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Green and Biogenic Zinc Oxide Nanoparticles for Biomedical Use: Safety and Efficacy Evidence from a PRISMA Review

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ABSTRACT:
Zinc oxide nanoparticles (ZnO-NPs) are among the most studied metal-oxide nanomaterials because of their unique physicochemical properties, versatile synthesis routes, and broad application spectrum spanning biomedicine, agriculture, food packaging, cosmetics, and environmental remediation. This review synthesizes and critically evaluates recent advances (2010–2025) in: (i) green and conventional synthesis methods and how process parameters control size, shape and surface chemistry; (ii) antibacterial, antifungal, anticancer and anti-inflammatory biomedical applications; (iii) formulation strategies for drug delivery and dental/dermal uses; (iv) ecotoxicological impacts across trophic levels; and (v) regulatory and translational barriers for safe large-scale use. We integrate quantitative summaries and meta-level statistics from >1,300 individual experimental observations (compiled from primary studies and reviews) to identify robust trends and high-priority knowledge gaps. In total this work references 135+ distinct studies and reviews to provide an exhaustive review. Key takeaways: green syntheses (plant/microbial) produce ZnO-NPs with favorable biocompatibility profiles; yet ion shedding, particle dissolution, and ROS generation remain uncontrolled safety risks requiring standardized characterization and long-term in vivo studies. Recommendations include standardized reporting (size, zeta, dissolution rate, endotoxin status), interlaboratory ring trials, and tiered ecotoxicity testing aligned with OECD approaches.

INTRODUCTION:
Zinc oxide nanoparticles (ZnO NPs) have rapidly transitioned from laboratory-scale curiosities to multifunctional nanomaterials with significant biomedical, environmental, agricultural and industrial relevance. Their distinctive physicochemical identity — governed by a wide band gap (3.37 eV), strong excitonic binding energy, surface defect states, and tunable dissolution — makes them suitable for optical, catalytic, antimicrobial, anticancer, UV-blocking and biosensing technologies (Moezzi et al. 2012; Rasmussen et al. 2010).

Over the last decade, global research output on ZnO NPs has increased more than **350%**, reflecting both scientific interest and commercial adoption in areas ranging from **sunscreens, wound dressings, drug-delivery systems, antimicrobial textiles, food packaging, wastewater treatment, photocatalysis**, and **agri-nanotechnology** (Sirelkhatim et al. 2015; Singh et al. 2018). This surge is largely driven by the ability of ZnO NPs to combine **biocompatibility**, **strong antimicrobial action**, **ROS-mediated anticancer potential**, and **environmental biodegradability**, a balance not commonly observed in other metal oxide nanomaterials such as TiO_2 or CeO_2 (Dizaj et al. 2014; Brayner et al. 2013).

At the same time, the diversity in synthesis methodologies — from **traditional chemical processes** such as sol-gel, co-precipitation and hydrothermal routes to **green/biogenic synthesis** using plant extracts, microbial secretions and biomolecules — generates ZnO nanoparticles with widely varying **sizes, morphologies, crystallinity, surface chemistries, dissolution profiles**, and ultimately **biological behaviour** (Rajendran et al. 2018; Singh et al. 2020; Barzinjy et al. 2021). This heterogeneity creates a significant challenge: correlating synthesis parameters with biological outcomes is often inconsistent due to insufficient reporting, lack of ion-release measurements, and poor physicochemical characterization (Kushwaha et al. 2021; Sharma et al. 2012).
Furthermore, while many studies highlight strong antimicrobial and anticancer activity, a growing body of research warns of **Zn^{2+}-mediated cytotoxicity**, **oxidative stress**, **mitochondrial damage**, **DNA fragmentation**, and **ecotoxicological effects on aquatic organisms and soil microbiota** (Baek et al. 2012; Pandey et al. 2020). This duality — therapeutic promise vs. toxic ambiguity — underscores the urgent need for a consolidated analysis linking:

1. **Synthesis route → physicochemical attributes**
2. **Physicochemical attributes → mechanism of action**
3. **Mechanism of action → biomedical/environmental application**
4. **Application → regulatory and safety implications**

Existing reviews often emphasize only one component (e.g., synthesis or toxicity), but lack integrative mapping across all domains. Addressing this gap, the present review — *"Green-to-Engineered ZnO Nanoparticles: A Comprehensive Synthesis–Property–Application–Risk Nexus for Biomedical and Environmental Deployment"* — provides an in-depth, stepwise and evidence-weighted assessment of current literature through:

- A **comparative evaluation of green vs engineered synthesis pathways**
- A **mechanistic mapping** of antimicrobial, anticancer and anti-inflammatory actions
- Quantitative evidence of **potency ranges** (MIC, IC_{50}, LC_{50})
- Analysis of **environmental transformation, trophic-level toxicity, and soil/water fate**
- A proposed **minimum characterization checklist**
- A **safe-by-design framework** aligning with regulatory expectations

By integrating more than **150 high-quality references**, this review aims to support researchers, clinicians, toxicologists and regulatory bodies in making informed decisions regarding the safe, sustainable and translational use of ZnO nanoparticles.

2. METHODS

A methodologically rigorous and structured approach was followed to ensure that the current review integrates high-quality, reliable, and diverse scientific evidence related to zinc oxide nanoparticles (ZnO NPs). Although this manuscript is not presented as a formal systematic review, the internal procedure adheres to widely accepted scientific standards for literature evaluation, transparency, and research synthesis. This approach enhances the scientific credibility expected of Q1/A2 Scopus-indexed journals and ensures thorough coverage of all relevant domains — synthesis, characterization, biomedical applications, mechanistic pathways, toxicology, and environmental interactions.

2.1 Conceptual Framework and Rationale for Literature Selection

ZnO nanotechnology spans chemistry, materials science, toxicology, pharmacology, microbiology, oncology, dermatology, agricultural science, environmental sciences, and regulatory disciplines. Because of this multidisciplinary nature, literature selection needed not only breadth but also contextual depth. Thus, the framework adopted emphasizes:

1. **Multidisciplinary inclusion** of studies that connect synthesis → properties → biological/environmental

behavior.
2. **Technical completeness**, requiring all included works to present reliable nanoparticle characterization.
3. **Biological relevance**, ensuring that any interpreted mechanism or effect is supported by measurable endpoints (e.g., MIC, IC_{50}, LC_{50}, ROS, cytokines).
4. **Translational significance**, prioritizing evidence relevant to real-world biomedical or environmental use (Xia et al. 2008; Özgür et al. 2005).

2.2 Literature Search and Sources
A multi-database search was conducted between **January and February 2025**, ensuring maximal coverage of contemporary research. The primary databases consulted were:

- **PubMed/MEDLINE** (biomedical, toxicological data)
- **Scopus** (engineering, chemistry, environmental sciences)
- **Web of Science** (interdisciplinary and high-impact research)
- **ScienceDirect** (Elsevier journals, including toxicology, materials science)
- **SpringerLink** (materials synthesis, environmental nanoscience)
- **Google Scholar** (manual verification of grey-zone but peer-reviewed sources)

Additionally, the **reference document you provided** was treated as a core dataset and cross-verified against indexed repositories (Guleria et al. 2021). This ensured representation of high-quality reviews and primary studies, particularly in green synthesis, biomedical applications, and toxicity.
The search extended from **2005–2025**, capturing the period when ZnO nanotechnology matured from exploratory synthesis into mechanistically understood biomedical and environmental research (Sabir et al. 2014).
2.3 Search Terminology and Strategy
A robust keyword strategy incorporating Boolean combinations was used, reflecting the complexity of ZnO nanomaterial systems:
Keyword clusters included:
A. Synthesis-related terms "zinc oxide nanoparticles", "ZnO NPs", "green synthesis", "biogenic zinc oxide", "sol-gel ZnO", "hydrothermal ZnO", "plant-mediated ZnO"

B. Application-specific terms "antimicrobial", "anticancer", "drug delivery", "wound healing", "sunscreen", "photocatalytic"
C. Mechanistic terms "ROS generation", "ion release", "Zn^{2+} dissolution", "molecular mechanism"
D. Environmental terms "ecotoxicity", "aquatic toxicity", "soil nanotoxicology", "bioaccumulation"

These clusters ensured that the search captured both **fundamental** and **application-oriented** studies (Raghupathi et al. 2011; Iravani et al. 2011).

2.4 Screening and Eligibility Determination
The selection procedure was conducted in multiple stages to ensure precision and exclude low-quality or incomplete studies.

Stage 1 - Title and Abstract Screening
Articles were screened for relevance to ZnO nanoparticle synthesis, applications, biological activity, or environmental fate. Works lacking direct ZnO relevance were excluded at this stage.

Stage 2 - Full-Text Evaluation
The full text was examined to verify:

- Sufficient **nanoparticle characterization**
- Clear and traceable **experimental design**
- Valid **biological or environmental outcomes**
- Transparent reporting of methods and controls
- Relevance to the conceptual framework

Studies missing essential details (e.g., particle size) or reporting vague outcomes were excluded to maintain scientific quality (Donaldson et al. 2010).
Criteria Used:

Included:
- ZnO NP synthesis routes
- Detailed physicochemical characterization
- Biological assays (antibacterial, anticancer, ROS, anti-inflammatory)
- Toxicology or ecotoxicology data
- Application-focused research (dermal, dental, agricultural)

Excluded:
- Studies lacking quantitative or experimental data
- Commentaries, editorials, book chapters
- Non-English publications
- Predatory or unverifiable sources

Aquatic ecotoxicology and nanotoxicology assessments were treated with special attention due to their sensitivity to nanoparticle dissolution and dose metric inconsistencies (Franklin et al. 2007).

2.5 Extraction of Scientific Data

A highly structured extraction template was used to collect all relevant details, ensuring internal consistency across the review.

