Synthesis And Characterization of Biofunctionalized ZnO Nanoparticles and Their Biophysical Impact on Cancer Cells

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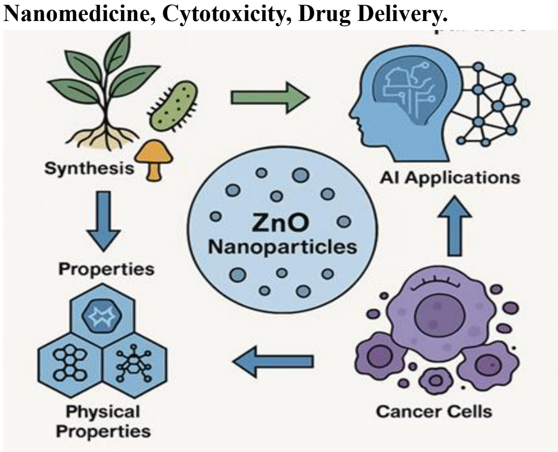
Abstract - Zinc oxide nanoparticles (ZnO NPs) exhibit unique physicochemical and biological properties, making them promising candidates in oncology. These characteristics make them good candidates for cancer diagnosis and treatment. Their small size, large surface area, and semiconducting properties allow them to interact effectively with cells. Additionally, their natural ability to produce reactive oxygen species (ROS) leads to selective toxicity against cancer cells. Current research heavily focuses on their synthesis. Green and biogenic methods are becoming more popular because they are eco-friendly, cost-effective, and safe for living organisms. These methods use plant extracts, microbes, or biomolecules, which reduce the need for harmful chemicals and improve the safety of ZnO NPs, thus lowering overall toxicity. In cells, ZnO NPs work against cancer by creating oxidative stress, disrupting mitochondrial function, and activating apoptosis pathways. Moreover, attaching proteins, polysaccharides, and therapeutic agents to ZnO NPs improves their stability, targeting ability, and drug delivery potential. The use of artificial intelligence (AI) and computer modeling has also transformed their applications, allowing for accurate predictions of how nanoparticles interact with cells, optimizing dosages, and developing personalized treatment strategies.

***Index Terms-* Zinc Oxide Nanoparticles, Precision Nanomedicine, Cytotoxicity, Drug Delivery.**



GRAPHICAL ABSTRACT: Role of ZnO Nanoparticle

I.INTRODUCTION

Nanotechnology has become an important area in biomedical research, offering new methods for diagnostics, treatments, and targeted drug delivery (Hegde, 2024). Among the different nanomaterials, zinc oxide (ZnO) nanoparticles have attracted significant attention because of their special physical, chemical, and biological properties (Parihar et al., 2018). These traits make them very useful in medical applications. Their wide band gap, photoluminescence, piezoelectric behavior, and semiconductor nature enable them to interact well with biological systems (Botello-Méndez et al., 2010). In addition, their ability to break down naturally and their relatively safe interactions with living organisms give them an edge over other metal oxide nanoparticles (Siddiqi et al., 2018).

In cancer research, ZnO nanoparticles are being studied as possible anticancer agents due to their ability to selectively kill cancer cells (Bisht & Rayamajhi, 2016). Their tendency to gather in tumors, generate reactive oxygen species (ROS), and activate cell death pathways helps them effectively stop the growth of cancer cells (Aalami et al., 2020). Furthermore, scientists can modify the surfaces of ZnO nanoparticles through functionalization or green synthesis, improving their stability, targeting ability, and effectiveness while reducing side effects (Alghamdi et al., 2023).

The investigation of ZnO nanosystems also includes biogenic and plant-based synthesis methods. These approaches lower chemical risks and add bioactive surface coatings that work together with the natural cancer-fighting properties of ZnO (Sportelli et al., 2022). Additionally, studying their interactions with animal models like *Drosophila melanogaster*, *C. elegans*, *Danio rerio*, and mice offers important information about their effectiveness, how they work in living organisms, and their safety (Brun et al., 2014).

