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Copper-Based Nanoparticles in Cancer Therapy: Integrating Synthesis, Biological Interactions, Mechanistic Insights

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Abstract

Copper-based nanoparticles (CuNPs) have emerged as a versatile and cost-effective platform in cancer nanomedicine, driven by their intrinsic redox activity and tunable physicochemical properties. Their anticancer activity largely involves amplification of reactive oxygen species (ROS), which impair mitochondrial functions, damages DNA, delays cell division and induces programmed cell death. Interestingly, the cause of selective cytotoxicity of CuNPs over normal tissues is the high basal oxidative stress in cancerous cells. The stability, selectivity, and functional performance of CuNPs have been enhanced lately by the new synthesis methods, surface engineering and the hybrid nanostructures. In preclinical investigations, dose-dependent anti-cancer activity has been demonstrated in a wide range of tumor models and biosafety issues suggest the significance of dose control of oxidative stress and the unwarranted consequences. In comparison to other metal nanoparticles such as silver and gold, CuNPs can provide a favorable balance among therapeutic activity, scalability, and cost-effectiveness. Regardless of these benefits, there are oxidative instability, reproducibility, and limited clinical translation. The development of the future will rely on using CuNPs in combination regimens and customized nanomedicine, and rigorous in vivo validation and clinical evaluation. Altogether, CuNPs are a potentially powerful but immature nanotherapeutic platform that has a high chance of being used to treat cancer in the next generation.

Keywords: Copper nanoparticles; Cancer therapy; Oxidative stress; Apoptosis; Nanomedicine.

1. Introduction

Cancer remains one of the leading causes of morbidity and mortality in the world, and it is one of the major global health burdens, despite substantial improvements in early diagnoses and



treatment measures [1]. The global cancer statistics also show that about 19.3 million new cancer cases and almost 10 million deaths related to cancer were reported worldwide in 2020 and its rate is expected to keep increasing with the population growth, aging, and environmental risks (such as smoking, alcohol consumption, etc.) [2]. The traditional therapeutic approaches such as surgery, chemotherapy, and radiotherapy have been significantly effective in increasing the survival of most malignancies, but their clinical performance is usually curtailed by extreme systemic toxicity, multidrug resistance, recurrence of the tumor and nonselectivity to malignant cells. Also, chemotherapeutic agents often cause harm to normal tissues that rapidly proliferate, causing unwanted effects like immunosuppressive effects, gastrointestinal toxicity, and dysfunction of organs that may undermine compliance with treatment and quality of life in patients [3]. Further, inherent and acquired pathways of resistance in tumor cells including increased DNA repair, drug efflux pump, and apoptotic evasion persist as significant impediments to long-term treatment delivery and still represent significant hindrances in oncology [4]. There is a growing interest in alternative and complementary approaches to treatment, especially nanotechnology-based approaches, which have the potential of targeted delivery, improved therapeutic indices, and reduced systemic toxicity in managing cancer [5].

The rapid development of nanotechnology has created new opportunities to enhance the diagnosis and treatment of cancer, providing some new approaches to improve treatment accuracy and effectiveness [6,7]. Nanomaterials have distinct physicochemical properties such as high surface to volume ratio, size tunability, and surface chemistry that allow easy drug loading, targeted delivery and cellular internalization [7,8]. In cancer, the nanoparticle-based systems have also shown improved tumor accumulation via the enhanced permeability and retention (EPR) effect and could be less systemic toxic than traditional chemotherapeutics [6,9]. Moreover, nanotechnology facilitates active drug delivery, enhanced bioavailability, and formation of multifunctional theranostic system incorporating imaging and therapy in a singular system [8,10]. Together, these benefits make nanomedicine an attractive approach to address the existing shortcomings in the field of cancer treatment and to develop more accurate and efficient treatment options.

Increasingly popular metal-based nanoparticles as cancer therapies have shifted away from classic and expensive noble metals like gold and silver to cheaper and redox-active metals like copper nanoparticles (CuNPs). Although gold and silver nanoparticles have shown great potential in both therapeutic and diagnostic applications, their sensitivity and high cost combined with low redox properties have led to the

utilization of other transition metals with superior catalytic and oxidation capabilities [11,12]. CuNPs, in particular, are reported to have a high redox cycling capacity and are able to catalyze the formation of reactive oxygen species, which makes the use of such nanoparticles as potential anticancer nanomaterials promising [13,14]. All these properties coupled with their comparative abundance and low cost of production make CuNPs good candidates to be used as next generation nanotherapeutic agents in cancer studies [10,15].

In comparison with gold and silver nanomaterials, CuNPs are highly catalytic and oxidative species that can be effective in the generation of reactive oxygen species which can induce cancer cell apoptosis to prevent tumor proliferation [8,16]. They are also quite inexpensive and naturally abundant which further increase their use in large-scale biomedical applications and translational research [8]. In addition, new methods of green synthesis and surface functionalization have also improved the stability and biocompatibility of CuNPs, increasing their therapeutic advantages in cancer [17]. These properties, when combined, have rendered CuNPs attractive agents in the next-generation anticancer nanotherapeutics, and worthy the growing interest in studies of their mechanistic and biomedical applications [18,19].