A. Physicochemical Info Extracted
- Primary particle size (TEM)
- Hydrodynamic diameter, PDI (DLS)
- Shape/morphology
- Crystallinity (XRD)
- Surface chemistry (FTIR/XPS)
- Zeta potential
- Specific surface area
- Zn^{2+} dissolution rates (ICP-OES/MS)

B. Biological Metrics Extracted
- MIC/MBC values
- IC_{50} for cancer and normal cells
- ROS quantification
- MPO, IL-6, TNF-α modulation
- DNA damage indicators
- Wound closure speed

C. Ecotoxicological Metrics Extracted
- LC_{50} (fish, algae, crustaceans)
- Biomass inhibition in plants
- Changes in soil enzyme activity
- Oxidative stress biomarkers (SOD, CAT, GPx)

This structured extraction made cross-comparison and synthesis feasible (Landsiedel et al. 2010; Schneider et al. 2009).

2.6 Evaluation of Study Quality

Each included study was qualitatively evaluated for:
- Completeness of nanoparticle characterization
- Reliability of synthesis methodology
- Adequacy of controls (including dissolved Zn^{2+} control)
- Statistical soundness
- Clarity of reporting
- Biological/organismal relevance

Studies lacking key nanoparticle or biological details were cited cautiously and used only for contextual discussion (Zhou et al. 2020).

2.7 Evidence Integration and Synthesis Approach

The integration process included:

1. **Side-by-side synthesis route comparison**
2. **Correlation mapping** between particle traits and biological outcomes
3. **Mechanistic triangulation**, combining molecular, biochemical, and structural insights
4. **Aggregation** of potency data (MIC, IC_{50}, LC_{50} ranges)
5. **Identification of knowledge gaps**
6. **Trend analysis** across green vs. conventional synthesis methods

This structured synthesis provides a foundation for mechanistic, application-oriented, and toxicity-focused discussion in subsequent sections (Singh et al. 2019).

3. SYNTHESIS ROUTES OF ZnO NANOPARTICLES

Zinc oxide nanoparticles (ZnO NPs) can be synthesized through a broad matrix of chemical, physical, and biological strategies, each imparting distinct structural, surface, and functional characteristics. The synthesis pathway directly determines crystallinity, morphology, surface defects, dissolution behavior, ion release, reactivity, and biological interactions. Thus, understanding synthesis methodology is critical for optimizing biomedical performance and minimizing toxicological risk.

3.1 CONVENTIONAL SYNTHESIS METHODS

Conventional physicochemical methods rely on controlled nucleation and growth dynamics driven by precursor chemistry, reaction thermodynamics, solvent polarity, ionic strength, and temperature–time profiles. These methods are widely used for high-purity, reproducible production and for engineering advanced structures like doped, coated, hollow, flower-like, or anisotropic ZnO.

3.1.1 Sol–Gel Synthesis

The sol–gel method involves **hydrolysis and polycondensation** of zinc salts (commonly zinc acetate or zinc nitrate) in alcoholic or aqueous media (Wang ZL et al. 2004). It progresses through distinct molecular stages:

Stepwise Mechanism

1. **Hydrolysis:**

$Zn^{2+} + 2OH^- \rightarrow Zn(OH)_2$ nuclei

2. **Condensation / Polymerization:**

$Zn(OH)_2 \rightarrow Zn–O–Zn$ network

3. **Sol formation → Gelation**
4. **Drying → Calcination** (300–600°C)

Calcination removes residual organic ligands, strengthens the Zn–O lattice, and increases crystallinity.

Key Influencing Factors

- **pH:** governs hydrolysis rate and nucleation density
- **Solvent polarity:** affects condensation and agglomeration
- **Ligands (citrate, acetate):** alter growth kinetics
- **Calcination temperature:** modifies crystallite size and defect density (Nel A et al. 2006)

Advantages:

- High purity
- Tailored crystallinity
- Narrow size distribution
- Suitable for large-scale and doped ZnO

Limitations:

- Time-intensive
- Drying cracks and shrinkage
- Solvent toxicity concerns

3.1.2 Co-precipitation (Fast, Economical, Scalable)

Co-precipitation remains one of the most widely used methods for biomedical-grade ZnO due to its simplicity, low energy demand, and aqueous chemistry (Gawande et al. 2016).

General Reaction

$Zn^{2+} + 2OH^- \rightarrow Zn(OH)_2 \rightarrow ZnO + H_2O$

Critical Parameters

- **pH (~9–12):**

High pH accelerates nucleation → smaller NPs but more agglomeration

- **Temperature:**

25–90°C influences growth and aging

- **Hydroxide agent:**

NH_4OH gives slower nucleation; NaOH gives faster, less controlled nucleation

Modifiers

- Complexing agents (EDTA, citrate)
- Alcoholic cosolvents
- Surfactants (CTAB, SDS, PVP)

Outcome

Particles vary from **10–80 nm**, depending on reaction rate and precursor strength. Rapid nucleation often leads to broad size distribution unless properly moderated.

3.1.3 Hydrothermal and Solvothermal Synthesis

Hydrothermal processes occur in sealed autoclaves at temperatures above boiling point, enabling *in situ* crystallization under pressure. This method is superior for producing **morphology-controlled ZnO** including rods, flowers, needles, and tubes (Zhang et al. 2012).

Mechanistic Foundation

- Superheated water enhances solubility of metal hydroxides
- Anisotropic growth along the (002) plane yields rod-like structures
- Surfactants dictate crystal facet exposure and orientation

Advantages:

- Excellent crystallinity
- Precise morphological control
- Low defect concentration
- Suitable for high-performance biomedical coatings

Limitations

- Reaction time (3–24 hours)
- Requires autoclaves
- Limited batch size

Hydrothermal ZnO often displays lower Zn^{2+} dissolution and reduced cytotoxicity compared to sol–gel ZnO (Singh et al. 2013).

3.1.4 Vapor-Phase and Physical Synthesis Routes

These methods include:

- **Chemical Vapor Deposition (CVD)**
- **Physical Vapor Deposition (PVD)**
- **Thermal evaporation**
- **Laser ablation in liquids (LAL)**

Vapor-phase synthesis produces extremely pure, crystalline, defect-minimized ZnO structures ideal for electronic, optical, and antimicrobial coating applications.

Laser Ablation Mechanism

High-power pulsed lasers strike a Zn target submerged in liquid, generating a plasma plume that rapidly cools to form ligand-free ZnO nanoparticles (Bala et al. 2015).

Advantages:

- No chemical contaminants
- Ultra-stable colloids
- High crystallinity

Disadvantages:

- Lower yield
- Expensive equipment

3.2 DOPING & SURFACE ENGINEERING STRATEGIES

Modifying the ZnO lattice or surface drastically alters optical, catalytic, antimicrobial, and biological responses.

3.2.1 Metal Doping (Ag, Cu, Mn, Co, Al, Fe)

Doping can:

- Narrow energetical band gaps
- Enhance charge separation
- Increase ROS generation
- Modify ion release profiles
- Change antimicrobial spectrum

Examples

- **Ag-doped ZnO:** dual antimicrobial activity via Ag^{+} + Zn^{2+} release (Parthangal et al. 2007).
- **Cu-doped ZnO:** improved photocatalytic and anticancer effects (He et al. 2015).
- **Al-doped ZnO:** reduced defect states, enhanced electron mobility (Chen et al. 2011).
- **Mn-doped ZnO:** altered magnetic and optical properties (Kundu et al. 2014).

Doping >5% often results in lattice distortion and defect clustering.

3.2.2 Non-Metal Doping (N, F, S, P)

Non-metal doping tailors:

- Band structure
- Oxygen vacancies
- Photocatalytic absorption

N-doping introduces localized states increasing visible-light activity (Rajiv et al. 2013).

3.2.3 Organic, Polymer & Biopolymer Coatings

Coatings critically influence stability, toxicity, and biomedical suitability.

Common Coatings

- **PEG** (stealth behavior)
- **PVP** (dispersibility)
- **Chitosan** (mucoadhesion, antibacterial action)
- **Gelatin** (biocompatibility)
- **Dextran** (anti-fouling)

PEGylation reduces nonspecific protein interactions in blood, improving circulation half-life (Safi et al. 2011). Chitosan-coated ZnO exhibits enhanced wound healing properties and increased epithelial cell migration (Kumar et al. 2019).

3.2.4 Ligand & Targeting Molecule Functionalization

Surface grafting with ligands such as:

- Folate
- Hyaluronic acid
- RGD peptides
- Antibodies
- Aptamers

enables selective recognition of cancers, bacteria, or inflamed tissues (Sharma et al. 2019). Ligand-specific targeting increases internalization and therapeutic indices.

3.3 GREEN & BIOGENIC SYNTHESIS ROUTES

Biogenic synthesis is a sustainable, ecofriendly method using biological entities as reducing, capping, and stabilizing agents. They produce bio-functionalized ZnO suitable for wound healing, antimicrobial applications, and tissue regeneration.

3.3.1 Plant Extract–Mediated Synthesis

Plant extracts (leaf, stem, flower, peel, fruit) are rich in:

- Polyphenols
- Flavonoids
- Alkaloids
- Terpenoids
- Saponins
- Enzymes

These compounds act as **reducing, capping, and templating agents** (Dwivedi et al. 2015).

Mechanistic Stages

1. Reduction of Zn^{2+} by phytochemicals
2. Nucleation of ZnO clusters
3. Bio-capping by phenolics or proteins
4. Morphology stabilization

Advantages include low toxicity, bioactive surfaces, and excellent biocompatibility. However, batch variability and complex phytochemical interactions limit precise control.

3.3.2 Microbial-Mediated Synthesis

Microbial synthesis involves extracellular or intracellular formation of ZnO using metabolites or enzymes.

Examples

- **Fungi (Fusarium, Aspergillus)**: high-yield extracellular NPs (Murali et al. 2023)
- **Bacteria (Bacillus, Lactobacillus)**: reductase-mediated synthesis (Jayaseelan et al. 2013)

Microbial ZnO displays enhanced stability due to protein capping layers.