Recent progress in computational biology and artificial intelligence (AI) has broadened the research opportunities for ZnO nanoparticles. These advancements allow for predictive modeling of toxicity, improvements in drug delivery systems, and applications in precision medicine (Mazumdar et al., 2025). Such integrated methods are crucial for addressing current challenges in turning lab discoveries into effective cancer treatments.

II. STRUCTURAL AND PHYSICO CHEMICAL PROPERTIES OF ZnO NANOPARTICLES.

2.1 Chemical Characteristics of Zinc Oxide Nanoparticles:

1.Amphoteric Nature:

Zinc oxide nanoparticles exhibit amphoteric behavior, meaning they can react both as an acid and as a base. They react with strong acids such as hydrochloric acid (HCl) to form zinc salts like $ZnCl_2$, while in the presence of strong bases such as sodium hydroxide (NaOH), they form zincate ions such as (Zn(OH)4)2−. This amphoteric nature contributes to their versatility in chemical and biological environments (Parihar et al., 2018).

2. Redox Activity:

ZnO nanoparticles participate in oxidation–reduction reactions, particularly under ultraviolet (UV) irradiation. During these reactions, reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydroxyl radicals ($OH\bullet$), and hydrogen peroxide (H_2O_2)are generated. These ROS play an important role in antimicrobial and anticancer activities of ZnO nanoparticles (Siddiqi et al., 2018).

3. Surface Reactivity:

The surface of ZnO nanoparticles contains abundant hydroxyl groups, which enable interactions with biomolecules, pollutants, and microbial cell membranes. This high surface reactivity enhances their catalytic, antibacterial, and environmental remediation properties (Sportelli et al., 2022).

4. Photocatalytic Chemical Behavior:

ZnO nanoparticles exhibit strong photocatalytic properties when exposed to UV or visible light. Under light irradiation, electron–hole pairs are generated on the nanoparticle surface, leading to the production of reactive oxygen species that degrade organic dyes, pollutants, and microbial biofilms. This photocatalytic activity makes ZnO nanoparticles useful in environmental purification and biomedical applications (Siddiqi et al., 2018).

2.2 Structural and Physical Characteristics of Zinc Oxide Nanoparticles:

Zinc oxide (ZnO) nanoparticles possess several unique physical characteristics that make them highly valuable in biomedical, environmental, and technological applications. These properties arise mainly due to their nanoscale dimensions, crystal structure, and electronic configuration. The reduction in particle size to the nanometer scale significantly increases the surface area and alters the optical, electrical, and magnetic behavior of the material compared with bulk ZnO (Parihar et al., 2018).

1.Crystal Structure:

ZnO nanoparticles typically exhibit a hexagonal wurtzite crystal structure, which is considered the most stable form under normal environmental conditions. In this structure, each zinc ion is tetrahedrally coordinated with four oxygen atoms, forming a highly ordered lattice. This structural arrangement contributes to the material’s stability, high electron mobility, and efficient charge transport properties, which are important for semiconductor and biomedical applications (Parihar et al., 2018).

2. Particle Size and Surface Area:

The size of ZnO nanoparticles generally ranges between 1 and 100 nm. At this nanoscale range, the particles possess a very high surface area-to-volume ratio, which enhances their surface reactivity and catalytic efficiency. The increased surface area allows ZnO nanoparticles to interact more effectively with biological molecules, pollutants, and microbial cells, making them useful in drug delivery, biosensing, and antimicrobial applications (Siddiqi et al., 2018).

3. Optical Properties:

ZnO nanoparticles exhibit remarkable optical properties, including strong photoluminescence and high transparency in the visible light region. These properties are mainly attributed to their wide band gap energy of approximately 3.37 eV and high exciton binding energy. Because of these features, ZnO nanoparticles are widely used in optoelectronic devices, ultraviolet (UV) detectors, biosensors, and imaging systems in biomedical research (Botello-Méndez et al., 2010).

