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(Review Article)

Recent advances in green-synthesized zinc oxide nanoparticles: Synthesis strategies, physicochemical characterization, antibacterial activity, toxicological aspects and biomedical applications: Review article

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Abstract

Green Synthesis of nanoparticles (NPs) using plant products results in green-synthesized zinc oxide nanoparticles ZnO NPs that are functional as much as the nanoscale form would be, and also referred to green synthing due to its advantages on environmental aspect. ZnO Nano-particles (NPs) own significant physicochemical properties such as large surface-area-to-volume ratio, ultraviolet absorption ability copy tholec with catalytic activity under UV light vacuum chloride plapasticty and considerable anti-microbial functionality. These characteristics into them show great potential to be acted as promising materials for the applications like antibacterial coating, wound healing, drug delivery system, biosensing probes and sensors systems, cosmetics agriculture; a food packaging material various environmental related purpose.

Green synthesis provides a relatively safer and greener alternative compared to many traditional chemical and physical methods. Various types of biological materials like plant extracts, bacteria, fungi and algae as well as natural polymers are employed for supporting the formation of nanoparticles by acting with various functions: reducing agents; stabilizing agents (preventing agglomeration); capping agent (lead to shape control). Of these routes, plant-mediated synthesis is particularly attractive because it is simple, low-cost and scalable with the added benefits that plants are rich in phytochemicals (e.g. phenolics/flavonoids/proteins/organic acids/polysaccharides). Finally, at the nanomaterial level these compounds can affect a plethora of factors such as nucleation, particle growth and morphology changes to uncover varying surface chemistry that gives rise to differences in colloidal stability (final ground state) or biological activity.

This review presents an overview of the recent advances achieved in green-synthesized zinc oxide (ZnO) nanoparticles over last two decades, encompassing comparative synthesis strategies and their physicochemical characterization results when blended with other biocommunicating nanomaterials elucidating its antibacterial mechanisms while discussing said toxicological limitations that serve as associative evidentiary basis for imparting multi-faceted biomedical applications towards a potential bio-nanomedicine. The properties of ZnO NPs are significantly influenced by different synthesis parameters (e.g., precursor concentration, ratio of extract to the metal salt or no. Consequently, establishment of standardization and comprehensive reporting can be beneficial to enhance reproducibility between studies for future comparison.

Integrated characterization approach (using XRD, UV–Visible spectroscopy, FTIR, SEM and TEM for microscopic examinations and DLS along with zeta potential analysis) is also discussed in the review. The antibacterial activity is primarily related to reactive oxygen species generation, Zn2+ ion release, membrane damage and oxidative stress disruption of the bacterial cellular functions. Nevertheless, to explain specific antibacterial mechanisms inhibition-zone assays alone are insufficient and need a stronger quantitative test.

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Despite their outstanding biomedical potential, the safety of green-synthesized ZnO NPs must be thoroughly evaluated. What should be considered is the fact that a green synthesis in itself does not imply biocompatibility, because under some conditions ZnO NPs can exhibit dose-dependent cytotoxicity; there may also occur oxidative stress or inflammation and genotoxicity. In conclusion, green ZnO nanoparticles are highly efficient and sustainable nanomaterials; however their practical use requires standardization of the synthesis process combined with a complete characterization not only physicochemical properties but also content related to antibacterial performance as well as comprehensive toxicological evaluation towards human health through standardized in vitro/in vivo methodologies.

Keywords: Zinc Oxide Nanoparticles; Green Synthesis; Plant Extracts; Antimicrobial Mechanisms; Reactive Oxygen Species; Zn2+ Release; Cytotoxicity; Biomedical Applications

1. Introduction

One of the most examined metal oxide nanomaterials with respect to structural, optical, chemical and biological characteristics is zinc oxide nanoparticles which can find applications in dozens of scientific and industrial domains up to now. ZnO is a wide-bandgap semiconductor characterized by powerful ultraviolet absorption, high exciton binding energy, photocatalytic action, simple chemical stability, low cost and comparatively large biological compatibility. Zinc oxide made in the nanoscale has a larger surface region per particle volume, which can improve features such as surface reactivity and contact with light energy, electro catalysis or ion discharge and contact with biological systems (Khodarev & Brand mussel2012). While at the nanoscale its properties can be explained by these nanoscale features, ZnO nanoparticles have found widespread application in cosmetics, sunscreens, sensors, photocatalysis, antimicrobial coatings (AgNPs -ZnO), food packaging systems as antioxidants and antibacterial agents for fresh-food preservation in agriculture [1], [2], [6], [10], [12] including (although not exclusively) environmental remediation of heavy metals and dyes from wastewater effluents using ZnO/PVA nanofibers produced with 90% lower concentrations than reported approaches [15],[31],[36],[83]-[93].