Despite the growing number of review articles on CuNPs for cancer therapy, most primarily summarize published findings without providing a comprehensive critical evaluation of how nanoparticle physicochemical characteristics, synthesis strategies, and structure-activity relationships collectively influence therapeutic efficacy, selectivity, and biosafety. Furthermore, emerging mechanisms beyond ROS-mediated apoptosis and the major challenges associated with clinical translation remain insufficiently integrated into a unified framework. Therefore, there is a need for an updated critical review that integrates recent evidence, identifies current knowledge gaps, and systematically connects these aspects to support the rational development of CuNP-based cancer nanotherapeutics.

This review critically examines recent advances in CuNPs as emerging nanomaterials for cancer therapy, with emphasis on their physicochemical characteristics, synthesis strategies, and molecular mechanisms of anticancer activity, particularly oxidative stress induction and apoptosis. The overall conceptual framework of the discussed topics is summarized in Fig. 1. Furthermore, this review integrates current experimental evidence, critically compares published findings, and discusses existing challenges and future research priorities to facilitate the rational development and clinical translation of CuNP-based anticancer nanomedicines.

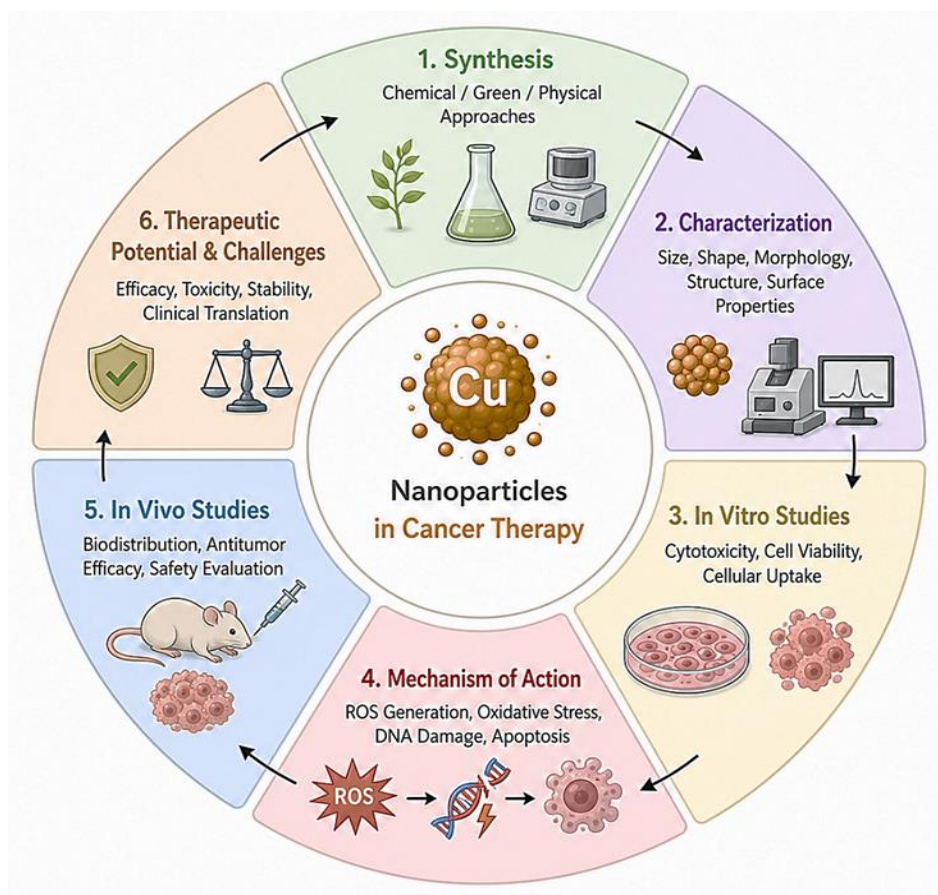


Figure 1. Schematic overview of the main aspects of CuNPs in cancer therapy, including synthesis approaches, physicochemical characterization, biological evaluation (in vitro and in vivo), and underlying anticancer mechanisms.

2. Synthesis Strategies of CuNPs

2.1. Chemical synthesis methods

2.1.1. Chemical reduction

One of the most established and controllable methods for producing CuNPs is chemical reduction, due to the relative simplicity of its operation and the ability to perform large scale production of CuNPs with adjustable physicochemical properties [20,21]. Under this approach, copper ions (Cu^{2+}) present as precursor salts like copper sulfate or copper chloride are reduced chemically to produce metallic copper (Cu^0), by strong reducing agents, including sodium borohydride, hydrazine or ascorbic acid [20]. The nanoparticle system is usually reduced through a nucleation step, then a growth step, whereby the rapid transformation of Cu^{2+} to Cu^0 produces initial atomic clusters which appear as clusters of different sized atom clusters then merge with other entity clusters to form stable and nanostructured nuclei of copper [22]. Reaction kinetics, precursor concentration, and temperature control the growth of these nuclei into distinct nanoparticles, whereas stabilizing or capping agents, such as polyvinylpyrrolidone (PVP) or citrate inhibits any further aggregation and surface oxidation by creating a coating around

the surface of the nanoparticles [8,23]. Moreover, it is possible to control the particle size distribution, morphology, and crystallinity of particles with great accuracy by optimizing the parameters in relation to the pH, weakening of reducing agents, and reaction time, which are the key aspects that determine the biological performance and anticancer activity [24]. The reproducibility, scalability, and structure control granted by chemical reduction procedures render them quite rewarding to the development of CuNPs to be utilized in biomedical and oncological settings [20]. The chemical reduction process, including precursor reduction, nucleation, controlled growth, and stabilization by capping agents, is schematically illustrated in Fig. 2.