3.3.3 Biomolecule & Amino Acid Assisted Synthesis

Biomolecules (albumin, starch, casein, cysteine, glutathione) offer controlled nucleation and gentle stabilization. Amino acids provide charge-specific binding, enabling pH-responsive behavior and selective drug loading (Singh et al. 2018).

3.4 COMPARATIVE SYNTHESIS OUTCOMES

Table 1: Comparative Synthesis Outcomes

Parameter	Conventional ZnO	Green/Biogenic ZnO
Crystallinity	High	Moderate–High
Size Distribution	Narrow	Broader
Surface Chemistry	Clean, inorganic	Bio-functionalized
ROS Potential	High	Moderate–Low
Biocompatibility	Moderate	High
Environmental Safety	Lower	Higher
Reproducibility	Excellent	Moderate

Key Insight: The synthesis method dictates biomedical performance. Green ZnO is superior for **wound healing & antimicrobial gels**, while conventional ZnO is preferred for **photocatalytic & anticancer applications** requiring strong ROS induction.

4. INTERPRETATION

4.1 To meet Q1/A2 journal standards, characterization must not simply "report values," but **explain why they matter**, what they indicate, and how they influence biological or environmental outcomes.

4.2 Table 2: Integrated Interpretation Table

Technique	What It Measures	Detailed Interpretation	Why It Matters for Biology

TEM / HRTEM (Wang et al. 2003)	Primary particle size, shape, lattice fringes	TEM is the only technique that reveals the actual crystalline domain size (not affected by hydration shell). HRTEM provides lattice spacing, showing defect density, twin boundaries, stacking faults. These structural defects strongly influence ROS generation.	Smaller ZnO dissolves faster → higher Zn^{2+} release. Rod- and plate-shaped ZnO interact differently with cell membranes. Defects increase oxidative stress, influencing cytotoxicity and antimicrobial action.
SEM(Alvarez et al. 2016)	Surface morphology, topography	SEM reveals overall shape, texture, anisotropy, hierarchical architectures (flowers/rods/clusters). These architectures indicate growth direction and stability.	Nanorods penetrate bacterial membranes more effectively. Hierarchical structures release Zn^{2+} slower due to reduced surface accessibility.
AFM(Zhang et al. 2017)	Surface roughness and mechanical properties	AFM identifies nanoscale roughness, adhesive forces, elasticity, hydrophilicity. These properties influence protein adsorption and NP–cell interactions.	Rougher ZnO surfaces bind more proteins → thicker corona → reduced cytotoxicity but increased immune modulation.
XRD(Yadav et al. 2018)	Crystal phase, crystallite size, lattice strain	XRD peak widening indicates smaller crystals or higher strain. Peak shifts reveal doping or lattice distortions. Rietveld refinement provides detailed crystal parameters.	Higher lattice strain often → higher defect density → more ROS → stronger antimicrobial effect but higher toxicity.
Raman(Cui et al. 2011)	Defect signatures, oxygen vacancies	Raman E2(high) peak broadening indicates disorder. Defect-related peaks (LO phonon modes) reveal oxygen vacancies, crucial for photocatalytic and ROS behavior.	More oxygen vacancies → stronger antibacterial action but possible eukaryotic toxicity.
FTIR(Sajan et al. 2020)	Organic and biomolecule capping	FTIR identifies phytochemicals, proteins, polymers capping ZnO. Capping thickness can be quantified from peak intensities.	Capping reduces dissolution, reduces cytotoxicity, increases compatibility, but may weaken antimicrobial effects.
XPS(Park et al. 2019)	Surface chemical states	XPS distinguishes Zn^{2+} vs Zn–OH vs Zn–O–C. O1s deconvolution reveals oxygen vacancy concentration. Surface carbon indicates plant/polymer residues.	More Zn–OH → lower ROS. More vacancies → photocatalytic ROS. Surface contamination modifies protein corona.
DLS(Akhtar et al. 2015) (Bhattacharjee et al. 2016)	Hydrodynamic diameter	Shows actual NP size in liquid (not dry). Aggregation dramatically increases hydrodynamic radius.	Size in biological media is the key determinant of cellular uptake — not TEM size.
Zeta Potential(Salvador-Morales et al. 2009)	Surface charge and stability	Determines electrostatic repulsion. Strong negative/positive charge prevents aggregation; near-zero charge → agglomeration.	Charge dictates membrane interaction: positive ZnO binds strongly to bacteria; negative ZnO binds more weakly.
UV–Vis(Dutta et al. 2013)	Band gap, excitonic absorption	Band gap narrowing indicates doping, defects, strain. Absorption peak shift indicates particle size or surface states.	Band gap affects ROS production and photocatalytic antibacterial mechanisms.
Photoluminescence (PL) (Singhal et al. 2012)	Defect emissions	Green emission = oxygen vacancies. Blue/yellow = intrinsic defects. High PL intensity = high defect levels.	Defects = stronger antimicrobial ROS, but also higher toxicity toward mammalian cells.
TGA/DSC(Karthik et al. 2018) (Patel et al. 2020)	Thermal stability	TGA shows % weight loss — indicating capping layer thickness. DSC peaks reflect energetic transitions.	Capped ZnO is more biocompatible; thermal stability influences shelf life of formulations.
ICP-OES/MS(Richardson et al. 2019)	Zn^{2+} dissolution	Measures real-time Zn^{2+} release — the most critical determinant of toxicity.	Higher Zn^{2+} = higher oxidative stress → greater antibacterial but higher cytotoxicity.

4.3 INTERPRETATION OF SURFACE CHEMISTRY

Surface chemistry is often **more important than size or morphology** because **biological systems interact primarily with the surface**, not the inorganic core. Surface groups determine how ZnO binds lipids, proteins, DNA, or cellular receptors. (Kim et al. 2019)

4.3.1 Interpretation of FTIR Peaks

Common FTIR bands and their meaning: (Huang et al. 2020)

- **3300–3500 cm^{-1} (O–H stretch):** Indicates surface hydroxyl groups; high OH content → increased hydrophilicity but increased dissolution in acidic media.
- **1600–1700 cm^{-1} (C=O stretch):** Associated with flavonoids and phenolics in green ZnO; thicker capping layer dampens ROS generation.
- **1000–1100 cm^{-1} (C–O–C stretch):** Indicates polysaccharide-based capping; forms a steric barrier around ZnO (good for drug delivery).
- **460–550 cm^{-1} (Zn–O):** Primary ZnO fingerprint.

Interpretation:
Presence of organic components:
- Reduced dissolution
- Improved biocompatibility
- Lower cytotoxicity
- Lower antimicrobial activity

4.3.2 Interpretation of XPS
XPS allows understanding how surface composition affects biological outcomes:
Key XPS Signals:
- **Zn 2p3/2 ≈ 1021.5 eV:** Zn^{2+} in ZnO
- **Peak shift >0.4 eV:** doping (Cu, Ag, Al)
- **O 1s deconvolution:**
  - 530.1 eV → lattice oxygen
  - 531.2 eV → oxygen vacancies
  - 532.5 eV → OH groups

Interpretation:
- High oxygen vacancies → strong ROS production.
- High OH content → lower cytotoxicity.
- Dopant peaks → modified antimicrobial or optical properties.

4.4 COLLOIDAL BEHAVIOR INTERPRETATION
4.4.1 Why DLS Values Differ From TEM
TEM shows *dry size*.

DLS shows *hydrated, solvated, sometimes aggregated* size.

Reasons for DLS > TEM:
1. **Solvation layer:** water molecules adhere to surface
2. **Protein corona:** increases apparent diameter
3. **Electrostatic double layer**
4. **Aggregation in salts/media**

Meaning for biology:
Cells interact with the **DLS size**, not the TEM size. (Saptarshi et al. 2013).

4.4.2 Table 3: Zeta Potential Interpretation by Biological Function

Zeta Potential	Interpretation	Biological Meaning
+25 to +40 mV	Highly positive	Strong binding to bacterial membranes → excellent antimicrobial action
+5 to –5 mV	Near neutral	High aggregation → unpredictable toxicity
–15 to –30 mV	Moderately negative	Stable → desirable for intravenous applications
–30 to –50 mV	Highly negative	Minimal aggregation → anti-inflammatory and safer

4.5 OPTICAL PROPERTIES
4.5.1 Band Gap Analysis
Band gap determines whether ZnO is: (Mahmoudi et al. 2013) (Sajjad et al.2017).
- Photocatalytic
- Photo-bactericidal(Li et al. 2018)
- Fluorescent

Band Gap Narrowing Means:
- More defects
- More visible-light activity
- Higher ROS under physiological conditions
- Stronger antibacterial effect (but higher toxicity)

4.5.2 PL Peak Interpretation Table

Table 4: PL Peak Interpretation Table

PL Emission	Origin	Meaning
~380 nm	Near-band-edge	High crystallinity
~450–470 nm	Zn interstitial defects	Moderate ROS
~520–540 nm (green)	Oxygen vacancies	Strong ROS, strong antibacterial
~600–650 nm	Deep-level defects	High cytotoxic risk

4.6 THERMAL ANALYSIS

Interpretation of TGA Curves(Rao et al. 2018), (Kumar et al. 2020).

Weight Loss Regions:

- **<150°C:** moisture loss
- **150–300°C:** degradation of plant/polymer capping
- **350–500°C:** removal of organic residues
- **>500°C:** crystalline restructuring

Meaning:

Thicker capping = higher biocompatibility but reduced antimicrobial potency.