The interest in ZnO nanoparticles is particularly increased especially for biomedical and antimicrobial research because they are able to act on microbial cells by multiple paths. Their antibacterial activity has been reported against Gram-positive and Gram-negative bacteria; however, the efficacy varies due to bacterial structure, nanoparticle size, morphology, concentration, charge hydrophobicity/philicity state and conditions of exposure. We suggest that ZnO nanoparticles kill bacteria ultimately by generating reactive oxygen species, releasing of Zn2+ ions, disrupting cell membranes and changing membrane permeability, damaging proteins as well as interfering with intracellular metabolic pathways. Because of these mechanisms that might happen at the same time, ZnO nanoparticles are commonly considered to be potential candidates for antimicrobial surfaces, wound-care systems, coatings, textiles, and packaging materials [38], [51], [52] [66]–[68] [86]-87.

Various methods of ZnO nanoparticle synthesis have been reported to date including precipitation, sol-gel processing, hydrothermal synthesis, solvothermal synthesis, combustion synthesis (one-pot), vapor deposition as well as other physical and chemical routes. Although these methodologies generate nanoparticles with controlled size, morphology and degree of crystallinity, they often require high temperature synthesis, strong reagents, toxic solvents, extended reaction times or expensive instruments. These limitations have prompted to design more environmentally friendly synthesis methods seeking to minimize environmental impact, simplify preparation and use safer biological materials as stabilizing- and capping agents [20], [21], [24]–[30], [42]–[44], [72].

Green synthesis depends on biological sources (plant extract, bacteria, fungi, algae, enzyme and biopolymer) for the stabilization of nanomaterials. These biological systems are endowed with molecules that can respond to metal ions and regulate nucleation and growth. The continuous search for plant extracts is due to being cheap, abundant and ease of preparation, chemical diversity. Members of the class of plant secondary metabolites may include phenolics, flavonoids, terpenoids, alkaloids, proteins and amino acids, sugars, organic acids and polysaccharides in leaves, seeds, fruits or their peels, roots and flowers. Compounds from l-cysteine-type family can be reducing agents, complexing agents, also stabilizing and morphogenic agents in the process of ZnO nanoparticle synthesis [8], [13], [14], [27], [32]–[41], [44]–[48],[74],[84],[89].

However, without critical assessment such benefits of green synthesis should not be overestimated. Biological extracts are chemically complex and their composition can vary according to plant species, plant part, geographical origin, harvesting season, extraction solvent, extraction temperature, extraction time and storage conditions. Therefore, studies using the same plant species can result in ZnO nanoparticles of different sizes, morphologies, surface chemistry and antibacterial activity. One of the main challenges constraining the reproducibility and mass application of green-synthesized ZnO nanoparticles is this variability [18], [19], [34], [37], [38], [67], [68], [86], 87.

An additional big challenge in this field is insufficient documentation on synthesis conditions. Several articles reported positive production of ZnO NPs through visual color change, UV–Visible absorption peaks, XRD patterns and FTIR bands, microscopy pictures and inhibition-zone dilution tests. Nonetheless, critical experimental parameters are often omitted or only briefly discussed. These factors are the molarity of precursor, extract-to-precursor ratio, pH, reaction temperature and time, stirring rate, drying temperature,(calcinations temperature), washing method and conditions (centrifugation conditions) storage medium final yield. Once these details are finally lost, it becomes near impossible to reproduce the synthesis, make biological comparisons or elucidate the impact of once-detailed synthetic conditions on nanoparticle properties [8], [24]–[28], [43], [44], 72, 88, 89.

Integrated physicochemical characterization is essential for a dependable study of ZnO nanoparticles. X-ray diffraction, usually employed to establish the phase purity of crystalline materials and specifically for wurtzite ZnO, The crystallite size obtained from XRD therefore does not always represent the ultimate particle size measured optically. Though UV–Visible spectroscopy gives some information related to optical absorption and band-gap-related behavior, it does not confirm morphology or purity. It is worth noting that FTIR provides some details about the organic functional groups from the plant or microbial capping agents, but as a standalone experiment, it cannot be used to show complete chemical composition. SEM and TEM provide information regarding morphology and primary particle size, while DLS and zeta potentials measurements give us information about hydrodynamic size, aggregation behavior, polydispersity, and colloidal stability in liquid media [1], [4], [5], [9], [31]–[36], [43], [44].