4. Electrical and Semiconductor Properties: ZnO nanoparticles function as n-type semiconductors due to intrinsic defects such as oxygen vacancies and zinc interstitials present within their crystal lattice. These defects contribute to enhanced electrical conductivity and electron transport. Such semiconductor behavior makes ZnO nanoparticles useful in the fabrication of sensors, transistors, and nanodevices for biomedical and environmental monitoring applications (Parihar et al., 2018).

5. Magnetic Properties:

Although bulk ZnO is generally considered non-magnetic, ZnO nanoparticles may exhibit weak magnetic properties when structural defects or impurities are present in the crystal lattice. These defect-induced magnetic characteristics can influence electronic and catalytic behavior and may have potential applications in spintronics and advanced biomedical technologies (Garcia et al., 2007).

Overall, the structural and physical characteristics of ZnO nanoparticles play a crucial role in determining their functionality and performance in various scientific and industrial applications. Their nanoscale dimensions, combined with unique optical, electronic, and structural properties, make them promising materials for future developments in nanomedicine and biotechnology (Siddiqi et al., 2018).

2.3 Biological Interactions and Biophysical Behavior of Zinc Oxide Nanoparticles:

Zinc oxide (ZnO) nanoparticles possess remarkable biophysical characteristics that influence their interaction with biological systems. Due to their nanoscale size, high surface area, and unique physicochemical properties, these nanoparticles can easily interact with biomolecules, cell membranes, and intracellular structures. Such interactions play a crucial role in determining their biological effects, including antimicrobial activity, anticancer potential, and biomedical compatibility. The ability of ZnO nanoparticles to penetrate biological barriers and interact with cellular components has made them an important subject of study in nanomedicine and biotechnology (Siddiqi et al., 2018).

1.Interaction with Cellular Membranes:

ZnO nanoparticles can attach to and interact with cellular membranes because of their small size and high surface reactivity. These interactions may alter membrane permeability and facilitate cellular uptake through mechanisms such as endocytosis. Once internalized, the nanoparticles can influence several cellular processes including metabolic activity and signal transduction pathways. Such membrane interactions play a vital role in determining the biological responses of cells exposed to ZnO nanoparticles (Ng et al., 2017).

2. Generation of Reactive Oxygen Species (ROS):

One of the most significant biophysical behaviors of ZnO nanoparticles is their ability to produce reactive oxygen species (ROS). These include superoxide radicals, hydroxyl radicals, and hydrogen peroxide. The generation of ROS can cause oxidative stress within cells, leading to damage of important biomolecules such as DNA, proteins, and lipids. This oxidative stress mechanism is widely considered responsible for the antimicrobial and anticancer activities of ZnO nanoparticles (Aalami et al., 2020).

3. Cellular Uptake and Intracellular Distribution:

ZnO nanoparticles can enter cells through various pathways such as endocytosis, phagocytosis, or passive diffusion. After internalization, they may accumulate in different cellular compartments including the cytoplasm, mitochondria, lysosomes, or nucleus. Their presence within these organelles can influence mitochondrial function, enzyme activity, and cellular signaling pathways, which ultimately affect cell survival or programmed cell death (Murali et al., 2021).

4. Cytotoxic and Anticancer Mechanisms:

The interaction of ZnO nanoparticles with cancer cells often results in selective cytotoxicity. These nanoparticles can induce oxidative stress, disrupt mitochondrial function, and activate apoptotic signaling pathways. Such mechanisms lead to the inhibition of tumor cell proliferation and the destruction of malignant cells. Because of these properties, ZnO nanoparticles are widely investigated as potential nanotherapeutic agents for cancer treatment and targeted drug delivery (Bisht & Rayamajhi, 2016).