4.7 DISSOLUTION & Zn^{2+} RELEASE

4.7.1 Real Biological Meaning of Dissolution (Kreuzer et al. 2021) (Liu et al. 2016).

Zn^{2+} release rate determines:

- Antibacterial power (Kim D et al. 2022)
- Oxidative stress induction (Huang R et al. 2019)
- Inflammatory response (Monopoli SR et al. 2012)
- Toxicity toward cancer cells (Lynch M et al. 2008)
- Toxicity toward normal cells (Janotti M et al. 2009)

Slow dissolution = safe but weaker activity

Fast dissolution = strong activity but potential toxicity

4.7.2 ZnO Dissolution in Different Media (Rasmussen et al. 2010) (Kumar S et al. 2016)

Table 5: ZnO Dissolution in Different Media

Medium	Dissolution Level	Biological Meaning
Water (neutral)	Very low	Stable, low toxicity
PBS	Moderate–high	Phosphate chelation increases Zn^{2+} release
DMEM/FBS	High	Amino acids bind Zn^{2+} → unpredictable toxicity
Gastric fluid (pH 1.5)	Complete	Relevant for oral ZnO supplements
Tumor pH ~6.5	High	Useful for cancer-targeted ZnO release

4.8 MINIMUM REPORTING CHECKLIST

4.8.1 Checklist Table (Azizi et al. 2016) (Borm et al. 2006) (Mudunkotuwa et al. 2015)

Table 6: Checklist Table of Minimum requirement

Characterization	Minimum Requirement	Why It Is Mandatory
TEM	100+ particles measured	Avoid false claims of size
XRD	Scherrer + phase purity	Confirm crystallinity
FTIR	All organic groups analyzed	Surface determines toxicity
XPS	O1s, Zn2p deconvolution	Identify defects/toxicity
DLS	Size + PDI + media stability	Biological relevance
Zeta Potential	pH-dependent	Predict membrane interactions
ICP-OES	Dissolution curves	Essential for toxicity
PL	Defect analysis	Predict ROS
BET	Surface area	Predict dissolution
AFM	Roughness	Predict protein corona

5 BIOMEDICAL MECHANISMS & THERAPEUTIC APPLICATIONS OF ZnO NANOPARTICLES

5.1 Overview of Biomedical Interactions of ZnO Nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) interact with biological systems across multiple hierarchical levels — from the molecular (protein corona, ion release, defect sites) to the cellular (membrane disruption, organelle dysfunction), tissue (angiogenesis, wound repair) and systemic (immune modulation, biodistribution) scales. Their versatility stems from combined properties such as **pH-sensitive dissolution**, **defect-mediated redox**

reactivity, and **easily tunable surfaces**, which together enable ZnO-NPs to act as antimicrobial agents, cancer therapeutics, regenerative stimulants, and diagnostic probes (Prasad et al. 2021; Singh et al. 2020; Yousef et al. 2019). Because malignant and microbial cells frequently operate under higher baseline oxidative stress and altered pH, ZnO exploits these vulnerabilities enabling selective activity in pathological microenvironments (Prasad et al. 2021; Chan et al. 2021).

5.2 Zinc Ion (Zn^{2+})–Mediated Biological Mechanisms

5.2.1 pH-Responsive Dissolution and Localized Zn^{2+} Bursts

ZnO dissolves more rapidly at acidic pH, a behavior that is central to its selective action in tumors, infected wounds and endo-lysosomal compartments. This acid-triggered dissolution ($ZnO + 2H^+ \rightarrow Zn^{2+} + H_2O$) leads to localized Zn^{2+} bursts that perturb metal homeostasis, inhibit essential enzymes in microbes, and trigger cell death pathways in cancer cells (Prasad et al. 2021; Kreuzer et al. 2006). The dissolution rate is strongly dependent on particle size (smaller → faster), surface defect density (more vacancies → faster), presence of coordinating anions (phosphate accelerates dissolution), and the nature of surface coatings (which typically slow ion release) (Liu et al. 2015; Kreuzer et al. 2006). Controlled design of these parameters allows ZnO to be tuned for desired therapeutic windows — for instance, relatively slow-dissolving, polymer-capped ZnO for dermal uses vs. small, defect-rich ZnO for tumor ablation (Prasad et al. 2021; Wang et al. 2019).

5.2.2 Zn^{2+} as a Biological Regulator and Therapeutic Effector

Zn^{2+} is not merely a toxicant — it is a biologically active micronutrient that functions as a structural cofactor for zinc-finger proteins, modulates antioxidant defenses (via metallothioneins and Cu/Zn-SOD), and influences signaling pathways (MAPK, PI3K/Akt). Local Zn^{2+} release thus has dual effects: at controlled concentrations it supports wound healing, cellular proliferation and barrier function; at high local concentrations it disrupts protein structure, interferes with DNA processes, and overwhelms antioxidant systems in pathogens and tumor cells (Prasad et al. 2021; Noor et al. 2022). This duality underpins many translational strategies where ZnO is formulated to supply therapeutic Zn^{2+} to healing tissues while acting cytotoxically toward pathogens or cancer cells (Li et al. 2021; Wang et al. 2019).

5.3 Defect-Driven ROS Generation

Defect states (oxygen vacancies, Zn interstitials, surface traps) are potent enhancers of ZnO's redox activity. Such defects reduce band-gap recombination rates and facilitate electron transfer to molecular oxygen, producing superoxide, hydrogen peroxide and hydroxyl radicals even under low light (Singh et al. 2020; Sajjad et al. 2017). Increased ROS leads to oxidative damage to lipids, proteins and nucleic acids, with particularly severe impacts on organisms already experiencing oxidative stress (e.g., cancer cells, infected tissues) (Singh et al. 2020; Arakha et al. 2015). Carefully engineered defect densities can thus be used to tune ZnO for stronger antimicrobial or anticancer activity, whereas reduction of defects (by annealing or coating) can reduce off-target toxicity for benign applications (Singh et al. 2020; Sajjad et al. 2017).

5.4 Direct Membrane Interaction

Beyond chemistry, ZnO exerts **physical** effects on cell membranes. Positively charged ZnO or sharp morphologies (rods, needles) interact electrostatically with negatively charged phospholipid membranes, promote adsorption and mechanical destabilization, and can physically perforate membranes, provoking leakage and cell lysis (Yousef et al. 2019; Raghupathi et al. 2011). In bacteria, membrane disruption is often the initial event that allows ROS and Zn^{2+} to further compromise intracellular physiology; in cancer cells, membrane interactions facilitate nanoparticle uptake that is followed by intracellular ion release and organelle damage (Yousef et al. 2019; Chan et al. 2021).

5.5 Intracellular Trafficking and Organelle-Specific Damage

Once internalized by endocytosis (clathrin/caveolin/macropinocytosis), ZnO accumulates in endo/lysosomal vesicles whose acidic milieu triggers accelerated dissolution. Resulting Zn^{2+} floods the cytosol and mitochondria, causing mitochondrial membrane depolarization, ATP depletion, and activation of intrinsic apoptotic cascades (cytochrome-c release, caspase-9/-3 activation) (Chan et al. 2021; Arakha et al. 2015). Lysosomal membrane permeabilization (LMP) secondary to ZnO dissolution can also release cathepsins and trigger apoptosis or necrosis depending on dose and cell type (Chan et al. 2021; Sato et al. 2019; Zhou et al. 2020).

5.6 Anti-Cancer Mechanisms

ZnO selectively targets cancer through a combination of localized dissolution in acidic microenvironments, heightened ROS generation relative to normal tissues, and preferential uptake by rapidly dividing cells. After internalization and dissolution, Zn^{2+} and ROS cause DNA damage, cell-cycle arrest (commonly at G2/M), and mitochondrial apoptosis. Additional anti-tumor mechanisms include inhibition of angiogenesis (VEGF downregulation), impairment of metastatic machinery (MMP suppression), and synergy with conventional cytotoxics due to oxidative sensitization (Arakha et al. 2015; Wang et al. 2019). Importantly, well-designed ZnO-based nanocarriers (e.g., drug-conjugated or polymer-coated systems) can provide targeted drug release with intrinsic ZnO cytotoxicity as an adjunctive mode of action (Wang et al. 2019; Li et al. 2021).

5.7 Antimicrobial and Antifungal Mechanisms

ZnO exerts broad-spectrum antimicrobial effects via combined modes: ROS generation, Zn^{2+} toxicity, membrane disruption, enzyme inhibition and interference with nutrient transporters (Raghupathi et al. 2011; Yousef et al. 2019). The multi-hit nature of ZnO's action reduces the likelihood of resistance development and makes ZnO a useful candidate for coatings (catheters, implants), topical formulations (wound dressings), and packaging applications. For fungi, ZnO impairs ergosterol biosynthesis, spore germination and hyphal growth — processes particularly sensitive to oxidative damage and metal-induced enzyme inhibition (Raghupathi et al. 2011; Bano et al. 2020; Mendes et al. 2020; Park et al. 2016).

5.8 Immunomodulatory and Anti-Inflammatory Mechanisms

At therapeutic doses, ZnO modulates immune responses by attenuating pro-inflammatory cytokines (TNF-α, IL-6) and enhancing anti-inflammatory mediators (IL-10), often via modulation of NF-κB and STAT signaling cascades (Gupta et al. 2022; Noor et al. 2022). ZnO also influences macrophage polarization, favoring an M2 (pro-healing) phenotype in many wound-healing studies, which accelerates re-epithelialization and restores tissue integrity (Gupta et al. 2022; Bano et al. 2020). These immunoregulatory properties make ZnO attractive for chronic wound contexts where prolonged inflammation impedes regeneration.

5.9 Wound Healing Mechanisms

ZnO contributes to all wound healing phases: it decreases infection risk via antimicrobial action; reduces excessive inflammation by modulating cytokine signaling; promotes proliferation and migration of fibroblasts and keratinocytes (via Zn^{2+}-mediated activation of Akt/ERK pathways); and supports angiogenesis through VEGF upregulation (Bano et al. 2020; Li et al. 2021). ZnO-containing polymeric dressings have demonstrated improved tensile strength, more organized collagen deposition, and significantly accelerated wound closure in animal models (Bano et al. 2020; Li et al. 2021).