In biological studies, the difference between primary particle size and hydrodynamic size is especially relevant. While TEM may identify small nanoparticles, DLS sizes will often be larger as particles in suspension may be protected by layers of solvent, surrounded by biological capping molecules or in the form of aggregates. Indeed, this difference can have a predominant effect on antibacterial activity, cellular uptake, toxicity and biological distribution. Consequently, rather than being treated as equivalent quantities in high-quality studies crystallite size, primary particle size, aggregate size and hydrodynamic diameter should be well defined to avoid confusion [19], [34], [36], [65], [83].

Antibacterial testing should also be performed upon certain considerations. Historically, agar diffusion and inhibition-zone assays have been popular since they are easy to perform and straightforward to interpret. One caveat on these methods, though, is their limited application on nanoparticles since it can be so that the latter easily sediments or diffuse poorly through agar. A bigger inhibition zone does not mean that the activity of the nanoparticles is higher and a smaller one does not imply inactivity. Hence, quantitative assays such as minimum inhibitory concentration, minimum bactericidal concentration, time-kill curves, antibiofilm assays, bacterial membrane leakage tests and ROS detection and dissolved zinc-ion measurement should be exclusive-based methods used for the antibacterial evaluation. This type of assay has thus been used as a stronger indicator of antibacterial mechanisms or to better compare studies [14], [37], [38], [51], [52], [55]–[60], [94].

Analysis of toxicity is another key domain in regards with target research on ZnO nanoparticles. While green-synthesized ZnO nanoparticles are frequently termed eco-friendly or biocompatible, safety cannot be predicted based solely on synthesis route. ZnO nanoparticles can undergo alteration in their structure and release zinc ions, produce reactive oxygen species (ROS), ultimately modulating mitochondrial bioenergetics activity, impacting membrane integrity that may further activate inflammatory signaling pathways and cause genotoxicity under specific circumstances. It is because their biological effects rely on particle size, morphology, surface charge and chemical or physical coating, concentration, exposure time, dissolution rate in fluids and swelling in tissues/agglomeration [7], [10], [19], [34], [38], [67], [83],[86],[93].

In terms of its possible use in biomedical applications, although research has demonstrated the strong potential for ZnO nanoparticles to be used in such areas it is essential that a realistic assessment of their merits is carried out. Emphasis on local applications (e.g., antibacterial coatings, wound dressings, antimicrobial textiles, surface-protective materials) more close to sense of feasible via controlled exposure. Stronger evidence related to selectivity, biodistribution, clearance, immune response, dose control and long-term safety are needed for facilitating more complex applications such as systemic drug delivery, anticancer therapy and bioimaging [6]. Thus,translating mushrooms as drug should be cautiously balanced against antibacterial activity and mammalian-cell compatibility [10], [12], [15]–[17], [35], [65], [83], [84].

In this review, we therefore present a concise and integrated overview of the state-of-the-art for green-synthesized ZnO nanoparticles. It covers synthesis strategies, physicochemical characterization, antibacterial mechanisms, toxicology and biomedical applications. It aims not only to summarize the merits of green ZnONp, but also to pinpoint the challenges that impede reproducibility and translation. Potential shortcomings include non-standardization of extract chemistry, incomplete reporting of synthesis conditions, overinterpretation of minimal characterization data, continued

reliance on inhibition-zone assays, mechanisms of action are rarely investigated [19], [34], [36], testing for antibacterial activity is not usually performed in more mechanistic detail and long-term toxicological evidence isnon-existent [38], [43], [83].

Correspondingly well-edited reviews on green-synonymised ZnO nanoparticles among the totally syntactic synthesis of material and biological function. It ought to describe how the size, shape, crystallinity, surface chemistry, colloidal properties as well as antibacterial activity and toxicity are determined by the synthesis parameters. And it should differentiate preliminary biological activity from true readiness for translation. This review focuses on standardization, mechanism, safety and application-specific design in order to guide the field of green ZnO nanoparticle research from descriptive synthesis studies toward reproducible, safe and application-ready nanomaterials [8], [14], [19], [23], [36], [38], [43], [44], [84], [85].

Table 1 Review logic and critical value.