2.1.2. Stabilizing and capping agents

Surface stabilization is crucial aspect during the synthesis of the CuNPs as newly formed copper nanoclusters possess high surface energy, and it is highly susceptible to oxidation and aggregation in aqueous media. To overcome this, various stabilizing/capping reagents i.e. polymers, surfactants, and biomolecules are employed to create protective coverage of the nanoparticle surfaces thereby enhancing colloidal stability and blocking unregulated growth of nanoparticles during the

nucleation and maturation stages [25]. These surface modifiers interact with copper atoms through electrostatic or coordination bonding, forming steric or electrostatic barriers that control nanoparticle dispersion, morphology, and crystallinity [26]. In addition to increased physicochemical stability, appropriate surface functionalization also affects significantly biological processes, including cellular internalization, biodistribution, and cytotoxic selectivity of the cancer cell [27]. As recent reports suggest, the therapeutic efficacy of CuNPs can be enhanced by developing engineered surface coatings that improve biocompatibility and minimize nonspecific toxicity without affecting their oxidative stress mediating anticancer effects, thereby enhancing their biomedical and oncological potential [28,29].

CuNPs are formed in a series of reduction-nucleation-growth steps as illustrated in Fig. 2. Copper ions are transformed into metallic nuclei, and then, the formation of particles is regulated in specific reaction conditions. In this mechanism, molecular species available in the medium of reaction are adsorbed to the surface of the nanoparticle, regulating the growth rate and suppressing interparticle fusion. The thermal treatment that follows, then allows crystallization and formation of necks between the particles, which results in a structural consolidation during the sintering process. This mechanistic process regulates the development of particle size and structural integrity during the synthesis procedure. In other words, as shown in Fig. 2 surface-active species regulate nanoparticle growth and prevent aggregation, while post-synthesis thermal treatment contributes to crystallization and structural consolidation.