5.10 Antioxidant and Cytoprotective Roles

In controlled concentrations, ZnO enhances antioxidant defenses in normal tissues by inducing metallothionein expression and stabilizing antioxidant enzymes such as SOD, catalase and glutathione peroxidase. This contributes to cytoprotective effects against secondary oxidative injury during tissue repair (Noor et al. 2020; Prasad et al. 2021). Thus, careful dosing and design enable ZnO to be both pro-oxidant (against pathogens/tumors) and protective (for healthy tissue).

5.11 ZnO as Drug Delivery Platforms

ZnO's high surface area, pH-responsive dissolution, and modifiable surface chemistry make it an excellent platform for drug loading, controlled release and imaging. Drug–ZnO hybrids leverage ZnO's dissolution in acidic microenvironments to effect targeted payload release, while surface functionalization (PEGylation, ligands) improves circulation and targeting (Wang et al 2019; Huang et al. 2020, (Fernandez et al. 2018), (O'Neill et al. 2017), . ZnO has been combined with chemotherapeutics, antibiotics, nucleic acids and growth factors to produce synergistic therapeutic effects (Wang et al. 2019; Li et al. 2021).

5.12 Biosensing and Diagnostic Applications

ZnO nanostructures (especially nanowires and defect-engineered forms) are effective transducers in electrochemical and optical biosensors because of favorable electron mobility, surface reactivity and photoluminescence. (Kim et al. 2018; Al-Juboori et al. 2022). These properties permit ultrasensitive detection of biomarkers (troponin, glucose), nucleic acid targets and enzymatic substrates, enabling point-of-care diagnostics and intraoperative imaging when combined with ZnO's intrinsic fluorescence (Huang et al. 2020; Li et al. 2021).

6. TOXICOLOGY, SAFETY, BIOCOMPATIBILITY AND RISK ASSESSMENT OF ZnO NANOPARTICLES

Zinc oxide nanoparticles (ZnO-NPs), despite their broad biomedical potential, exhibit complex toxicological behaviors influenced by **particle size**, **surface chemistry**, **defect structure**, **dose**, **exposure duration**, and most critically, **dissolution dynamics**. Their dual nature - protective at physiological concentrations yet cytotoxic at higher levels - necessitates rigorous safety evaluation. Toxicity is not intrinsic but **highly context-dependent**, shaped by physicochemical properties and biological environments. This section comprehensively analyzes ZnO-NP safety, toxicity mechanisms, exposure pathways, in vitro/in vivo outcomes, dose thresholds, biodistribution, and regulatory considerations.

6.1 Determinants of ZnO-NP Toxicity

ZnO toxicity is governed by interplay between **Zn^{2+} dissolution**, **ROS overproduction**, and **nanoparticle–cell interactions**. Unlike inert nanomaterials, ZnO undergoes environmental transformation, releasing ions that contribute substantially to biological responses (Johansson et al. 2020). The toxicological profile varies with:

- **Primary particle size** (smaller → stronger dissolution and ROS)
- **Surface coatings** (polymer, protein, phytochemical capping reduces toxicity)
- **Morphology** (nanorods penetrate membranes more strongly than spheres)
- **Defect density** (more defects → more ROS)
- **Agglomeration behavior** (large agglomerates reduce uptake but induce localized inflammation)
- **Protein corona composition** (determines immune and cellular responses)

Understanding these factors enables tuning ZnO for safer biomedical applications.

6.2 In Vitro Toxicity Mechanisms

ZnO-induced cytotoxicity in vitro varies dramatically across cell lines. Immune cells, hepatocytes, and lung epithelial cells tend to be more sensitive, while fibroblasts and keratinocytes often tolerate moderate exposures.

6.2.1 Oxidative Stress and ROS Overload

ROS overgeneration is a primary driver of ZnO cytotoxicity. Excess ROS leads to:

- **Lipid peroxidation** → membrane leakage
- **Protein carbonylation** → enzyme dysfunction
- **DNA fragmentation** → activation of p53, PARP
- **Collapse of antioxidant defenses** (GSH depletion)

Local ROS production is amplified in low-antioxidant cell types, explaining differential toxicity across tissues (Rosenkranz et al. 2017).

6.2.2 Zn^{2+} Ion Toxicity in Cells

High intracellular Zn^{2+} disrupts:

- Mitochondrial membrane potential
- Calcium homeostasis
- Protein folding
- DNA-binding transcription factors

Ion-induced stress triggers both intrinsic and extrinsic apoptotic pathways (Ma et al. 2019).

6.2.3 Lysosomal Rupture and Organelle Stress

Lysosomal dissolution of ZnO causes:

- Lysosomal membrane permeabilization (LMP)
- Cathepsin release
- Secondary mitochondrial damage

Leading to apoptosis or necrosis depending on dose intensity (O'Neill et al. 2017).

6.2.4 Inflammatory Signaling Activation

High doses of ZnO induce NF-κB activation, upregulate IL-1β and TNF-α, and may stimulate inflammasome pathways in macrophages (Park et al. 2016).

6.3 In Vivo Toxicity and Organ-Specific Effects

6.3.1 Biodistribution

ZnO-NPs exhibit organ distribution primarily driven by protein corona identity, aggregation, and route of exposure.

Major Sites of Accumulation
- Liver (Kupffer cell uptake)
- Spleen (RES system)
- Lungs (after inhalation)
- Kidneys (ion clearance)
- Intestinal tissues (after oral intake)

Surface-modified ZnO shows reduced hepatic accumulation compared to bare ZnO (Peterson et al. 2021).

6.3.2 Hepatic Toxicity

High Zn^{2+} flux in hepatocytes causes:
- Mitochondrial swelling
- Elevated liver enzymes (ALT/AST)
- Oxidative stress
- Inflammation

However, low-dose ZnO supports hepatoprotective effects via antioxidant modulation (Al-Juboori et al. 2022).

6.3.3 Pulmonary Toxicity (Inhalation Route)

Inhaled ZnO induces:
- Oxidative injury to alveolar epithelium
- Inflammation (neutrophil influx)
- Edema
- Altered lung function

High-dose inhalation exposure in occupational settings is linked to “metal fume fever” (Johansson et al. 2020).

6.3.4 Gastrointestinal Effects (Oral Route)

Most orally ingested ZnO dissolves in gastric acid, forming bioavailable Zn^{2+}.

At Moderate Doses
- Supports intestinal repair
- Enhances mucosal immunity
- Improves nutrient absorption

At High Doses
- Alters gut microbiota
- Causes gastric irritation
- Induces oxidative stress in enterocytes (Martínez et al. 2020)

6.3.5 Renal Handling and Nephrotoxicity

Kidneys clear Zn^{2+} effectively; hence renal toxicity is usually mild unless high systemic doses or prolonged exposure are involved. Ion-mediated tubular stress and oxidative injury occur at excessive levels (Xiong et al. 2019).

6.4 Genotoxicity and DNA Damage Analysis

ZnO’s genotoxicity arises mainly from ROS and Zn^{2+} overload.

Mechanistic Sources of DNA Damage
- Oxidative base modifications
- Single- and double-strand breaks
- Telomere shortening
- Centrosome disruption
- Chromosomal aberrations

In vitro studies confirm DNA fragmentation in macrophages and epithelial cells at high nanoparticle doses (Rosenkranz et al. 2017). Proper surface coatings drastically reduce genotoxicity (Ma et al. 2019).

6.5 Immunotoxicity and Inflammatory Outcomes

ZnO can have **anti-inflammatory** or **pro-inflammatory** effects depending on:

- Concentration
- Surface coating
- Exposure duration
- Cell type

Pro-Inflammatory Outcomes

High NP doses elevate:

- TNF-α
- IL-8
- IL-6
- ROS in immune cells

Primarily through oxidative stress-triggered NF-κB signaling (Park et al. 2016).

Anti-Inflammatory Effects

Low doses suppress inflammatory cytokines, favoring M2 polarization and wound healing (Gupta et al. 2022).

6.6 Oxidative Stress–Linked Systemic Toxicity

Excessive ROS generated in vivo can lead to:

- Lipid peroxidation in liver and kidneys
- Cardiovascular oxidative stress
- Hematological dysregulation
- Activation of apoptotic pathways across tissues
- Systemic inflammatory responses

Dose–response curves demonstrate that toxicity is nonlinear — **very low doses may be beneficial**, moderate doses neutral, and high doses detrimental (Al-Juboori et al. 2022).

6.7 Safe Doses, Thresholds, and Regulatory Guidelines

Safe Exposure Levels *(Approximate ranges; highly context-dependent)*

- **In vitro (mammalian cells):** 1–10 µg/mL generally safe
- **In vivo (rodents):** 2–50 mg/kg/day well tolerated depending on coating
- **Oral supplementation:** ZnO approved as GRAS within defined Zn limits

Regulatory Bodies Involved

- US FDA
- EMA
- OECD
- ISO 10993 biological evaluation standards

Risk assessment frameworks emphasize **dissolution rate**, **surface coatings**, and **protein corona identity** as key predictors of toxicity (Peterson et al. 2021).

6.8 Strategies to Reduce ZnO Toxicity

6.8.1 Surface Coating Approaches

- PEGylation
- Protein coating
- Polysaccharide (chitosan, dextran)
- Lipid bilayers
- Plant extract capping

Coatings reduce dissolution and ROS, improving biocompatibility (Ma et al. 2019).

6.8.2 Doping and Defect Engineering

Doping with Mg, Fe, or Si reduces defect density and ROS, lowering cytotoxicity (Fernandez et al. 2018).

6.8.3 Particle Size Optimization

Larger ZnO (>80–100 nm) dissolves slowly → lower toxicity, suited for wound dressings and topical uses.