5. Biocompatibility and Biological Safety:

Although ZnO nanoparticles can induce cytotoxic effects in harmful or abnormal cells, they can also demonstrate relatively good biocompatibility under controlled concentrations. Their biodegradability allows them to dissolve gradually into zinc ions, which are essential trace elements required for several biological processes. This characteristic makes ZnO nanoparticles promising materials for biomedical applications such as biosensors, tissue engineering, and therapeutic drug delivery systems (Hegde, 2024).

III. GREEN AND BIOGENIC SYNTHESIS OF ZnO NANOPARTICLES

Green and biogenic synthesis of zinc oxide (ZnO) nanoparticles has gained considerable attention in recent years as an environmentally friendly and sustainable alternative to conventional physical and chemical synthesis methods. Traditional nanoparticle synthesis techniques often involve toxic chemicals, high energy consumption, and complex procedures that may pose risks to human health and the environment. In contrast, green synthesis utilizes biological materials such as plant extracts, microorganisms, and natural biomolecules as reducing and stabilizing agents, making the process safer, cost-effective, and eco-friendly (Melese et al., 2024).

Plant-mediated synthesis, also known as phytosynthesis, is one of the most widely used approaches for the green production of ZnO nanoparticles. In this method, plant extracts obtained from leaves, roots, stems, flowers, or fruits contain various bioactive compounds such as flavonoids, phenolics, alkaloids, terpenoids, and proteins. These phytochemicals play an important role in the reduction of zinc ions into zinc oxide nanoparticles and also act as stabilizing or capping agents that prevent aggregation of nanoparticles (Elumalai et al., 2015). For example, extracts from plants such as *Celosia argentea*, *Lepidium sativum*, and *Pandanus odorifer* have been successfully used for the synthesis of ZnO nanoparticles with enhanced antioxidant, antibacterial, and anticancer activities (Alghamdi et al., 2023; Meer et al., 2022; Hussain et al., 2019).

Microbial-mediated synthesis is another important biogenic method used for the production of ZnO nanoparticles. Various microorganisms such as bacteria, fungi, and algae have the ability to synthesize nanoparticles through enzymatic reactions and metabolic processes. Microorganisms like *Aeromonas hydrophila* and *Lactobacillus* species have been reported to produce ZnO nanoparticles with significant antimicrobial properties (Jayaseelan et al., 2012; Suba et al., 2021). In microbial synthesis, enzymes and proteins secreted by the microorganisms facilitate the reduction of metal ions and control the size and shape of the nanoparticles.

Biogenic synthesis methods offer several advantages compared with conventional synthesis techniques. These methods generally occur under mild reaction conditions such as room temperature and neutral pH, reducing the need for high energy input. Additionally, the biological molecules present in plant extracts or microbial systems act as natural capping agents, improving nanoparticle stability and preventing agglomeration. This results in nanoparticles with better dispersion, controlled morphology, and enhanced biological activity (Sportelli et al., 2022).

Another important advantage of green synthesis is its potential to enhance the biomedical properties of ZnO nanoparticles. The bioactive compounds from plant extracts may remain attached to the surface of nanoparticles, forming a natural coating that can improve their antimicrobial, antioxidant, and anticancer properties. Such biofunctionalized nanoparticles have shown promising results in biomedical applications including drug delivery, biosensing, and cancer therapy (Dwivedi et al., 2024).

Overall, green and biogenic synthesis methods provide a sustainable and efficient approach for the production of ZnO nanoparticles. These approaches reduce environmental impact, eliminate the use of hazardous chemicals, and produce nanoparticles with enhanced biological compatibility. As research in nanobiotechnology continues to advance, green synthesis techniques are expected to play an increasingly important role in the development of safe and effective nanomaterials for biomedical and environmental applications.

Figure 1. systhesis of ZnO Nanoparticles

IV. ANTICANCER ACTIVITIES OF ZnO NANOPARTICLES

Zinc oxide (ZnO) nanoparticles have attracted considerable attention in recent years for their potential applications in cancer therapy. Due to their unique physicochemical properties, such as a high surface area, semiconductor nature, and the ability to generate reactive oxygen species (ROS), ZnO nanoparticles exhibit promising anticancer activities (Bisht & Rayamajhi, 2016).