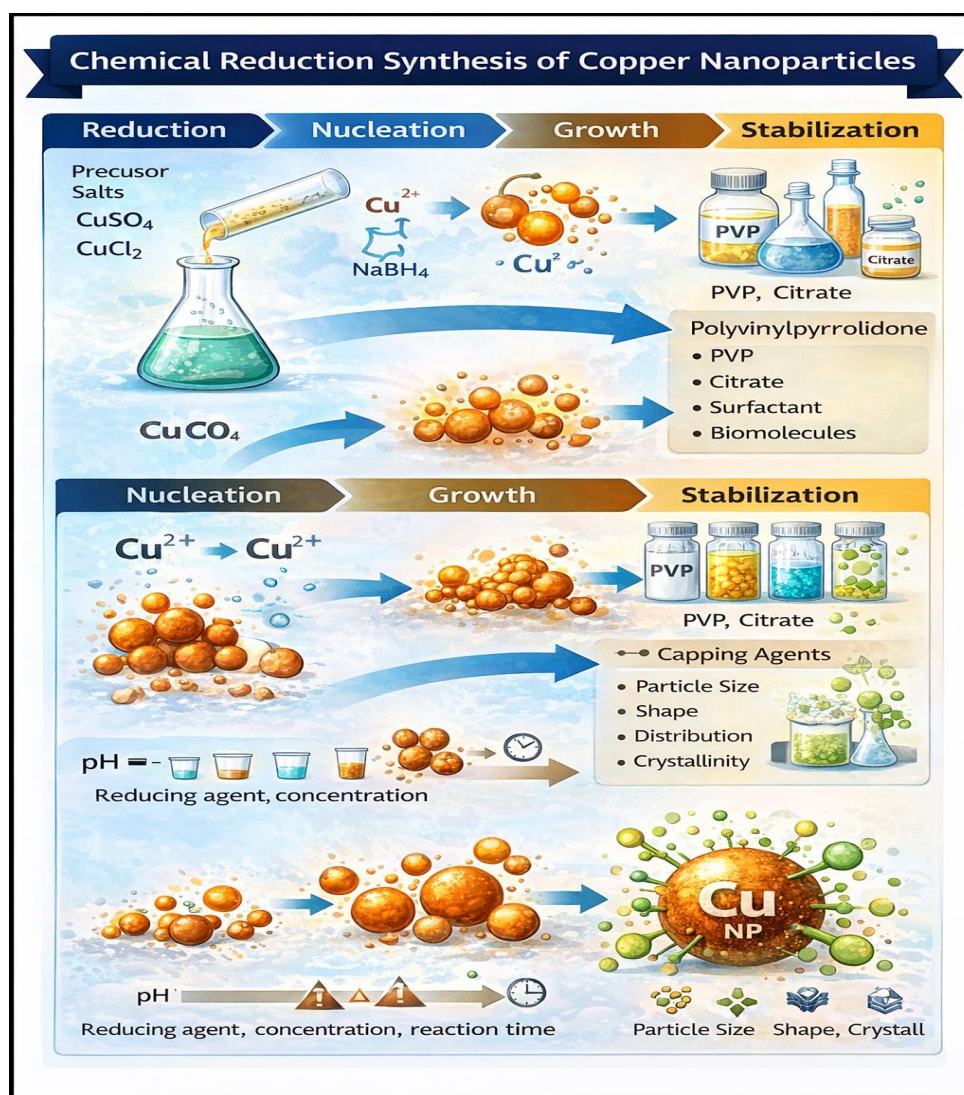


Figure 2. Schematic illustration of the chemical reduction synthesis of CuNPs, highlighting the sequential stages of precursor reduction (Cu^{2+} to Cu^0), nucleation, and particle growth under controlled reaction conditions, based on relevant literature [30,31]. The figure also depicts the role of stabilizing and capping agents (e.g., PVP, citrate, surfactants) in preventing aggregation and surface oxidation, thereby ensuring colloidal stability. Key synthesis parameters, including pH, temperature, reducing agent strength, and reaction time, govern particle size, morphology, and crystallinity. Post-synthesis processes such as thermal treatment further contribute to structural consolidation and nanoparticle integrity.



2.2. *Green synthesis approaches*

Green synthesis of CuNPs has also received growing interest as a more sustainable alternative to traditional physicochemical methods of fabricating nanoparticles. In this method, plant extracts, microorganisms, and natural biomolecules are used as reducing and stabilizing agents to allow the formation of nanoparticles at mild and environmentally friendly conditions [32]. Green routes, as compared to traditional chemical synthesis, use fewer dangerous reagents, use less energy, and are more biocompatible in general, which makes them especially appealing in biomedical and environmental use [33]. The primary effect of bioactive compounds present in natural extracts improves controlled nucleation and growth of CuNPs which promote colloidal stability and functionality of the constructs [34,35]. These advantages have made the green-synthesized CuNPs to have a promising future in multiple aspects which include antimicrobial systems, catalytic reactions and novel anticancer nanomedicine [36]. As a result, green synthesis is now being considered as among the main approaches in safe and versatile developing nanomaterials based on copper. The general processes of plant-mediated green synthesis of CuNPs are graphically represented in Fig. 3, showing the reduction of copper ions with phytochemicals, nucleation and growth of the nanoparticles, and subsequent stabilization and ROS-mediated anticancer effects.

2.2.1. *Plant extracts*

Plant extracts are a promoted bioresource to sustainable production of CuNPs due to the naturally assemblies of bioactive

metabolites which play out the production of nanoparticles. Unlike entirely chemical reduction systems, plant extracts contain a very diverse repertoire of phytochemicals (flavonoids, phenolics, terpenoids, alkaloids, proteins, polysaccharides) that can be deployed to reduce metal ions, as well as to stabilize the resulting nanostructures. With the use of functional groups such as the hydroxyl and carbonyl groups, these compounds will react with copper ions and allow the aggregation and growth to be regulated and the organic capping layers to be obtained on the nanoparticle surface along with nanoparticle being able to be more colloidally stable [37] [38].

Together with nanoparticles formation regulation, plant-derived biomolecules play an effective role both on the physicochemical properties and on the biological behavior of the produced nanoparticles. The phytochemical variations between plant species could play a major role in the distribution of particle sizes, morphology and the surface properties and ultimately determine the catalytic and antimicrobial behavior of the resultant nanoparticles [39]. Furthermore, surface-bound phytochemicals can have added bioactivity and greater biocompatibility over nanoparticles prepared through traditional chemical methods [40]. Due to their synergistic interaction, plant metabolites and nanoparticle surfaces, plant-mediated synthesis has also been a key approach toward the generation of environmentally friendly nanomaterials with prospective biomedical and catalytic uses. Table 1 summarizes the representative plant sources that were used in the synthesis of the CuNPs, their major phytochemicals, and their reported biological activities.

