 2** provides a schematic representation of the green synthesis technique utilized to create ZnO NPs.

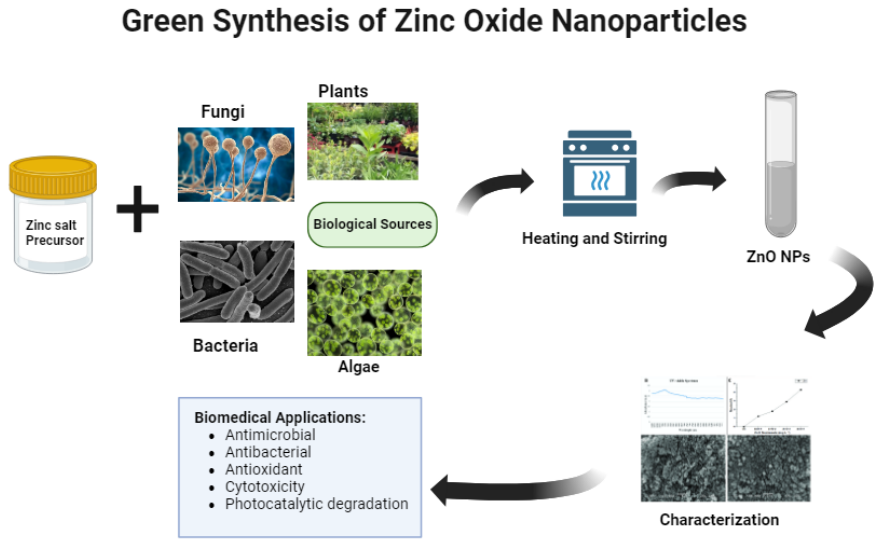


Figure 2. Green Synthesis Method of ZnO NPs

Biomolecules known as "green sources" serve as reducing and capping agents when NPs are being synthesized, further solidifying and enhancing their properties. Numerous phytoconstituents and secondary metabolites found in plants facilitate the bio-reduction activity during the production of nanoparticles[43]. These molecules also eliminated the need for additional chemical capping and reducing agents. In contrast, microbial-mediated synthesis of ZnO NPs has an advantage over plant-mediated synthesis due to the ease of replication of microorganisms. Numerous biomolecules and enzymes that are made in the suspension or growing medium by microorganisms are crucial for the bioreduction of nanoparticles (NPs) and for the formation of a variety of morphologies with both mono- and polydispersed NPs. However, as synthesis techniques are time-consuming, there are several drawbacks related to the isolation or screening of prospective microorganisms, the use of chemicals for the growth medium, and the cost-effectiveness of synthesis procedures. Plants are the most viable options for green synthesis since they produce more stable versions of the same molecules than bacteria[44]. During the green synthesis process, many biomolecules derived from plants or other environmentally friendly sources are mixed to produce stable, nontoxic ZnO NPs[45]. Sources for producing ZnO NPs from green sources can be found in **Table 3**.

Table 3: Biological Origins Employed in the Production of Zinc Oxide Nanoparticles

Biological Origin	Size(nm)	Physical Appearance	Applications	References
Plants				
Cassia fistulia	2.6	Globular	Antibacterial	[46]
Citrus limon	38-40	Spherical	Antibacterial	[47]
Pongamia pinnata	30-40	Spherical wurtzite	Antimicrobial	[48]
Oak fruit hull	34	Spherical	Photocatalytic degradation	[49]
Deverra tortuosa	9.2-31.1	Hexagonal wurtzite	Cytotoxicity	[50]
Bacteria				
Staphylococcus aureus	10-50	Acicular	Antimicrobial	[51]
Psedomonas aeruginosa	35-80	Spherical	Antioxidant	[52]
Serratia ureilytica	170-600	Varied	Antibacterial	[53]
Lactobacillus sporogens	145	Hexagonal	Antimicrobial	[54]
Yeast				
Xylaria acuta	34-54	Hexagonal	Antimicrobial	[55]
Pichia kudriavzevii	10.2-60	Hexagonal wurtzite	Antioxidant	[56]
Fungi				
Aspergillus fumigates	1.3-6.9	Oblate spherical	Agriculture	[57]
Aspergillus terreus	54.2-82	Spherical	Antifungal	[58]
Algae				
Ulva lactuca	10.5-50.5	Rod shaped	Photocatalytic activity	[59]
Alginate	20-40	Cubical or rod	Antibacterial	[60]
Other Biomolecules				
Soluble starch	50	Spherical	Antimicrobial	[61]
Plasmid-DNA	32	Tetrapod	-	[62]
Alanine, threonine and glutamine	16	Hexagonal wurtzite	Antimicrobial	[63]