6.8.4 Controlled Release Formulations

Embedding ZnO in hydrogels, liposomes, or polymeric matrices moderates Zn^{2+} release and limits systemic

exposure (Li et al. 2021).

6.9 Environmental Toxicity and Ecotoxicology

ZnO impacts aquatic organisms due to ion dissolution.

Reported Effects

- Gill damage in fish
- Oxidative stress in algae
- Growth inhibition of plankton
- Soil microbial dysbiosis

However, coated ZnO shows substantially reduced ecotoxicity (Martínez et al. 2020).

6.10 Risk–Benefit Assessment for Clinical Applications

Despite toxicity risks at high exposures, ZnO remains one of the **most clinically promising metal oxides** because:

- It dissolves into a biologically essential ion
- Toxicity can be tuned via coatings and formulation
- It exhibits strong therapeutic benefits (antimicrobial, anticancer, wound healing)
- It shows limited long-term accumulation due to Zn clearance mechanisms

With proper design, ZnO-NPs demonstrate an excellent **risk–benefit ratio** for topical, injectable, and oral biomedical formulations (Peterson et al. 2021; Noor et al. 2022).

7. Clinical Applications, Translational Potential & Future Prospects

Zinc oxide nanoparticles (ZnO-NPs) possess a unique balance of **biocompatibility**, **multimodal therapeutic activity**, **structural tunability**, and **ion-based degradability**, placing them at the forefront of emerging biomedical technologies. Unlike inert metal oxides, ZnO exhibits a dynamic biological behavior shaped by pH-dependent dissolution, surface defect chemistry, and adaptable nanostructuring. These properties translate into potent **antimicrobial**, **anti-inflammatory**, **anticancer**, **wound-healing**, **biosensing**, and **drug delivery** applications that have reached both preclinical and early clinical evaluation (Chang et al. 2021; Park et al. 2022).

7.1 Clinical Maturity and Therapeutic Relevance of ZnO Nanoparticles

ZnO-NPs are among the very few metal-based nanomaterials already incorporated into **commercial dermatological products**, **wound dressings**, **oral supplements**, and **implant coatings**, owing to their favorable safety profile and FDA-recognized GRAS status for specific uses (Chang et al. 2021). Their clinical value stems from three properties:

1. **Intrinsic therapeutic activity** (antibacterial, anticancer, anti-inflammatory)
2. **Biodegradability into essential Zn^{2+} ions**
3. **Tunability through surface engineering and dopant modification**

Unlike silver or titanium nanoparticles, the degradability of ZnO into a biologically necessary ion enhances regulatory acceptance and reduces long-term accumulation concerns.

7.2 Advanced Clinical Applications in Wound Healing and Dermatology

7.2.1 Next-Generation Wound Dressings

ZnO-NPs address multiple pathological barriers to wound healing:

- **Rapid bacterial clearance** including drug-resistant strains
- **Reduction of early inflammatory burden**
- **Stimulation of fibroblast proliferation** through Zn^{2+} signaling
- **Acceleration of collagen maturation and deposition**
- **Regulation of angiogenesis** via VEGF modulation

Composite dressings embedding ZnO within hydrogels, alginate fibers, polyurethane films, or chitosan matrices have shown **55–70% enhanced wound closure** and superior tissue regeneration in preclinical models (Hadi et al. 2020). Their multifunctionality reduces the need for systemic antibiotics, lowers infection recurrence, and promotes faster recovery.

7.2.2 Dermatological and Cosmetic Formulations

ZnO-NPs offer:

- **Anti-acne action** by inhibiting *Cutibacterium acnes*
- **Broad-spectrum UV protection** with high transparency
- **Anti-inflammatory benefits** for eczema and dermatitis

- **Antioxidant and anti-pigmentation effects** due to ROS modulation

Nano-ZnO formulations minimize skin whitening effects typical of bulk ZnO, enabling cosmetically elegant sunscreens and therapeutic ointments (Lee et al. 2021).

7.3 Medical Device and Implant Coatings

ZnO coatings are increasingly used to reduce device-associated infections, which account for nearly 50% of hospital-acquired complications. There:

- **Broad-spectrum antimicrobial activity**
- **Ability to resist biofilm formation**
- **Durability and compatibility with metals, polymers, and ceramics**
- **Stability under sterilization procedures**

make them powerful candidates for coating surgical and indwelling devices.

Clinically relevant applications include:

- Antimicrobial urinary catheters
- Orthopedic implant coatings to prevent osteomyelitis
- Dental implants with enhanced osseointegration
- Vascular stents with anti-inflammatory Zn^{2+} release
- Sutures with prolonged antimicrobial action

ZnO-coated titanium and PEEK implants have shown **significant biofilm suppression** and **enhanced osteoblast attachment**, indicating dual anti-infective and regenerative roles (Liu et al. 2021).

7.4 ZnO Nanoparticles in Cancer Theranostics

ZnO-NPs exhibit **intrinsic tumor selectivity**, primarily due to:

- Faster dissolution at acidic tumor pH
- Higher Zn^{2+} uptake through upregulated ZIP transporters
- Elevated oxidative stress sensitivity of cancer cells
- Preferential endocytosis by proliferative cells

7.4.1 Therapeutic Functions

ZnO participates in:

- **ROS-driven apoptosis**
- **Mitochondrial membrane depolarization**
- **Cell cycle arrest at G2/M**
- **DNA fragmentation**
- **Suppression of angiogenesis and metastasis**

ZnO-mediated cytotoxicity is particularly effective in breast, cervical, lung, ovarian, and glioblastoma cancer models (Rossi et al. 2021).

7.4.2 ZnO-Based Combination Therapies

Conjugation with chemotherapy drugs (doxorubicin, cisplatin, paclitaxel) or natural agents (curcumin, quercetin) yields:

- pH-triggered drug release
- Increased tumor accumulation
- Reduced systemic toxicity
- 2–3× improved therapeutic indices (Rossi et al. 2021; Zhang et al. 2022)

7.4.3 Theranostic Platforms

Defect-rich ZnO serves as:

- **Fluorescent imaging probe**
- **Drug carrier**
- **ROS inducer**

in a single nanostructure, enabling real-time monitoring of therapeutic response.

7.5 ZnO as a Drug Delivery Platform

ZnO offers unique advantages in drug delivery:

- **High loading capacity** due to mesoporous or defect-rich structure
- **Acid-responsive release** optimized for tumors and inflamed tissue
- **Fluorescent imaging** for real-time tracking
- **Ease of functionalization** (PEG, antibodies, peptides, aptamers)

Hybrid ZnO systems (ZnO–liposome, ZnO–hydrogel, ZnO–PLGA) provide controlled release profiles suitable for:

- Anticancer drugs
- Antibiotics
- Anti-inflammatory molecules
- Growth factors for regenerative medicine

(Zhang et al. 2022)

7.6 Dental and Oral Health Applications

Nano-ZnO enhances mechanical strength and antimicrobial performance of dental materials, benefiting:

- Restorative composites
- Endodontic sealers
- Denture liners
- Orthodontic adhesives
- Periodontal gels

ZnO-NP incorporation leads to:

- Reduced secondary caries
- Decreased fungal colonization (*Candida spp.*)
- Improved bonding and wear resistance

(He et al. 2020)

These innovations align with clinical needs for durable, antimicrobial dental biomaterials.

7.7 Biosensing, Diagnostic & Imaging Technologies

ZnO's high electron mobility, defect luminescence, and strong surface reactivity enable:

Electrochemical Applications

- Glucose sensors
- Cholesterol biosensors
- Cancer biomarker detection
- DNA hybridization assays

Optical Applications

- UV/visible fluorescence imaging
- pH-sensitive tumor imaging
- Real-time intracellular tracking

ZnO nanowires have been integrated into **wearable diagnostic platforms** for continuous monitoring (Alam et al. 2022).

7.8 Translational Challenges and Barriers

Despite promising results, several challenges remain:

7.8.1 Manufacturing and Reproducibility

Batch-to-batch variability in size, dissolution rate and defect levels affects biological consistency (Huang et al. 2022).

7.8.2 Regulatory Complexity

Nanomaterials require extensive ISO 10993 testing:

- Genotoxicity
- Hemocompatibility
- Sensitization
- Chronic toxicity
- Degradation profiling

7.8.3 Long-Term Biodistribution and Clearance

More data are needed on chronic exposure, accumulation, immune memory, and long-term metabolic effects.

7.8.4 Standardization of Characterization Protocols
Global harmonization is required to compare ZnO safety across labs and clinical settings.

7.9 Future Prospects: Next-Generation ZnO Nanomedicine
ZnO-NPs are expected to evolve into **smart, adaptive, and biologically integrated nanomedicines** through:
7.9.1 Precision and Personalized Medicine
- Patient-specific nanoformulations
- Targeting based on tumor acidity or receptor profiles

7.9.2 Intelligent Implants
ZnO-based coatings capable of:
- Releasing antimicrobials on infection signals
- Encouraging bone and tissue regeneration
- Real-time diagnostic feedback

7.9.3 Multifunctional Theranostic Systems
Coupling imaging, therapy, drug delivery, and real-time monitoring into single ZnO platforms.

7.9.4 Regenerative Engineering
ZnO-integrated scaffolds promoting osteogenesis, angiogenesis, and nerve repair (Guo et al. 2023).

7.9.5 Green Nanotechnology
Phytochemical- and microbe-derived ZnO exhibit improved safety, sustainability, and regulatory acceptance (Fatima et al. 2023).

8. Limitations, Knowledge Gaps & Future Research Directions
Despite the rapid evolution of ZnO nanoparticle (ZnO-NP) science, several important scientific, translational, regulatory, and clinical gaps must be addressed to establish ZnO as a fully validated biomedical platform. While ZnO possesses strong antimicrobial, anticancer, and regenerative capabilities, its multifunctionality also introduces complexity in toxicity prediction, product standardization, and clinical reproducibility. This section outlines major limitations, unresolved scientific questions, translational challenges, and future avenues necessary to enable safe and effective clinical adoption.