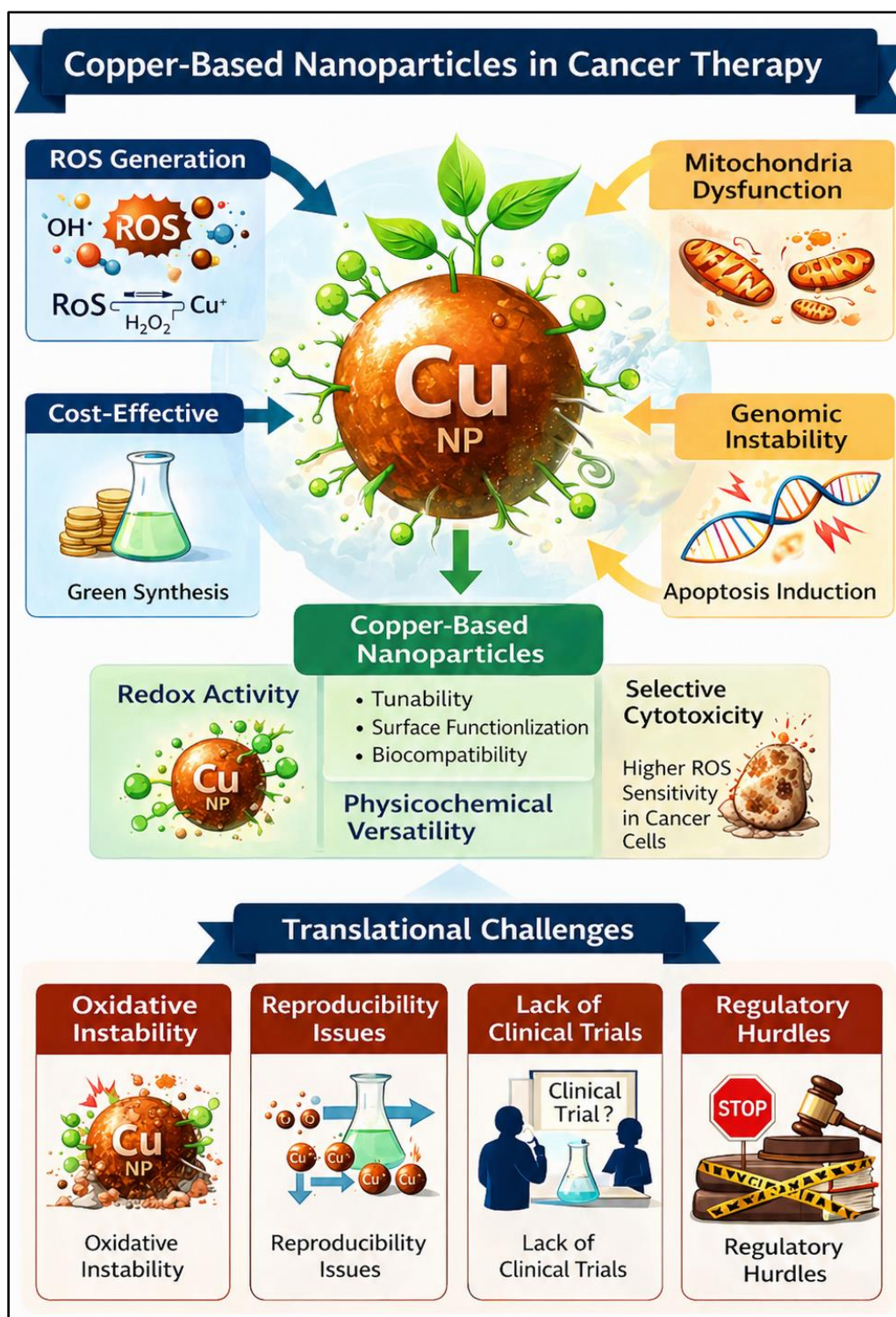


Figure 3. Schematic representation of the green synthesis of CuNPs mediated by plant-derived phytochemicals, which act as reducing and stabilizing agents, based on previous studies [13,41,42]. The synthesized nanoparticles subsequently induce anticancer effects via ROS-mediated oxidative stress, triggering mitochondrial dysfunction, DNA damage, and apoptosis.



Table 1. Plant-mediated green synthesis of CuNPs using different plant extracts. The table summarizes representative plant sources, plant parts used, particle size ranges, major phytochemicals involved in the reduction and stabilization processes, and the reported biomedical or antimicrobial activities of the synthesized nanoparticles.

Plant source	Plant part used	Particle size (nm)	Key phytochemicals involved	Biomedical activity	Ref.
<i>Azadirachta indica</i>	Leaves	18–80	Flavonoids, terpenoids, polyphenols (reducing & capping agents)	Antibacterial activity and stable CuNP formation	[43]
<i>C. sinensis</i> + <i>O. sanctum</i>	Leaves (aqueous extract)	12–55	Catechins, eugenol, phenolics, flavonoids	Enhanced antibacterial activity via synergistic phytochemicals	[44]
<i>Aloe barbadensis</i> (Aloe vera)	Leaf extract	60 nm (avg)	Phenolics, polysaccharides, and bioactive metabolites acting as reducing and stabilizing agents	Catalytic performance and potential biomedical applications	[45]
<i>Moringa oleifera</i>	Seeds	20–60	Proteins, amino acids, phenolics	Antimicrobial activity and environmental remediation potential	[46]
<i>Punica granatum</i>	Peel extract	30–100	Ellagic acid, tannins, polyphenols	Antimicrobial activity against opportunistic pathogens	[47]
<i>Allium sativum</i>	Bulb extract	25–75	Organosulfur compounds (allicin derivatives), flavonoids	In vivo antibacterial efficacy (lung infection model)	[48]

2.2.2. Microbial and biomolecule-mediated synthesis

The microbial systems as well as isolated biomolecules have become efficient biological platforms to synthesize CuNPs because of their capacity to reduce metal ions via the enzymatic and metabolic pathways. Copper ions can be reduced into nanoscale structures by a variety of microorganisms: bacteria, fungi, yeast, and algae using intracellular or extracellular processes that involve reductases, peptides, and electron-shuttling biomolecules. These biological components promote the nucleation process and the growth process and at the same time stabilize the form of nanoparticles by covering them with protein or polysaccharide which enhance colloidal stability and biocompatibility [49,50].

Cellular metabolites and extracellular polymeric substances are used in microbial synthesis pathways as natural reducing and capping agents and allow the formation of nanoparticles in the mild environmental conditions. The

mechanism enables microorganisms to produce nanoparticles of predictable morphology and functional surfaces which can be used to augment antimicrobial, catalytic or biomedical activity. As an illustration, the fungal-mediated synthesis with the by use of *Aspergillus niger* has been thus reported to synthesize spherical CuNPs with sizes of approximately 15 to 85 nm, and exhibits promising antimicrobial and antioxidant effects [51].