3. Properties of ZnO NPs

ZnO is a material with outstanding electrical, optical, mechanical, photocatalytic, antimicrobial, and antifouling characteristics, which is of great importance. ZnO NPs behave considerably more like TiO_2 in terms of both their characteristics and uses. ZnO NPs have antiseptic, genotoxic, and cytotoxic effects[64]. It is also used in medicines as an anticancer and antibacterial agent. There are two ways of looking at how ZnO NPs affect both tumor cells and normal cells' cytotoxicity. The enhanced antibacterial properties of ZnO contributed to a reduction in dirt accumulation on oil paintings and improved resistance to microbial growth. Furthermore, ZnO nanoparticles were utilized to inhibit the biodegradation and deterioration of oil paintings supported by paper[65]. The release of Zn^{2+} and generation of Reactive Oxygen Species (ROS) are related to the ZnO activities as shown in **Figure 3**. It is currently unclear from the research on the cytotoxicity and genotoxicity of ZnO NPs whether they just destroy tumor cells or also harm healthy cells.

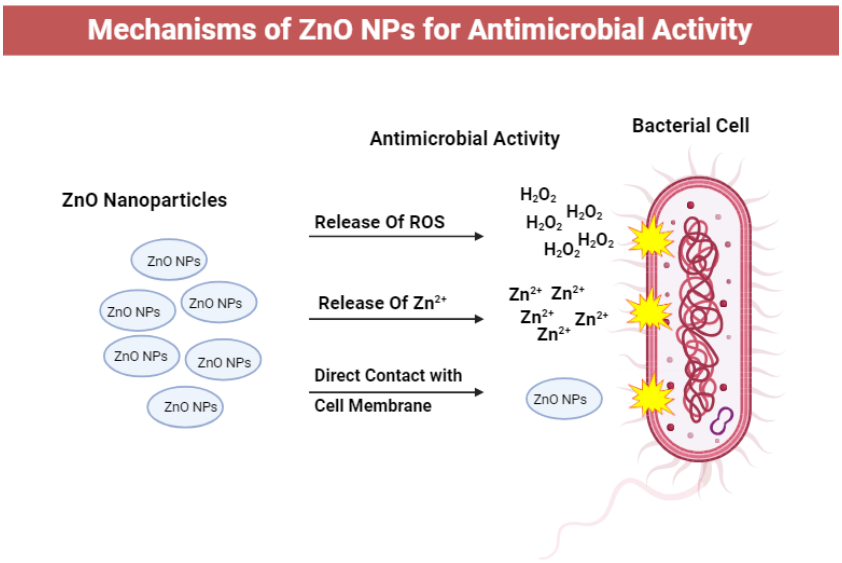


Figure 3. Mechanisms of ZnO NPs for Antimicrobial Activity

4. Limitation of ZnO Photocatalysts

ZnO nanostructures have many remarkable advantages, but they also have a few shortcomings that prevent them from functioning as effective semiconductor photocatalysts. ZnO nanostructures are first referred to as UV-light-driven semiconductors (UVLD) because of their huge band gap, which can only be triggered by UV radiation[66]. As UV light only makes up around 10% of solar radiation in total, its photocatalytic efficacy will be severely diminished when employed in the sunshine. Although UV light is regarded as hazardous, there will be less freedom when constructing photocatalytic reactors due to the low utilization of visible light in practical and industrial applications.

5. Characterization Of ZnO NPs

ZnO peaks are the only characteristic X-ray diffraction (XRD) peaks found in the synthesized nanopowder confirming that it was free of impurities. **Figure 4** shows the chemically and biologically synthesized ZnO nanoparticles X-ray diffraction pattern. When nanoscale particles are present in the manufactured material, it is evident from the XRD peaks, which considerably widen. This identified the hexagonal wurtzite phase of ZnO, which exhibits diffraction peaks at 31.84, 34.52, 36.33, 47.63, 56.71, 62.96, 68.13, and 69.18 with lattice constants of a = b = 0.324 nm and c.