8.1 Limitations in Synthesis, Standardization, and Physicochemical Control
One of the most persistent limitations in ZnO-based biomedical research is the lack of strict **standardization** across synthesis methods. Each route—chemical precipitation, hydrothermal, sol–gel, combustion, or green synthesis—produces distinct:
- morphologies
- surface defect profiles
- dissolution rates
- surface charge patterns
- ROS-generation potentials

These variations significantly alter biological behavior, making cross-study comparison difficult (Fernandes et al. 2022). For example, two ZnO samples with identical “30 nm average size” may differ radically in bandgap, lattice strain, or defect density, all of which dictate toxicological and therapeutic output.

Key limitations:
- Lack of consensus on “clinically acceptable” ZnO size or shape
- Variability in capping/functionalizing agents
- Poor reproducibility between laboratories

- Absence of standardized reporting formats for surface chemistry, polydispersity, and defect signatures

Without global characterization benchmarks, ZnO-based nanomedicines cannot progress smoothly through regulatory pipelines.

8.2 Biological Safety and Toxicological Uncertainties

Although ZnO degrades into biologically relevant zinc ions, toxicity concerns persist due to:

- ROS overproduction
- mitochondrial damage
- lipid peroxidation
- DNA damage under certain exposures
- inflammatory signaling activation

Moreover, ZnO-NPs exhibit **cell-type-dependent toxicity**, with cancer cells typically more susceptible than healthy cells (Agarwal et al. 2021). However, several knowledge gaps remain:

Unresolved safety questions:

- Long-term accumulation after repeated dosing
- Impact on reproductive and developmental systems
- Effects on microbiome composition
- Chronic exposure outcomes in immunocompromised patients
- Potential nanotoxic synergy when used simultaneously with other nanomaterials or drugs

These limitations indicate that safety must be evaluated in a **context-specific** manner rather than making universal toxicity assumptions.

8.3 Gaps in In Vivo Behavior, Pharmacokinetics & Biodistribution

Many studies rely heavily on **in vitro** toxicity or efficacy data, which do not fully replicate:

- dynamic protein corona formation
- degradation behavior in biological fluids
- immune clearance mechanisms
- tissue deposition
- renal vs. hepatic elimination
- biotransformation pathways

ZnO's dissolution rate drastically changes under biological conditions, but few studies quantify dissolution under **realistic physiological environments** (Niazi et al. 2022).

Critical knowledge gaps:

- Accurate pharmacokinetic (PK) models of Zn^{2+} release
- Chronic biodistribution and clearance profiles
- Comparative PK of bare vs. functionalized ZnO
- Influence of tumor acidity on dissolution variability
- Impact of corona proteins on targeting efficiency

Translational advancement requires more **systematic in vivo PK/PD studies** with standardized endpoints.

8.4 Limitations in Scaling, Manufacturing & Translational Feasibility

Large-scale production of ZnO nanoparticles remains limited due to:

- inadequate batch reproducibility
- high cost of dopant engineering
- challenges in controlling defect levels
- difficulties in large-volume green synthesis
- aggregation during storage

Long-term shelf stability is rarely reported, although it is essential for pharmaceutical deployment (Rehman et al. 2021).

Additionally, functionalized ZnO formulations (PEGylated, antibody-conjugated, peptide-targeted) raise challenges in:

- GMP-compliant manufacturing
- stability under sterilization

- maintaining biological function after lyophilization
- cost-effectiveness for industry-scale usage

8.5 Regulatory and Clinical Translation Barriers

ZnO is relatively underrepresented in clinical trials compared to gold or iron oxide nanoparticles.

Primary regulatory obstacles include:

- undefined nanoparticle-specific toxicity guidelines for ZnO
- concerns regarding ROS-related inflammatory events
- limited long-term human exposure data
- absence of standardized clinical-grade ZnO reference materials

Regulatory agencies such as FDA and EMA require extensive nanomaterial-specific data, including:

- hemocompatibility
- genotoxicity
- immunotoxicity
- chronic toxicity studies
- degradation product risk assessment

Most ZnO publications focus on efficacy rather than comprehensive safety profiles, making regulatory approval slower (Calderón-Jiménez et al. 2020).

8.6 Gaps in Mechanistic Understanding

Although ZnO's antimicrobial and anticancer activities are established, the **molecular pathways** remain partially described.

Key mechanistic gaps include:

- interactions between ZnO and cellular zinc transporters
- role of mitochondrial zinc flux
- oxidative stress signaling thresholds
- interplay between defect states and ROS generation
- immune-modulatory effects of ZnO on macrophages, T cells, and neutrophils

High-throughput transcriptomic and metabolomic studies are still scarce, limiting mechanistic clarity.

8.7 Environmental and Ecotoxicological Knowledge Gaps

ZnO-NPs pose potential environmental risks due to their release from:

- cosmetics
- sunscreens
- industrial waste
- textile coatings
- biomedical devices

ZnO can accumulate in soil, aquatic systems, and agricultural cycles (Tella et al. 2021).

Ecotoxicology gaps include:

- trophic-level transfer
- long-term soil–plant uptake
- accumulation in freshwater organisms
- chronic exposure outcomes in ecological systems

These concerns must be addressed to support sustainable large-scale use.

8.8 Future Research Directions

Although ZnO nanoparticles have demonstrated considerable therapeutic promise, their successful transition from laboratory research to clinical implementation depends on strategic, multidisciplinary advancements. Addressing these future directions will ensure the development of safer, more effective, and regulatory-compliant ZnO nanomedicines (Fernandes et al. 2022; Agarwal et al. 2021).

8.8.1 Development of Standardized, GMP-Grade ZnO Nanomaterials

The absence of standardized, pharmaceutical-grade ZnO nanoparticles remains a major limitation in current

biomedical research. Efforts must now focus on developing **Good Manufacturing Practice (GMP)**–compliant protocols to ensure batch-to-batch consistency and global regulatory acceptance (Sridharan et al. 2022).

A. Harmonized Physicochemical Benchmarks
Future studies should establish universal benchmarks for:
- particle size distributions,
- controlled morphologies (rods, spheres, tetrapods),
- lattice strain and crystalline defect intensity,
- hydrodynamic diameter in serum,
- zeta potential stability across physiological pH,
- dissolution profiles at varying acidity (4.5, 6.5, 7.4).

Such benchmarks will align ZnO nanomaterials with FDA-acceptable characterization standards (Gatto et al. 2021).

B. GMP-Compliant Production Pipelines
Key areas of development include:
- scalable biogenic reactors for plant/microbe-mediated synthesis,
- validated sterilization techniques (gamma radiation, aseptic filtration),
- lyophilization methods preserving nanoparticle integrity,
- ICH-guided stability testing for long-term shelf-life studies.

These steps will enable reliable production suitable for clinical trials (Roh et al. 2023).

8.8.2 Chronic In Vivo Safety, PK/PD & Biotransformation Profiling
Despite strong preclinical evidence, the long-term in vivo behavior of ZnO nanoparticles remains insufficiently understood, especially regarding **chronic exposure, biodistribution, clearance, Zn^{2+} release dynamics, and immune interactions** (Niazi et al. 2022).

A. Long-Term Toxicology Studies
Research should evaluate:
- multi-month tissue accumulation,
- systemic inflammation and cytokine profiles,
- reproductive and developmental toxicity,
- effects in immunocompromised and aged models.

Comprehensive assessments will support regulatory submissions (Martins et al. 2021).

B. Biotransformation Mapping
ZnO undergoes dynamic transformation inside biological fluids. Future work must map:
- protein corona evolution,
- enzymatic interactions,
- dissolution–aggregation cycles,
- formation of secondary nanospecies.

These processes fundamentally influence toxicity and efficacy (Keller et al. 2022).

C. Pharmacokinetic/Pharmacodynamic Modeling
ZnO requires unique PK/PD modeling to integrate both **nanoparticle behavior** and **Zn^{2+} ion release**, especially in acidic tumors and inflamed tissues (Rocha et al. 2021).

8.8.3 AI-Driven Predictive Toxicology, QSAR Modeling & In Silico Nanodesign
Artificial intelligence offers powerful tools to predict ZnO nanoparticle behavior and reduce reliance on animal testing.

A. AI-Driven Toxicity Prediction
Machine learning can model:
- ROS output thresholds,
- mitochondrial stress patterns,
- immune activation potential,

- safe exposure ranges.

These data-driven insights will guide rational material design (Jain et al. 2022).

B. QSAR (Quantitative Structure–Activity Relationship) Modeling

QSAR can link physicochemical parameters—size, surface defects, bandgap energy—with biological outcomes such as antimicrobial potency and cytotoxicity (Sane et al. 2021).

C. In Silico Optimization of Functional Nanostructures

Computational modeling can design ZnO nanostructures with optimized:

- dissolution rates,
- surface reactivity,
- tumor-targeting capability,
- ROS modulation capacity.

This accelerates development of safer nanoformulations (Rahmani et al. 2023).

8.8.4 Smart, Multi-Stimuli Responsive ZnO Systems

Next-generation ZnO nanocarriers must be engineered to respond precisely to biological triggers for controlled release and targeted therapy.

A. Multi-Stimuli Responses

Future ZnO systems may integrate responsiveness to:

- tumor acidity,
- enzymatic overexpression (MMPs, esterases),
- hyperthermia,
- oxidative microenvironments,
- external magnetic or ultrasound stimuli.

These intelligent platforms offer high therapeutic precision (Lin et al. 2022).

B. Theranostic Integration

Combining imaging, sensing, and therapy in one ZnO platform enhances clinical utility while reducing dosage and toxicity.

8.8.5 Personalized Nanomedicine Approaches

Personalized ZnO-based therapies could revolutionize treatment efficacy by tailoring nanoparticle design to each patient's molecular profile.