Due to these benefits, microbial and biomolecule-mediated methods are now being considered as scalable and environmentally friendly methods of synthesizing metal nanoparticles. Their capacity to work in aqueous media and biologically functionalize surfaces of nanoparticles provides special possibilities to design functional nanomaterials to work in biomedical, catalytic and environmental areas [52,53]. The general mechanism of microbial-mediated synthesis of CuNPs, including enzymatic reduction and nanoparticle formation by microbial metabolites, is schematically illustrated in Fig. 4.

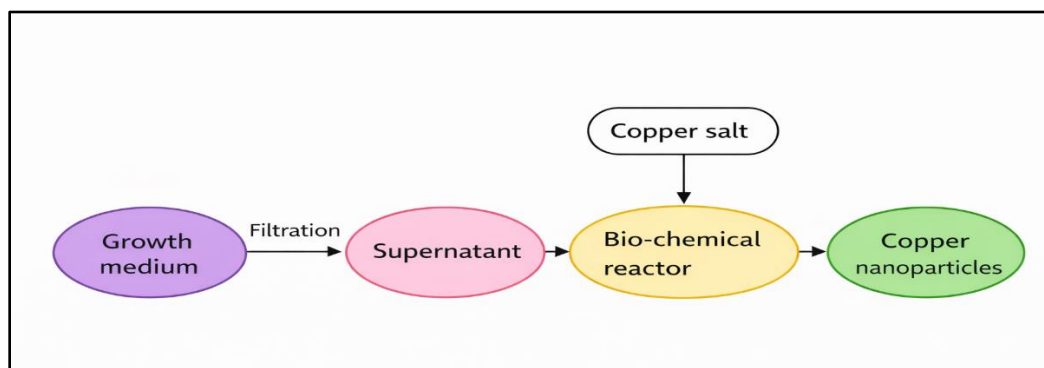


Figure 4. Schematic representation of the process of microbially mediated production of CuNPs. Bacteria and fungi reduce copper ions (Cu^{2+}) by enzymatic and metabolic routes, triggering the nucleation and growth of CuNPs, and the extracellular biomolecules act as natural capping and stabilizing factors [54].

2.2.3. Advantages of biogenic CuNPs

Biogenic production of CuNPs provides an alternative to traditional approaches, namely, biological systems help to obtain nanoparticles in non-hazardous conditions with low energy consumption and biogenic requirements, which increases environmental and biological safety [38,55].

The other outstanding benefit associated with biogenic CuNPs is that biological metabolites have an inherent role in the reduction and stabilization processes. Phytochemicals (polyphenols, flavonoids, proteins, polysaccharides) can be used as reducing and capping agents simultaneously, to obtain nanoparticles with enhanced colloidal stability and surface functionality without the need to add other synthetic stabilizers [56]. These surface-bound biomolecules tend to modify the physicochemical properties of CuNPs and potentially lead to improved biological action, such as antimicrobial, catalytic and biomedical actions [57]. Moreover, biosynthetic protocols often produce nanoparticles with well-regulated size distribution and active surfaces, which can be used to increase their biological system interaction. The features are particularly beneficial in the biomedical and environmental areas where the stability of nanoparticles, reduced toxicity and surface reactivity is required in the definition of performance and safety [58].

2.3. Challenges in CuNPs synthesis

Even though there has been an increase in interest regarding CuNPs, the production of the nanoparticles still faces several practical and scientific difficulties that might interfere with their stability, reproducibility, and mass production. Other issues, which continue to be significant to different synthesis methods, include aggregation, particle oxidation, and inability to control the size and morphology. Further, the consistency of the resultant nanostructures could be affected by fluctuations in the conditions of experiment and chemistry of the precursor. These are the specific problems that need to be addressed at the time to

improve the credibility of CuNPs fabrication and expand their application in technology and biomedicine.

2.3.1. Oxidation susceptibility of CuNPs

Despite numerous advantages of CuNPs, their high susceptibility to oxidation so easily in the case of exposure to air, moisture, or aqueous conditions is one of the most important disadvantages of this nanoparticles. Having the huge surface-volume ratio of copper at the nano scale, the metallic surface is reactive to oxygen at and easy to form copper oxides that cannot be resisted as in the case of Cu_2O or CuO . Such type of oxidative processes can significantly alter the physicochemical properties of the nanoparticles including electrical conductivity, catalytic activity and antimicrobial activity of the nanoparticles hence determining its stability and functionality in other application fields over time [56,59]. Aggregation of nanoparticles due to oxidation on the surface is also a phenomenon to be considered in most applications and performance of CuNPs in life science or catalytic conditions. To overcome this deficit, a number of such stabilization strategies have been proposed to include the functionalization of surfaces with polymers, organic ligands or biomolecules which may act as protective surfaces against exposure to oxygen. Other than the enhanced resistance to oxidation, the added coatings can enhance the dispersion stability and biocompatibility of the CuNPs making them more suitable in real application [60,61].

Oxidation, therefore, is one of the key factors that have to be controlled in the process of synthesis, storage, and use of copper-based nanomaterials. Recent research focuses on the application of green synthesis methods and biomolecule-based capping reagents to increase the stability of nanoparticles without altering their functions [62].