One of the major mechanisms responsible for the anticancer activity of ZnO nanoparticles is the generation of reactive oxygen species. When ZnO nanoparticles interact with cancer cells, they can produce ROS such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide. These reactive molecules induce oxidative stress inside the cancer cells, leading to damage of important biomolecules including DNA, proteins, and lipids. The excessive oxidative stress ultimately triggers apoptosis, or programmed cell death, which helps inhibit the proliferation of cancer cells (Aalami et al., 2020).

Another important factor contributing to the anticancer properties of ZnO nanoparticles is their ability to accumulate within tumor cells. Due to their nanoscale size, ZnO nanoparticles can easily penetrate cellular membranes and enter the intracellular environment through endocytosis. Once inside the cells, they may localize in various organelles such as mitochondria and lysosomes. This intracellular accumulation can disrupt mitochondrial function, interfere with cellular metabolism, and activate signaling pathways that lead to apoptosis or necrosis of cancer cells (Murali et al., 2021).

Surface modification and green synthesis techniques further enhance the anticancer efficiency of ZnO nanoparticles. When synthesized using plant extracts or biological materials, the nanoparticles may carry natural bioactive compounds on their surface. These compounds can improve the stability, targeting ability, and therapeutic performance of ZnO nanoparticles. Additionally, surface functionalization allows nanoparticles to be engineered for targeted drug delivery, enabling them to deliver anticancer drugs directly to tumor tissues while minimizing side effects on healthy cells (Alghamdi et al., 2023).

ZnO nanoparticles have also demonstrated significant cytotoxic effects against various types of cancer cells, including breast cancer, lung cancer, and cervical cancer cells. Experimental studies have shown that these nanoparticles can effectively inhibit tumor cell growth and proliferation through oxidative stress, mitochondrial damage, and activation of apoptotic pathways. Such findings highlight the potential of ZnO nanoparticles as promising agents for cancer diagnosis and therapy (Ng et al., 2017).

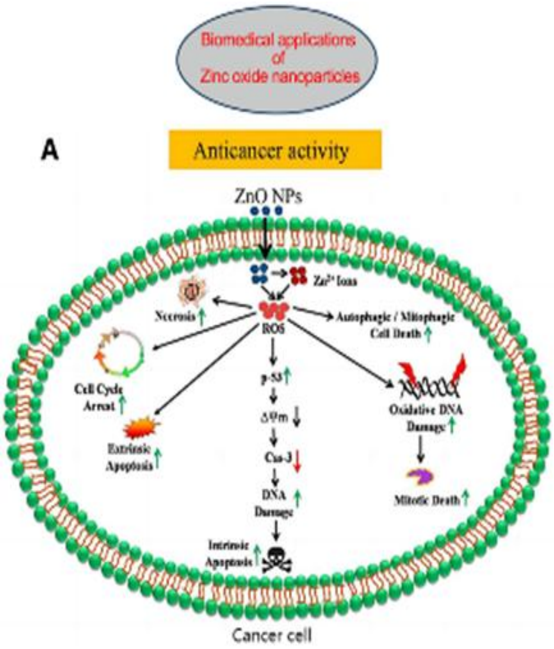


FIGURE 2: Biomedical application of Zinc Oxide Nanoparticles.

V. IN VIVO STUDIES OF ZnO NANOPARTICLES USING ANIMAL MODELS

In vivo studies play a crucial role in understanding the biological behavior, therapeutic potential, and safety of zinc oxide (ZnO) nanoparticles in living organisms. While in vitro experiments provide valuable preliminary information about the biological activity of nanoparticles, in vivo models allow researchers to evaluate their interactions within complex biological systems. Animal models such as mice, zebrafish (*Danio rerio*), fruit flies (*Drosophila melanogaster*), and nematodes (*Caenorhabditis elegans*) are widely used to study the toxicity, biodistribution, and biomedical applications of ZnO nanoparticles (Brun et al., 2014).