A. Tumor Microenvironment Profiling

ZnO formulations may be adjusted according to:

- pH gradients,
- metabolic signatures,
- angiogenesis status,
- redox balance.

B. Genetic Stratification

Differences in zinc transporter expression (ZIP1/ZIP4) influence ZnO uptake efficiency, enabling personalized anticancer treatment.

C. Immune-Based Personalization

ZnO dosing and surface engineering must be adapted for patients with chronic inflammation, autoimmune disorders, or suppressed immunity.

8.8.6 Green, Biogenic & Sustainable ZnO for Clinical Translation

While green-synthesized ZnO shows superior biocompatibility, several challenges remain.

A. Extract Standardization

Plant and microbial extracts vary widely in:

- polyphenol content,

- reductive capacity,
- stabilizing ligands.

Standardization is required to ensure reproducibility (Roh et al. 2023).

B. Biogenic Capping Advantages

Biomolecule-capped ZnO tends to show:

- reduced cytotoxicity,
- improved antioxidant properties,
- enhanced immune tolerance.

Future work must identify optimal phytochemical signatures for clinical use.

C. Scalability Barriers

Industrial-scale biogenic production still faces:

- biomass variability,
- inconsistent metabolite levels,
- need for GMP-approved organic sources.

8.8.7 Hybrid ZnO-Based Theranostic and Regenerative Platforms

Hybrid systems represent the frontier of ZnO nanomedicine.

A. Hybrid Theranostic Systems

Combining ZnO with gold, magnetite, or carbon nanomaterials can create platforms for:

- dual-mode imaging,
- magnetically guided targeting,
- synergistic ROS generation,
- real-time therapeutic monitoring.

B. Regenerative Medicine Integration

ZnO's intrinsic osteogenic and angiogenic properties make it ideal for:

- bone tissue scaffolds,
- vascular regeneration,
- neural repair frameworks.

C. Intelligent Implantable Devices

Future ZnO-enabled implants may offer:

- infection-responsive drug release,
- sensing and diagnostic feedback,
- wireless communication with external devices.

Such multifunctional systems could fundamentally redefine implant safety and longevity.

9 CONCLUSION & GLOBAL SUMMARY

Zinc oxide nanoparticles (ZnO-NPs) represent one of the most versatile, scientifically robust, and clinically promising nanomaterials in modern biomedical research. Over the past decade, advances in synthesis, functionalization, and mechanistic understanding have transformed ZnO from a classical semiconductor material into a **multidimensional biomedical agent**, exhibiting potent antimicrobial, anticancer, anti-inflammatory, regenerative, and biosensing capabilities (Hsu et al. 2022). Their intrinsic **pH-responsive dissolution**, **biodegradability into essential Zn^{2+} ions**, and **surface defect–driven biological activity** distinguish them from other metal oxide nanoparticles and elevate their translational appeal.

Clinical Promise Highlighted Across This Review

Evidence compiled across Sections 3–8 demonstrates that ZnO-NPs possess a unique combination of features ideal for precision medicine:

- **Broad-spectrum antimicrobial action** effective against multidrug-resistant pathogens
- **Intrinsic anticancer effects** driven by selective ROS generation and intracellular zinc disruption
- **Strong wound-healing acceleration**, including angiogenesis, fibroblast proliferation, and collagen deposition
- **Compatibility with medical implants**, enabling anti-infective and regenerative coatings

- **High drug-loading capacity** and multi-stimuli responsiveness for advanced drug delivery
- **Optoelectronic properties** suitable for imaging, biosensing, and theranostic systems

Together, these capabilities make ZnO a compelling candidate for next-generation biomedical interventions, especially in oncology, tissue engineering, infection control, and smart implant technologies (Morales et al. 2023).

Integrative Understanding & Central Insights

1. ZnO is both a therapeutic and a carrier.

Few nanomaterials combine intrinsic therapeutic effects with versatile drug-delivery functionality. ZnO's **dual role** allows synergistic treatments with reduced dosing, improved efficacy, and minimized toxicity (Sharma et al. 2022).

2. Surface engineering determines biological destiny.

Functionalization through polymers, peptides, antibodies, phytochemicals, or dopants dramatically alters toxicity, biodistribution, immune interaction, and targeting capability. The future of ZnO nanomedicine lies in **surface-tailored, precision-designed formulations** (Verma et al. 2021).

3. Stimuli-responsive and hybrid ZnO systems are the future.

Integrating ZnO with smart materials and hybrid nanostructures can yield ultra-sensitive diagnostic platforms and multi-modal therapies capable of imaging, sensing, and treating simultaneously (Rahman et al. 2022).

4. Biogenic and green synthesis is critical for clinical acceptance.

Green-synthesized ZnO demonstrates reduced toxicity, improved immunocompatibility, and enhanced surface functionality—aligning naturally with regulatory expectations for safe nanomedicine (Sen et al. 2023).

5. Translational barriers remain, but they are solvable.

Concerns regarding chronic toxicity, long-term biodistribution, batch variability, and scalability highlight the need for **rigorous standardization**, **comprehensive PK/PD studies**, and **GMP-compliant manufacturing systems** (Ortega et al. 2021).

A Unified Outlook: Where ZnO Nanomedicine Is Heading

The cumulative evidence suggests that ZnO is moving toward a clinically realistic future where:

- wound dressings accelerate healing more effectively than conventional therapies
- antimicrobial coatings drastically reduce implant-associated infections
- ZnO-based nanocarriers deliver chemotherapeutics with unprecedented precision
- theranostic nanoplatforms offer real-time imaging and targeted therapy
- regenerative scaffolds embedded with ZnO restore damaged tissue with superior outcomes
- personalized ZnO nanomedicine adapts treatment to patient-specific tumor biology and immune status
- Despite these hurdles, the scientific trajectory strongly supports ZnO's emergence as a **clinically transformative nanomaterial**—one with the potential to reshape the landscape of modern nanomedicine through smart, safe, personalized, and multifunctional therapeutic systems.

Such trends reflect the material's compatibility with the evolving paradigm of precision medicine and smart nanotherapeutics (Marques et al. 2022)

Key Insights	**Biomedical Relevance / Outcomes**	**Limitations & Challenges**	**Future Research Directions**
Chemical, sol–gel, hydrothermal, precipitation, microwave, green/biogenic synthesis	Enables controlled morphology, size tuning, and defect engineering; surface functionalization improves targeting	Batch variability, poor reproducibility, inconsistent defect states, polydispersity	Develop GMP-grade protocols, standardized characterization metrics, scalable biogenic synthesis
Zn^{2+} release, ROS modulation, bandgap tuning, lattice defects, high surface reactivity	Drives antibacterial potency, anticancer selectivity, imaging ability, pH-responsive behavior	Defect variability affects toxicity; dissolution depends on microenvironment	AI-modeling of structure–biological response, QSAR prediction tools
Broad-spectrum antibacterial, antifungal, anti-biofilm activity	Useful for wound dressings, implant coatings, catheters, surgical meshes	Cytotoxicity at high doses, potential microbiome disruption	Infection-responsive smart coatings, long-term in vivo infection models
ROS-driven apoptosis, mitochondrial dysfunction, Zn transporter modulation	Selective toxicity to tumor cells; synergy with chemotherapeutics; pH-triggered drug release	Dose optimization unclear; limited clinical trials	Personalized nanoformulations based on tumor acidity and transporter expression
Enhances collagen	Rapid tissue regeneration; improved	Chronic exposure studies lacking	Smart regenerative scaffolds,

formation, fibroblast proliferation, angiogenesis	wound closure; anti-inflammatory effects		ZnO-enabled 3D printed constructs
High loading capacity, tunable dissolution, inherent fluorescence, imaging potential	Targeted delivery, combination therapy, real-time imaging, biodiagnostic integration	Stability under sterilization, storage aggregation, immune interactions	Multi-stimuli responsive and hybrid ZnO nanocarriers
Antibacterial composites, endodontic sealers, antifungal coatings	Reduces secondary caries, inhibits biofilm formation, strengthens materials	Biofilm re-growth in acidic oral environments	Long-term clinical dental trials, smart dental composites
ROS generation, DNA damage potential, organ accumulation concerns	Potentially safe with proper surface engineering; biocompatible at low doses	Incomplete chronic toxicity data; unclear biodistribution	Long-term PK/PD, biotransformation profiling, systematic toxicology standards
Potential soil/water accumulation, trophic transfer	Important for safe large-scale deployment	Limited ecological data	Ecotoxicological modeling, safe-by-design nanomaterials
Requires ISO 10993 biocompatibility, nanotoxicology assessments	Essential for clinical translation	Lack of nanoparticle-specific guidelines	Develop regulatory-grade reference ZnO materials
AI-driven design, personalized nanomedicine, intelligent implants, green nanofabrication	Highly integrative future of ZnO nanomedicine	Cost, scalability, regulatory constraints	Smart biosensing implants, hybrid multifunctional systems, sustainable production pathways

Final Global Summary:

In summary, **ZnO nanoparticles embody the convergence of material science, nanotechnology, medicine, and biology**, offering multifunctional capabilities unmatched by many established biomedical nanomaterials. Their unique physicochemical features—ROS modulation, photonic behavior, pH-sensitive dissolution, surface defect engineering, and bioavailability of Zn^{2+}—enable broad-spectrum applications spanning oncology, infection control, wound healing, biosensing, and regenerative medicine.

However, widespread clinical adoption depends on addressing key challenges: standardized manufacturing, chronic toxicity evaluation, mechanistic clarity, environmental safety, and regulatory compliance. The future success of ZnO in clinical practice will require collaborative efforts across materials scientists, toxicologists, biomedical engineers, regulatory bodies, and clinicians.

Table 7. Consolidated Summary of ZnO Nanoparticles: Synthesis, Properties, Biomedical Applications, Safety Challenges, and Future Directions

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