2.3.2. Stability and reproducibility

The stability and reproducibility of CuNPs are some of the most important issues that determine the real-life applicability

of these nanoparticles in biomedical, catalytic, and environmental systems. Due to their high surface energy and strong tendency toward oxidation, CuNPs may undergo structural and chemical transformations during storage or application, leading to aggregation, surface passivation, or changes in functional properties. This uncertainty may influence the dispersibility of nanoparticles and their catalytic or antimicrobial activity especially in atmospheric conditions where copper is easily oxidized into oxide coating [63,64]. The other critical problem is that of reproducibility of the production of nanoparticles, particularly in the biological or green methodology of nanoparticles synthesis. Differences in the biology of different sources, extract composition, reaction conditions, and environmental conditions can have an effect on the nucleation and growth processes but lead to variations in the size, morphology, and surface chemistry of nanoparticles across

batches. Therefore, the stable nanoparticle features have continued to be one of the most important factors in guaranteeing dependability and scalability in the manufacture of nanomaterials [38,55].

To overcome these problems, recent research pays much attention to the cautious optimization and standardization of the conditions of synthesis such as precursor concentration, pH, temperature, and reaction time. Besides that, stabilizing agent or biomolecule-mediated capping layers have been reported to enhance the stability of nanoparticles and the dispersion results of nanoparticles have been demonstrated to last longer periods thus making them reproducible and easy to implement into technology and biomedical schemes [63]. The oxidation and aggregation processes affecting CuNPs stability are schematically illustrated in Fig. 5.

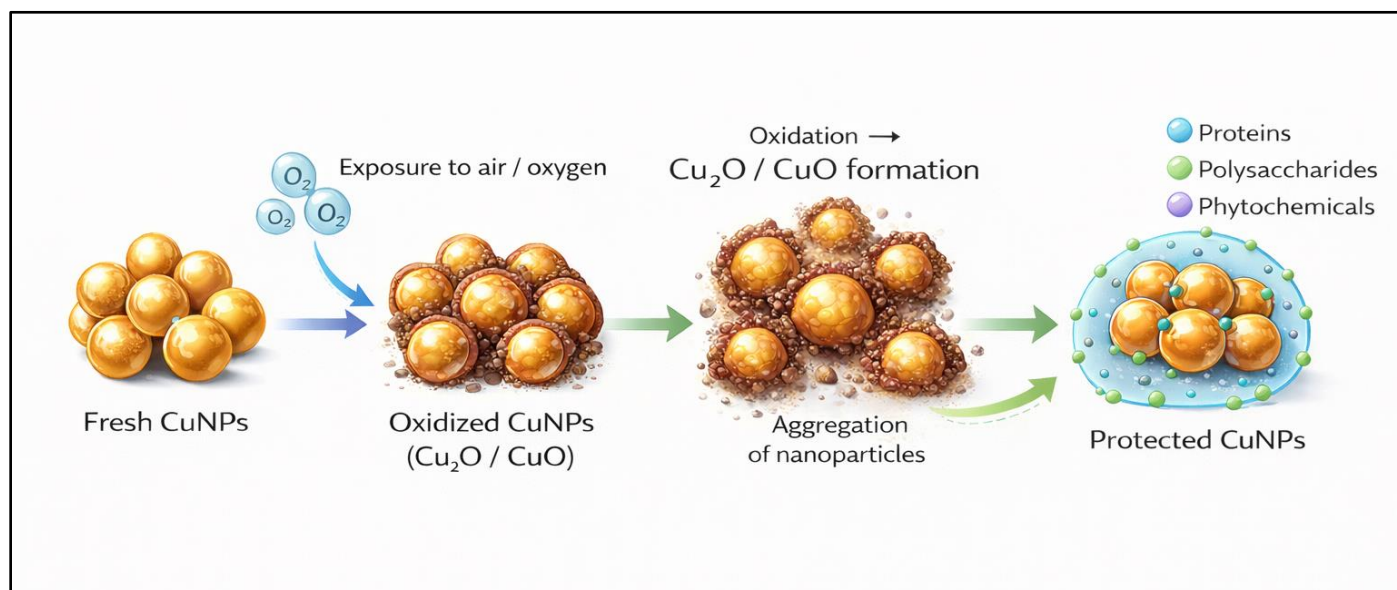


Figure 5. Schematic illustration of the instability mechanisms of CuNPs, highlighting oxidation to copper oxides ($\text{Cu}_2\text{O}/\text{CuO}$), nanoparticle aggregation, and stabilization through surface coatings or biomolecule-mediated capping agents [65,66].

3. Physicochemical Properties of CuNPs

The unique physicochemical properties of CuNPs are due to their nanoscale size and a large surface/volume ratio. These properties such as size and morphology, crystallinity, surface chemistry, and optical properties are the determining factors in the catalytic performance, antimicrobial performance, and biomedical applications. In addition, minor differences in the conditions of synthesis and stabilization methods may also have a strong impact on these parameters. As a result, the complete knowledge of the physicochemical properties of CuNPs is needed in order to customize its behavior and increase its range of application.

3.1. Size, shape, and surface characteristics

CuNPs physicochemical properties particularly the particle size, its morphology and surface properties are extremely worth considering in establishing the physicochemical stability as well as the biological performance of the nanoparticles. The size of the particle is another factor that creates significant difference with regards to the surface area and reactivity; small nanoparticles are somewhat more catalytically and antimicrobially active due to lack of separation between size and surface is high [38], as illustrated in Fig. 6.