(a) (b)

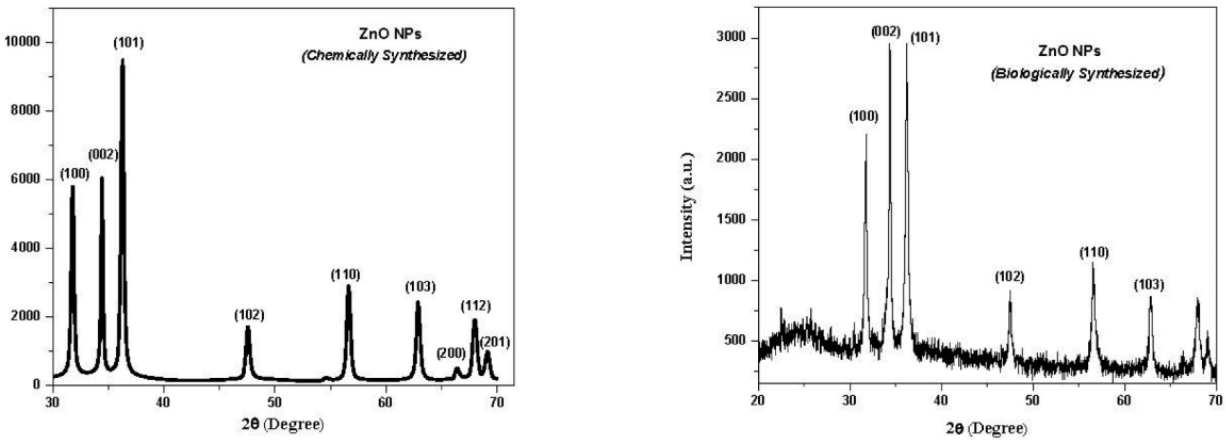
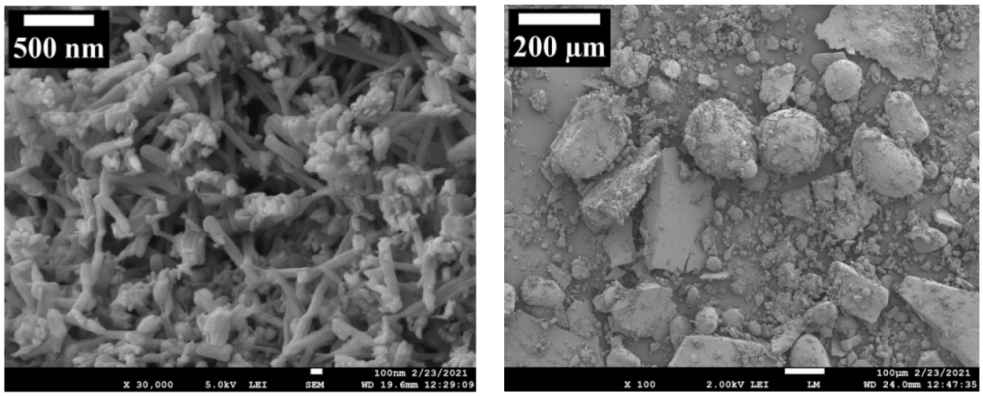


Figure 4. XRD Pattern for ZnO Nanoparticles (a) chemically synthesized ZnO NPs and (b) Green synthesized ZnO NPs by Ajay Kumar Tiwari[67] available under an open access Creative Common CC BY license, at https://www.mdpi.com/openaccess

The structure of the produced ZnO nanoparticles was examined using scanning electron microscopy. **Figure 5** displays the SEM image of ZnO obtained through both chemical and green synthesis methods. The SEM images demonstrate that the crystal morphologies are rounded and cubical for biologically manufactured ZnO NPs (**Figure 5b**) and are nanorods-like when chemically produced (**Figure 5a**).



(**a**) (**b**)

Figure 5. SEM image for ZnO NPs by (a) Chemically synthesized methods (b) Green Synthesis by Ajay Kumar Tiwari[67] available under an open access Creative Common CC BY license, at https://www.mdpi.com/openaccess

The hexagonal plane of the produced nanoparticles was confirmed by transmission electron microscope. Examining the optical characteristics of nano-sized particles using UV-visible absorption spectroscopy is a common activity. ZnO nanopowder absorption spectra show an apparent absorption band at 355 nm[68]. The wavelength of the excitonic peak of absorption for ZnO nanoparticles is around 258 nm, which is significantly shorter than the 358 nm band gap wavelength.