Dimension	Scientific value	Critical weakness
Synthesis	Connects biological chemistry to nucleation and capping	Extract composition is rarely standardized
Characterization	Confirms phase, morphology and colloidal state	Techniques are often interpreted separately
Antibacterial activity	Shows broad-spectrum potential	Assay conditions limit comparison
Toxicology	Defines safe dose window	Long-term evidence is limited
Translation	Connects material to product use	Scale-up and regulation remain weak

The manuscript is described in Table 1 as a critical Review instead of a descriptive applications list.[19], [34], [36], [43], [83]

1.1. Green synthesis routes

Of the various green syntheses, plant-mediated synthesis in which leaves, seeds, fruits, peels and roots are used as reducing agents is most frequently reported because they are cost effective and diverse in terms of essential phytochemicals. A common pathway involves zinc acetate or zinc nitrate with an aqueous extract from a plant, which is then adjusted to a pH, heated and dried, often calcined. The major modifiers are precursor concentration, molarity, extract ratio, pH, reaction time, temperature and calcination temperature. [8], [13], [14], [27], [32], [41], [44], [74], [84], [89]

Table 2 Comparative evaluation of green ZnO NP synthesis routes.

Route	Advantages	Limitations	Best use
Plant extract	Low cost, fast, phytochemical-rich	Seasonal variability	Large screening and low-cost production
Fungal	High enzyme production	Culture contamination risk	Antimicrobial fibers and coatings
Bacterial	Potentially biocompatible matrices	Requires sterile culture control	Biomedical and feed-related systems
Algal	Marine polysaccharide capping	Source variability	Photocatalysis and antibiofilm work
Biopolymer-assisted	Improved handling and release control	Harder comparison with bare ZnO	Wound dressings and composite coatings

Oriented microbial, fungal, algal, and biopolymer-assisted pathways may provide a relatively stronger biological control or higher degree of composite integration but present issues with biosafety due to managed culture conditions and batch-to-batch variability. These methods are particularly suited for applications where the final application is a coating,

textile, wound dressing or bioactive composite rather than merely a powder. [21], [30], [43], [45]-[50], [54]-[64], [75]-[82]

And here is why, this table shows synthesis choice should be made by the application in mind not assuming one green route always better than all others. [29], [35], [39], [40], [65], [70], [79]

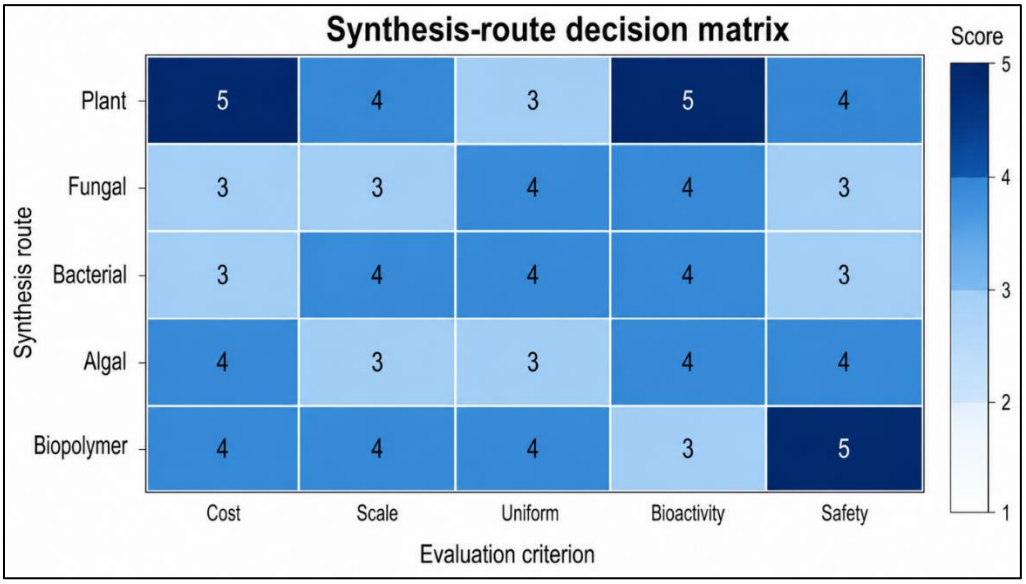

Figure 1 Synthesis-route decision matrix.

It takes very scattered statements of literature and puts it into a decision matrix system. It is NOT experimental, but rather interpretive and should be used to motivate discussion about which synthesis to select. [39], [40], [65], [70], [72]

2. Physicochemical characterization

A rigorous ZnO NP Review must convey that no technique is complete. XRD confirms crystalline phase; UV-Vis identifies optical behavior; FTIR provides evidence for capping-group assignment; SEM/TEM reveals morphology and primary size; DLS measures hydrodynamic size that are correlated to the stability of individual NPs in solution, and zeta potential estimates colloidal stability. The best studies connect these measures rather than treating them as independent numbers. [1], [4], [5], [9], [31]-[36], [43], [44]