One of the primary objectives of in vivo studies is to evaluate the toxicity and biocompatibility of ZnO nanoparticles. These nanoparticles may enter the body through different routes such as inhalation, ingestion, or injection. Once inside the organism, they can interact with tissues, organs, and cellular components. Studies conducted on mice have shown that prolonged exposure to ZnO nanoparticles may influence physiological processes such as mineral metabolism and organ function. These investigations help researchers determine safe dosage levels and understand the potential risks associated with nanoparticle exposure (Wang et al., 2016).

Zebrafish (*Danio rerio*) have emerged as an important model organism for studying the developmental and toxicological effects of ZnO nanoparticles. Due to their transparent embryos and rapid development, zebrafish provide a convenient system for observing nanoparticle distribution and biological effects in real time. Research has shown that exposure to ZnO nanoparticles can affect embryonic development, oxidative stress responses, and cellular metabolism in zebrafish, offering valuable insights into nanoparticle toxicity and environmental impact (Brun et al., 2014).

Another widely used model organism is the fruit fly (*Drosophila melanogaster*), which is frequently employed in genetic and toxicological studies. Investigations using *Drosophila* have demonstrated that ZnO nanoparticles can induce oxidative stress and DNA damage in cells through the generation of reactive oxygen species. These studies are important for understanding the molecular mechanisms underlying nanoparticle-induced toxicity and their potential effects on living organisms (Ng et al., 2017).

Similarly, the nematode *Caenorhabditis elegans* is used to study the environmental and biological effects of ZnO nanoparticles. This organism is particularly useful because of its simple anatomy, short life cycle, and well-characterized genetic system. Studies involving *C. elegans* have shown that ZnO nanoparticles can influence growth, reproduction, and survival rates depending on the concentration and exposure duration. These findings help researchers evaluate the ecological and biological safety of ZnO nanoparticles (Gupta et al., 2015).

In addition to toxicity evaluation, animal models are also used to investigate the therapeutic potential of ZnO nanoparticles. In cancer research, nanoparticle-based drug delivery systems are tested in animal models to assess their ability to target tumor tissues, reduce tumor growth, and improve treatment efficiency. These studies provide essential information about nanoparticle biodistribution, pharmacokinetics, and therapeutic effectiveness in living systems.

VI. ROLE OF ARTIFICIAL INTELLIGENCE IN ZnO NANOPARTICLE DRUG DEVELOPMENT

Artificial intelligence (AI) has emerged as a powerful tool in modern biomedical research, significantly transforming the field of nanomedicine. In the development of zinc oxide (ZnO) nanoparticle-based drug delivery systems, AI and computational approaches play an important role in improving the design, optimization, and evaluation of nanoparticles. These technologies help researchers analyze large datasets, predict nanoparticle behavior, and accelerate the discovery of effective nanotherapeutic strategies for various diseases, including cancer (Mazumdar et al., 2025).

One of the major applications of AI in ZnO nanoparticle drug development is in the prediction of nanoparticle properties and behavior. Machine learning algorithms can analyze experimental data to predict the physicochemical properties of nanoparticles such as particle size, surface charge, stability, and toxicity. These predictive models allow researchers to design nanoparticles with optimized characteristics suitable for drug delivery and therapeutic applications. By reducing the need for extensive experimental trials, AI significantly saves time and research costs (Mazumdar et al., 2025).

Another important contribution of AI is in the optimization of nanoparticle synthesis and formulation. In traditional nanoparticle development, researchers often rely on trial-and-error methods to determine the best synthesis conditions. However, AI-based models can evaluate multiple parameters such as temperature, pH, precursor concentration, and reaction time to identify optimal conditions for producing ZnO nanoparticles with desired properties. This computational approach improves reproducibility and efficiency in nanoparticle synthesis (Asmaz et al., 2024).