6. Applications Of ZnO NPs In Biomedical Engineering

Nanomedicine is a developing field of science that focuses on the use of smart materials for biomedical applications. Various nanoparticles are being studied and used in this aspect[69]. They have numerous applications in the field of biomedical engineering, agriculture, and the food industry. Since zinc oxide nanoparticles are biocompatible, have low toxicity, are sustainable, and are cost-effective, they have emerged as a popular metal oxide nanoparticle and garnered the attention of researchers worldwide. This is the reason ZnO nanoparticles have widespread applications. Among them are the most noteworthy uses of ZnO NPs in the coating, antimicrobial therapy, cosmetics, medicine, photocatalysis, insecticides, sunscreen materials, agriculture, and antimicrobial agents as shown in **Figure 6** ZnO NP's outstanding antibacterial potentials have helped with its various uses in antiseptic surgical tape applications, shampoos, calamine lotions, and creams[70].

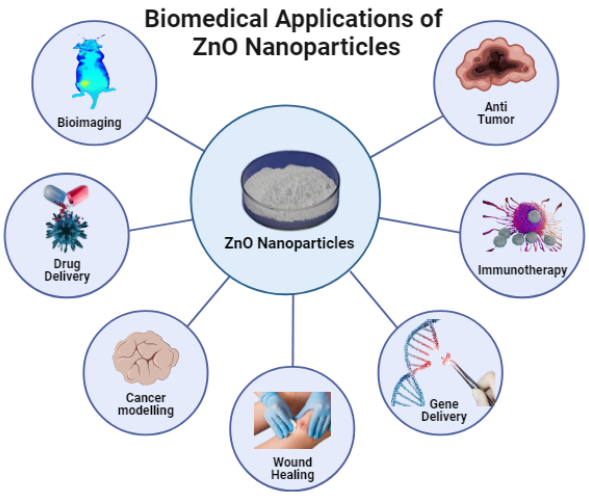


Figure 6. Applications of ZnO Nanostructures in Medicine

6.1 Bioimaging

Researchers have recently discovered that ZnO nanoparticles come in a variety of forms that can be used as bioimaging materials. The amphibious ZnO Quantum dots (QDs) with blue fluorescence were synthesized using hyperbranched polymers and used in bioimaging applications[71]. The surfaces of ZnO nanoparticles allow modification easily, and they can

maintain stability in an aqueous solution, as well as the QDs in it increase by roughly 30% after carefully considering the modification[72]. The best ZnO is water soluble and contains hyperbranched polyethylenemine molecules for bioimaging purposes. ZnO nanoparticles of pure n-type is also used for bioimaging applications with the use of conventional fluorescent techniques of microscopy[73]. In general, to generate emissions, the majority of nanoparticles need a UV source for excitation. Lowering the energy gap enables a 405 nm laser to adequately stimulate the nanoparticles to identify their emission throughout live-cell imaging experiments utilizing a confocal microscope. The study as summarized in **Table 4** establishes the groundwork for the application of these nanoparticles to various bioimaging objectives. It allows scientists to test the interlinkage between pure n-type ZnO nanoparticles and human cells via fluorescent imaging techniques.

Table 4: Applications of Zinc Oxide Nanoparticles in Bio-imaging

Material Type	Model	Reference
Nanoparticles of ZnO (26-30 nm)	Cells of rat liver and human skin	[74]
Nanoparticles of ZnO (15-30 nm)	Examination of cellular structures within skin tissues	[75]
Nanoparticles of ZnO (21 nm)	Skin structures	[76]
Nanoparticles of ZnO (10-300 nm)	Bioimaging of zebrafish blood cells, as well as shoots and roots of *Arabidopsis* plants	[77]
Nanoparticles of ZnO (2-200 nm)	Plant tissues with a focus on cell implosion events	[78]
Nanocrystals of ZnO (< 100 nm)	Bioimaging of KB cells	[79]

ZnO nanoparticles show great superiority over other metal nanoparticles in the field of bioimaging because of these unique characteristics. Fluorescence-based bioimaging benefits greatly from their high photoluminescence, which increases visibility. Because ZnO nanoparticles are naturally biocompatible, there is less cytotoxicity, which is important to keep cells viable throughout imaging processes[80]. Furthermore, targeted imaging via particular ligand attachments is made possible by the surface functionalization of ZnO nanoparticles[81]. ZnO nanoparticles moreover exhibit remarkable stability throughout time, guaranteeing regular and dependable imaging outcomes for prolonged monitoring. In contrast to alternative metal nanoparticles, ZnO nanoparticles are a better option for bioimaging applications because of these combined properties.