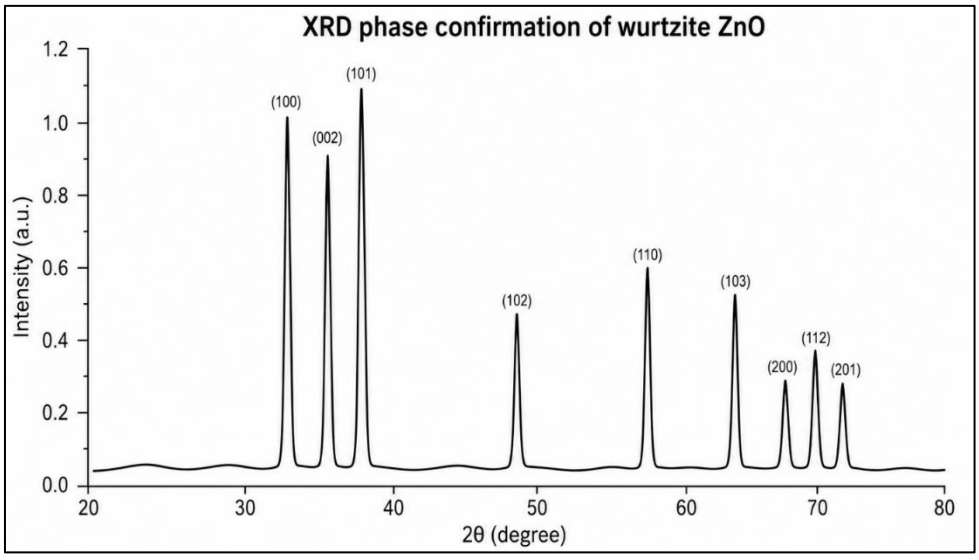

Figure 2 Representative XRD phase confirmation of wurtzite ZnO.

The labelled reflections correspond to common wurtzite ZnO planes. In a final submission, this placeholder should be replaced with experimental XRD data and matched to a recognized database card. [1], [4], [33], [36], [93]

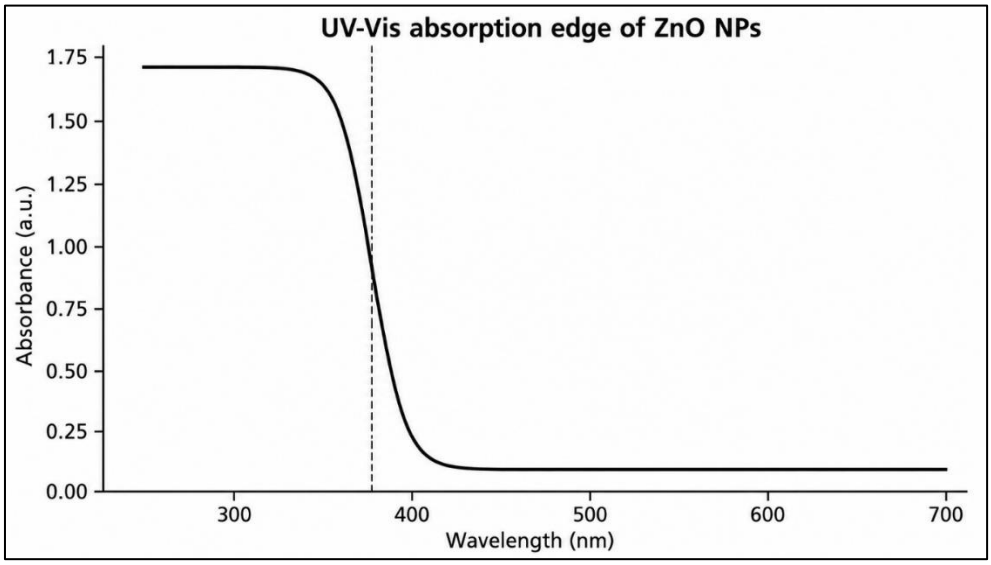

Figure 3 Representative UV-Vis absorption edge.