AI also plays a crucial role in the development of targeted drug delivery systems using ZnO nanoparticles. Through advanced data analysis and modeling techniques, AI can identify specific molecular targets associated with diseases such as cancer. This information helps researchers design nanoparticle-based drug carriers that selectively deliver therapeutic agents to diseased tissues while minimizing damage to healthy cells. Such targeted delivery systems improve treatment effectiveness and reduce side effects, which is a major goal in modern cancer therapy (Sengar, 2024).

In addition, computational biology and AI techniques are widely used for toxicity prediction and safety evaluation of ZnO nanoparticles. Before clinical applications, it is essential to assess the potential toxic effects of nanoparticles on biological systems. AI-driven predictive models can analyze biological data to estimate nanoparticle toxicity, biodistribution, and interaction with cells and tissues. This helps researchers identify potential risks at an early stage and design safer nanomaterials for medical applications (Mazumdar et al., 2025).

Furthermore, AI is increasingly being used to support precision medicine in nanotechnology-based therapies. By integrating patient-specific data such as genetic information, disease biomarkers, and clinical history, AI systems can help design personalized nanoparticle treatments. ZnO nanoparticle-based drug delivery systems can be optimized according to individual patient conditions, leading to more effective and personalized therapeutic strategies (Sengar, 2024).

VII. CONCLUSION

Zinc oxide (ZnO) nanoparticles have emerged as highly promising nanomaterials in the field of nanotechnology and biomedical research. Their unique physicochemical characteristics, including a wide band gap, large surface area, semiconductor behavior, and strong photocatalytic activity, make them suitable for a wide range of scientific and medical applications. These properties enable ZnO nanoparticles to interact effectively with biological systems, making them valuable in areas such as drug delivery, antimicrobial treatments, biosensing, and cancer therapy.

In recent years, significant advancements have been made in the synthesis and application of ZnO nanoparticles. While conventional physical and chemical synthesis methods have been widely used, green and biogenic synthesis approaches have gained increasing attention due to their environmentally friendly nature and reduced toxicity. Plant-mediated and microbial synthesis methods utilize natural biomolecules as reducing and stabilizing agents, producing nanoparticles with improved stability and biological compatibility. These eco-friendly methods also reduce the use of hazardous chemicals and provide sustainable alternatives for nanoparticle production.

The biomedical potential of ZnO nanoparticles is particularly evident in cancer research. Their ability to generate reactive oxygen species, induce oxidative stress, and activate apoptotic pathways allows them to selectively target and destroy cancer cells. In addition, surface modification and functionalization techniques enable ZnO nanoparticles to serve as efficient drug delivery carriers. These systems can deliver therapeutic agents directly to tumor tissues, improving treatment efficiency while minimizing harmful effects on healthy cells.

Studies using animal models have provided valuable insights into the biological interactions, distribution, and safety of ZnO nanoparticles within living systems. Research involving organisms such as mice, zebrafish, fruit flies, and nematodes helps scientists understand how nanoparticles behave in complex biological environments. These investigations are essential for evaluating the safety and therapeutic effectiveness of ZnO nanoparticles before their application in clinical settings.

Recent developments in computational biology and artificial intelligence have further expanded the potential of ZnO nanoparticle research. Advanced computational tools can assist in predicting nanoparticle properties, optimizing synthesis conditions, and designing targeted drug delivery systems. These technologies also help in evaluating toxicity and improving the efficiency of nanoparticle-based therapies.

Overall, ZnO nanoparticles represent versatile and powerful nanomaterials with significant potential in biomedical and therapeutic applications. Continued research focusing on safe synthesis techniques, detailed biological evaluation, and advanced computational strategies will further enhance their role in nanomedicine. With ongoing scientific progress, ZnO nanoparticles are expected to contribute greatly to the development of innovative diagnostic and therapeutic approaches for cancer and other diseases.

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