6.2 Drug Delivery

NPs made from zinc oxide have been investigated as a potential choice for targeted drug delivery because of their ability to be easily synthesized from inexpensive precursors, their improving drug stability, their biocompatibility, and their efficient ingestion by endocytosis into cells. Targeted drug delivery methods have effectively employed porous ZnO structures, including porous nanotubes, porous nanobelts, porous nanorods, and porous cages. The enhanced cytotoxic potential of ZnO NPs loaded with anticancer drugs is based on the pH-dependent discharge of the targeted medication and ZnO NPs into the cytoplasm, which happens through receptor-mediated endocytosis. Additionally, ZnO NPs produce an excess of ROS and Zn^{2+} ions, which causes cancer cells to undergo apoptosis[82]. The in vivo toxicity evaluation of biologically synthesized doxorubicin-loaded ZnO NPs using Borassus fabellifer extract showed low cytotoxicity in a murine model system and dose-dependent cytotoxicity against human cancer of the breast (MCF7) as well as cancer of the colon (HT-29) cell lines alongside an IC50 value of 0.125 μg ml^{-1}[83].

The advantages of a ZnO nanoparticles-based drug delivery system(DDS) are; that it reduces the possibility of systemic toxicity by preventing the early release of the bound medication into the bloodstream, it enhances the water solubility and pharmacokinetics of hydrophobic medications[84], by active targeting it aids in the drug's transportation to the target cells or organs. It has few to no harmful effects on normal, healthy organs and tissues. The majority of ZnONP-based drug delivery applications are executed there is minimal in vivo data on the administration of the hydrophilic anticancer medication doxorubicin[85]. Furthermore, the majority of DDS are noted to display a pH-responsive drug release pattern. Several drug delivery systems that

utilize ZnO NPs are summarized in **Table 5**.

Table 5: Zinc Oxide Nanoparticles based drug delivery systems

Material Type	Drug	Cellular Model	Reference
Chitosan-encapsulated ZnO quantum dots	DOX	-	[86]
Acid degradable ZnO quantum dots	Doxorubicin	HeLa	[87]
ZnO/PEG nanocomposite	Doxorubicn	Gram-positive bacteria	[88]
ZnO quantum dots-conjugated Au nanoparticle	Camptothecin (CPT)	Hela cells	[89]
ZnO NPs embedded carboxymethyl cellulose (CMC)	Curcumin	Normal (L929) and cancer (MA104) cells	[90]
ZnO quantum dots	-	HepG2 cells	[91]

6.3 Anticancer Activity

The deadly illness that results from uncontrolled cell development is cancer. It is projected that there will be 16.5 million deaths caused by cancer and 29.5 million new cases of cancer diagnosed annually by 2040[92]. Despite long-standing conventional treatment methods and technical advancements, cancer still exists. Its many drawbacks, such as low bioavailability, unfavorable health consequences, and high cost, restrict the use of these techniques. Anticancer medications have been shown to harm the electron transport chain in mitochondria, which has been linked to the development of reactive oxygen species (ROS) in cells. As a result, it is known that these treatments may cause noticeable levels of ROS to arise[93]. Overabundance of ROS can also damage mitochondria and disrupt protein function, which can lead to cell death. Because ZnO NPs are capable of releasing dissolved ions of zinc into the cells, boosting the generation of ROS, and triggering the apoptotic signaling pathway, they can be somewhat deadly to cancer cells[94].

ZnO NPs can also target specific cancer cells because of their unique electrostatic properties. An excess of anionic phospholipids attracts ZnO NPs to cancer cells electrostatically, which promotes the cancer cells to take in ZnO NPs and cause cytotoxicity[95]. ZnO NPs can work inside tumor cells more easily because of their small size, which also helps them permeate and reside inside the cells. The following are possible mechanisms for ZnO NPs' targeted cytotoxicity against cancer cells: in an alkaline intracellular environment, ZnO NPs dissolve and release Zn^{2+} ions, which causes cancer cells to produce more ROS than normal cells and start intrinsic apoptotic pathway in mitochondria, which kills cancer cells[96]. ZnONP cytotoxicity is caused by a molecular pathway that includes disruptions to cellular lipids and proteins, damage to DNA, and the formation of ROS, which significantly increases the level of oxidative stress.