The near-UV absorption edge is a signature of ZnO NPs, but optical shifts need to be interpreted with respect to XRD and microscopy, as defects, aggregation and capping can all affect spectra.[4], [5], [13], [32], [93]

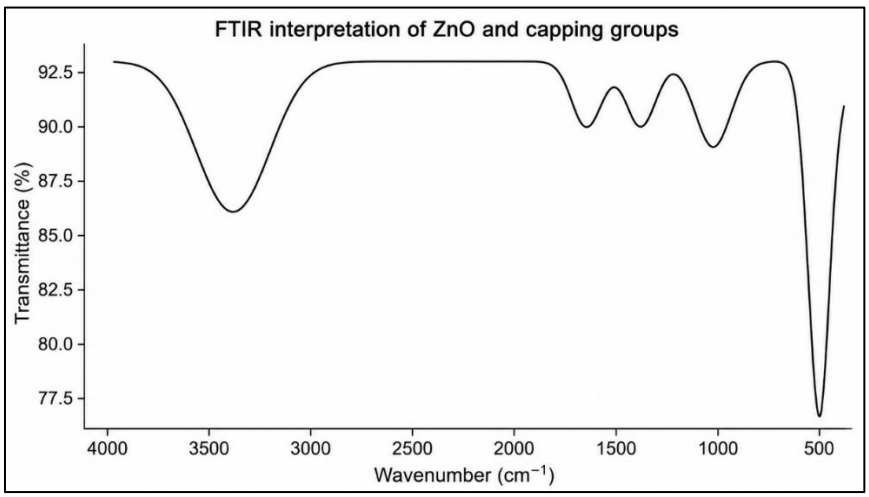

Figure 4 Representative FTIR interpretation**.**

While FTIR may indicate organic capping and Zn-O vibration, it should not be used as the sole proof of purity. XRD, EDS and ideally TGA or XPS are needed for solid interpretation. [24]-[28], [43], [44], [89]

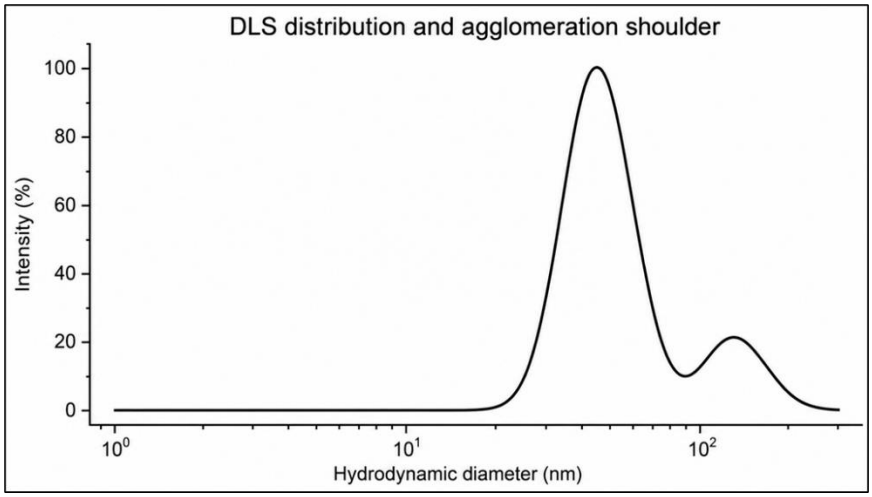

Figure 5 Representative DLS distribution.

Hydrodynamic diameter in DLS measurements is usually larger than size derived from TEM due to solvation and agglomeration. Any Review must clearly explain this difference. [19], [34], [36], [65], [83]

Table 3 Characterization technique map.

Technique	Output	Common mistake	Best practice
XRD	Phase/crystallite size	Equating crystallite with particle size	Report card, FWHM and assumptions
UV-Vis	Absorption edge	Using edge as size proof	Compare with XRD/TEM
FTIR	Capping groups/Zn-O	Claiming purity from FTIR alone	Combine with structural and elemental data
TEM/SEM	Morphology/primary size	Using too few particles	Add size histogram
DLS	Hydrodynamic size	Direct comparison with TEM	Report medium, pH, PDI
Zeta	Colloidal stability	Treating as toxicity proof	Report ionic strength and pH

Table 3 addresses one of the most common reviewer criticisms: over-interpretation of isolated characterization data. [19], [33]-[36], [65], [83]

3. Antibacterial mechanisms

The mechanisms involved in the process whereby oxidative stress is activated by ZnO NPs (synergism between ROS generation, release of zinc ions, direct contact with membrane cytocomponents and intracellular pressure) have been also reported to cause apoptosis in gingival fibroblast cells. ROS are capable of oxidizing lipids, proteins and nucleic acids; zinc ions canbring homeostasis disruption; contact with NP causes membrane integrity destruction. The most significant mechanism varies due to size, surface defects, light exposure, pH and concentration of the bacterial suspension, nutrient medium composition and type of bacteria envelope.[38], [51], [52], [66]-[68], [86], [87], [94], [95]