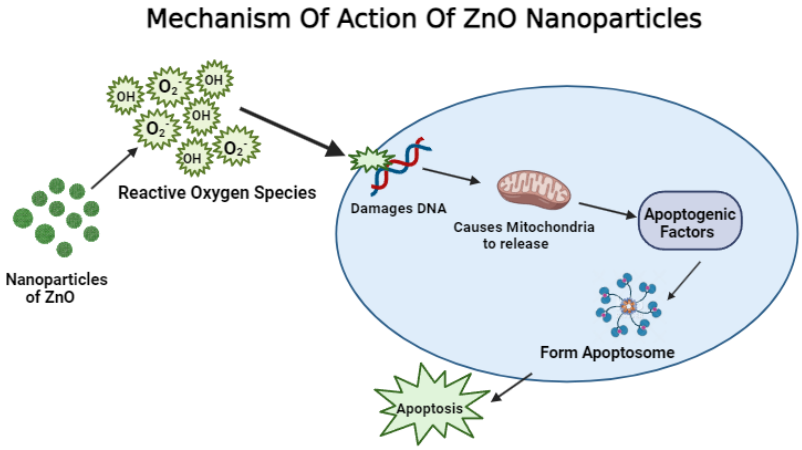


Figure 7. ZnO NPs' mechanism of action is as follows: DNA damage is caused by the ROS (reactive oxygen species) generated by ZnO NPs, and apoptogenic chemicals are released from the mitochondrial membrane to create apoptosomes, which in turn cause apoptosis.

ZnO NPs impacts and possible therapeutic processes were assessed using HepG2 human liver cancer cells. ZnO NPs were observed to enhance genotoxicity as well as cytotoxicity in HepG2 cell lines, which were attributed to mitochondrial malfunction brought on by ROS[97]. The activation of caspase enzymes and the release of apoptotic molecules of proteins into the cytosol, including cytochrome C, occur when the outer membrane pores open due to a loss of mitochondrial membrane potential. Due to their extreme potency and selectivity towards cancer cells, zinc oxide NPs, among many other NPs, are demonstrating promising applicability and efficacy in cancer therapy. ZnO nanoparticles are a promising anticancer agent because they can selectively target and kill cancer cells due to their enhanced permeability and retention (EPR) effect, electrostatic interaction, and selective cytotoxicity caused by increased ROS present in cancer cells[98].

6.4 Other Biomedical Applications

When applied as an ointment, green-synthesized ZnO NPs have shown a high degree of wound healing potential, according to various studies[99]. It has been shown that ZnO NPs formed biologically possess catalytic, enzyme-inhibition, anti-diabetic, and anticholinergic effects. ZnO NPs not only emit blue and near-UV light, but they also exhibit green or yellow fluorescence, which is indicative of oxygen vacancies. The nanoparticles of zinc oxide in the 20–80 nm size range are widely employed in commercial products like doping and catalysis because of their unique physicochemical characteristics[100]. ZnO NPs would affect the way certain cells and tissues function. The unique properties of ZnO NPs make them advantageous in a wide range of industries, including electronics, gas sensing, environmental remediation, catalysis, and biology. These applications also drive the use of ZnO NPs in commercial products.

7. Conclusion and Future Perspectives

This study examines the processes used in the synthesis, characteristics and uses of ZnO nanoparticles. These nanoparticles are made in a variety of ways and are notable for their strength and exceptional qualities. The utilization of green production of zinc oxide nanoparticles (ZnO NPs) in diverse biomedical applications was highlighted in this review, as a way to overcome the limitations of conventional chemical and physical procedures. Their structures are examined using procedures including XRD, and SEM. This review is expected to considerably advance the topic and make a contribution to the current study. To discover more about the most secure methods to synthesize ZnO NPs and their potential therapeutic uses, it is necessary to take into account the following points: toxic surfactants and costly precursors should not be used in the chemical synthesis of ZnO NPs, to produce ZnO NPs with excellent stability, alternative green synthesis techniques had to be used. It is important to thoroughly examine ZnO NP's anticancer properties utilizing suitable in vivo models. ZnO NPs have the potential to be highly effective drug delivery vehicles for the targeted distribution of hydrophilic, hydrophobic, and immunomodulatory medicines in cancer therapy; however, more study is needed to confirm this. In addition to PH responsiveness, possible medication release caused by light, ROS, and ultrasound should be investigated. To investigate the clinical usage of zinc oxide nanoparticles, additional risk studies through various exposure routes are required.

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Author's Contributions

The authors contributed equally to this work. All contributors played a substantial role in the conceptualization, design, and implementation of the review, including the examination and understanding of the data.

Conflict of Interest

The authors assert that they do not have any conflicts of interest concerning the content of this review article. There are no associations, either financial or personal, that might unduly affect or predispose the findings presented in this article.

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