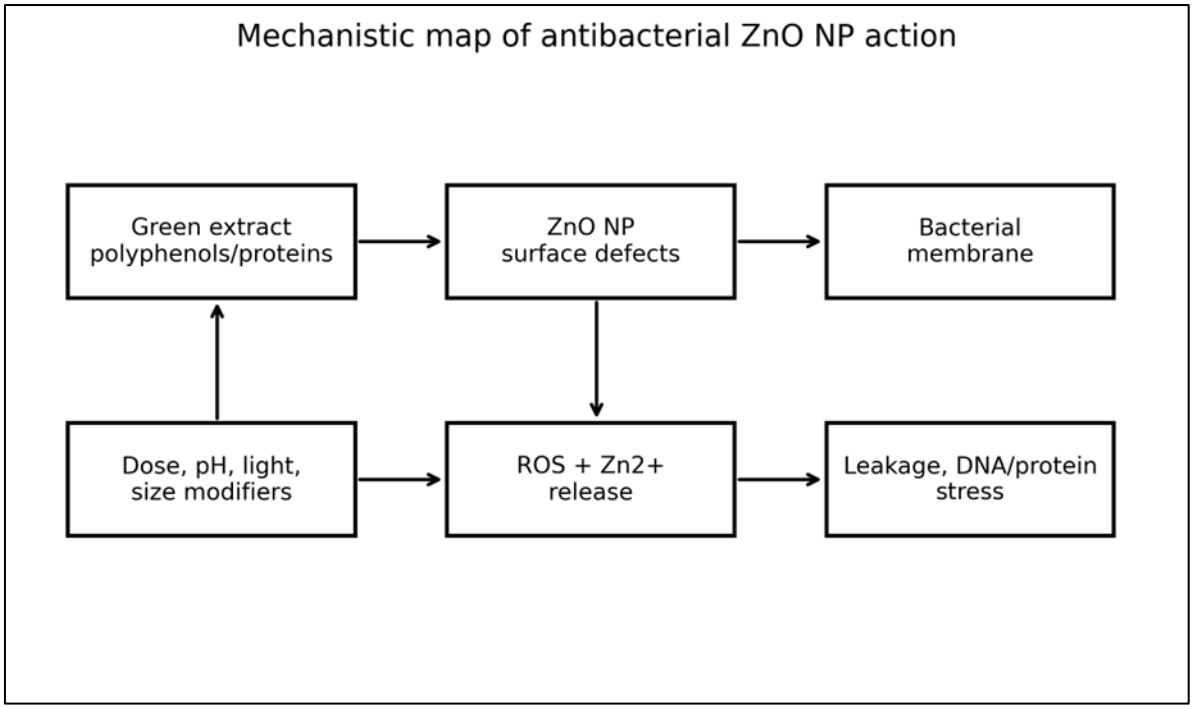

Figure 6 Mechanistic map of antibacterial ZnO NP action**.**

As illustrated in the figure, antibacterial activity should not be reduced to a single mechanism. An overview review that talks about ROS, Zn2+ release, membrane interaction, experimental conditions for assays and bacteria structure at the same time would be ideal. [38], [51], [52], [66], [68], [94]

Inhibition-zone results are poor for mechanism but useful when screening. Small molecules diffuse though agar, but this does not occur for nanoparticles and sedimentation can skew apparent activity. Zinc release Heterogeneity and quantified data of MIC, MBC, time-kill curves, antibiofilm assays, membrane leakage tests and ROS quantification give much more power.[14], [37], [38], [51], [52], [55]-[60], [94]

Table 4 Antibacterial mechanism-to-evidence map.

Mechanism	**Indicator**	**Strength**	**Concern**
ROS generation	ROS probes, ESR, oxidative markers	Strong if quantified	Light and medium confounding
Zn2+ release	ICP-OES/ICP-MS	Strong if controlled	Dissolution may not fully explain killing
Membrane damage	SEM/TEM bacteria, leakage assays	Moderate to strong	Images alone are qualitative
Biofilm inhibition	Crystal violet, CLSM	High clinical relevance	Must separate killing from anti-adhesion
Antibiotic synergy	FIC, checkerboard, time-kill	High translational value	Requires standardized design

4. Biomedical applications

This encompasses areas including biomedical applications such as antimicrobial dressings, wound-healing platforms as well as drug delivery, anticancer therapy, bioimaging, biosensing and antimicrobial coatings. The nearer term, more realistic applications are local applications (e.g., coatings and dressings in which exposure is easier to control than systemic delivery).[10], [12], [15]-[17], [35], [65], [83], [84]

ZnO NPs are described in many cancer-related studies as selective proliferating cell killers through oxidative stress and apoptosis [44]. This is a promising start but should not be oversold in the absence of controls using normal cells, apoptosis markers, mitochondrial assays, and preferably in vivo biodistribution data.[15]-[17], [46], [77], [85], [86]

Table 5 Biomedical application readiness.

Application	Evidence maturity	Required tests	Translation comment
Antibacterial coatings	High in vitro	Leaching, durability, repeated killing	Near-term if release is controlled
Wound dressings	Moderate-high	Cytocompatibility, inflammation, animal wounds	Promising because exposure is local
Cancer therapy	Moderate in vitro	Selectivity, biodistribution, clearance	Needs stronger in vivo safety
Drug delivery	Emerging	Loading, release, pH response	Needs surface functionalization
Biosensing/bioimaging	Emerging-moderate	Signal stability and interference	Application-specific

The table separates scientific promise from translational readiness and prevents exaggerated claims. [12], [15]-[17], [35], [65], [83]

5. Toxicology and safety

The biggest hurdle to real biomedical application is toxicity. At inappropriate doses, ZnO NPs can release Zn2+, generate ROS, alter mitochondrial function, activate inflammatory pathways, and induce genotoxicity. Toxicity is not constant: it depends on particle size, morphology, coating, agglomeration, exposure route, concentration and cell type. [7], [10], [19], [34], [38], [67], [83], [86], [93]

One of the biggest weaknesses in this literature is on a single viability assay at high in vitro concentrations. Nanoparticles can disrupt optical assays so safety assessment should avoid dependence on single endpoint and include: viability, membrane integrity, mitochondrial activity, ROS production, apoptosis markers inflammatory mediators and genotoxicity. [7], [19], [34], [58], [67], [86], [93]

Table 6 Recommended toxicity endpoints**.**

Endpoint	Assay family	Reason
Viability	MTT/CCK-8 plus confirmatory assay	Avoids single-assay bias
Oxidative stress	ROS probes, antioxidant markers	Links toxicity to mechanism
Membrane integrity	LDH, live/dead staining	Detects acute damage
Apoptosis	Annexin V/PI, caspase	Defines cell-death pathway
Inflammation	IL-6, TNF-alpha, NF-kB	Relevant for skin and wound exposure
Genotoxicity	Comet, micronucleus	Important for regulatory confidence

This table strengthens the Review by converting safety criticism into a practical testing framework. [7], [19], [34], [67], [86]

6. Critical gaps and future directions

The first gap is reproducibility. Unfortunately in many green ZnO NP studies, the extract chemistry, pH value, precursor-to-extract ratio, ionic strength of solutions drying conditions calcination profile and storage conditions were not

reported [4]. In the absence of these variables, it remains challenging to replicate particle size, morphology, crystallinity and biological response.[8], [24]-[28], [43], [44], [72], [88], [89]

The second gap is around quantitative comparability. Reviews should look to go beyond the inhibition zones list and also include MIC, MBC, biofilm inhibition, ROS generation, Zn2+ release particle size PDI zeta potential and mammalian-cell. This enables true structure-property-activity analysis.[38], [51], [52], [66]-[68], [94], [95]

The third gap is translation. While green synthesis offers more sustainable processes, it does not default to clinical readiness. Preclinical aspects that still require continued work before which medical claims start to be plausible are scale-up, sterilisation, stability and regulatory toxicology and then medium-term in vivo studies.[10], [15]-[17], [34], [35], [83], [84], [86]

Table 7 Reviewer-style checklist.

Item	Minimum expectation	Why it matters
Synthesis reporting	pH, molarity, extract ratio, time, temperature	Reproducibility
Structural data	XRD plus microscopy	Phase and morphology confirmation
Colloidal data	DLS, PDI, zeta potential	Exposure state
Biological assays	MIC/MBC, ZOI, biofilm, toxicity	Mechanism and safety
Statistics	Replicates, error bars, significance	Reliable interpretation
Critical discussion	Limitations and contradictions	Review-level quality

The checklist assists to translate the manuscript into a publishable Review, which can assist for expansions on numerical datasets extracted later.[19], [34], [36], [38], [83]

7. Conclusion

The scientific significance of green synthesized ZnO nanoparticles is attributed to their sustainable synthesis, versatile physicochemical properties, as well as demonstrated antibacterial and biomedical activity. The bottom-line conclusion is not "ZnO NPs are safe (or even clinically ready) across the board" but rather that ZnO NPs are potentially useful materials with performance characterized by how well they can be synthesized, surface chemistry, size distribution, colloidal state and dose at time of exposure. Thus, a high-quality Review ought to focus on mechanism, standardization, toxicity and translation. [8], [14], [19], [23], [36], [38], [43], [44], [84], [85]

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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