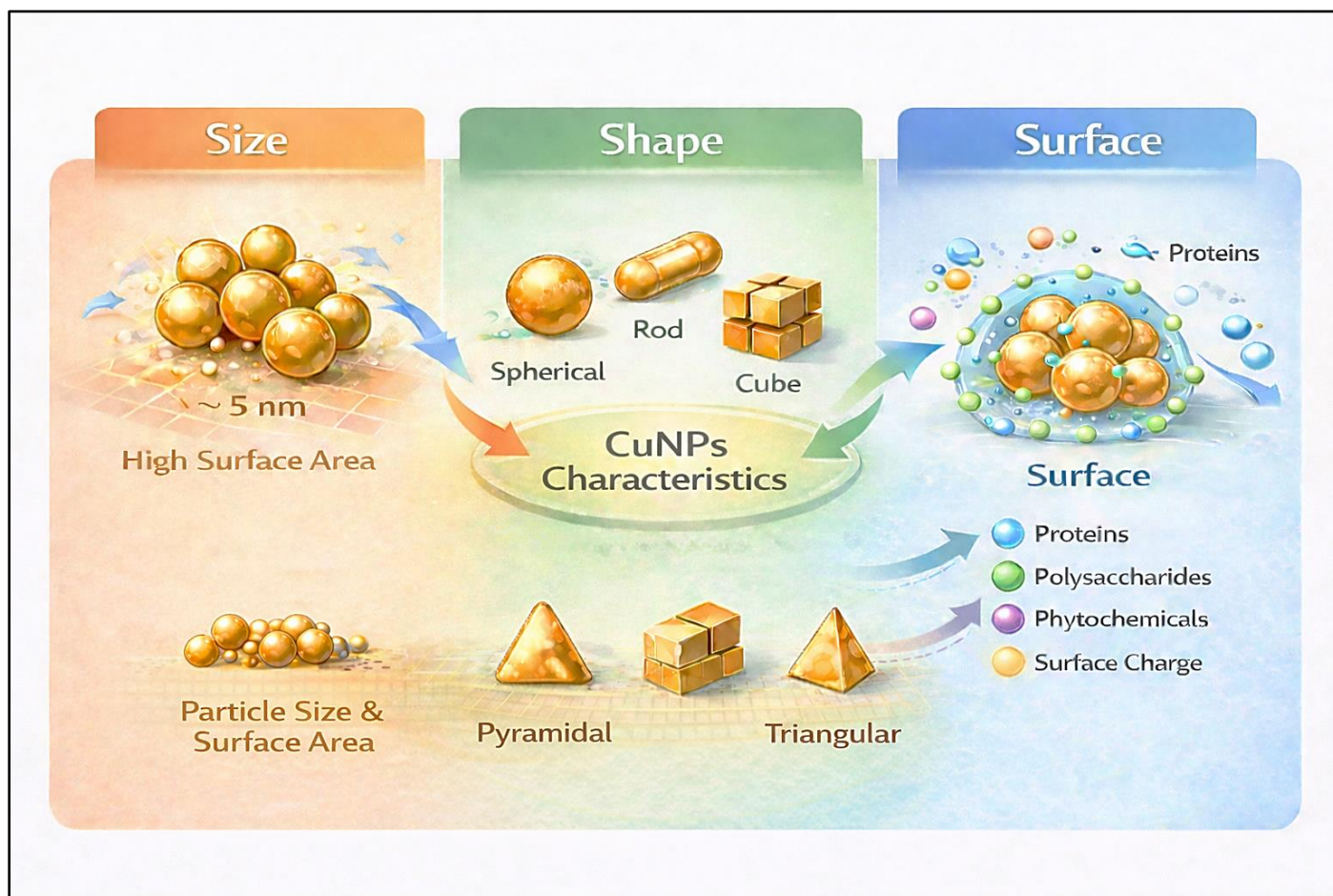


Figure 6. Systematized diagram of the major physicochemical properties of CuNPs, such as diameter, morphology (spherical, rod, cubic, pyramidal and triangular), and surface structures, based on [67,68]. The combination of these parameters affects the surface area, reactivity of the nanoparticles, the interaction with the biological systems, and the stability of the nanoparticles in general.

In addition to size, nanoparticle shapes such as spherical, rod-like, cubic or triangular can impact on the localization of the surface energy as well as expression through microbial cell membrane thereby, prosecuting their efficiency in function [55].

3.2. Redox behavior and ion release ($\text{Cu}^+/\text{Cu}^{2+}$)

As illustrated in Fig. 6, physicochemical properties of CuNPs, i.e. size of the particles, shape of particles, and surface properties do not only act as structural feature, but instead they directly serve to manipulate redox attributes and ion release kinetics [35]. The smaller size of the particle increases the surface to volume ratio, and therefore, accelerates the electron transfer rate and increases the reversal of Cu^0 , Cu^+ and Cu^{2+} [60]. At the same time, morphological differences (spherical, rod-like or cubic shapes) also modulate the surface energy distribution and the exposure of reactive faces and, as a result, both catalytic activity and reaction with the environment. The coating of the CuNPs using bio-molecules e.g., proteins, polysaccharides or phytochemicals is also a considerable aspect of stabilization of the CuNPs to avoid aggregation and the copper ion ($\text{Cu}^+/\text{Cu}^{2+}$)

release is controlled [69]. This is important in maintenance of biological activity, especially in the production of reactive oxygen species (ROS), with minimal occurrence of nonspecific toxicity [70]. Therefore, stability, reactivity and biological performance of CuNPs are dictated by the size, shape and surface chemistry ratio.

3.3. Optical and catalytic properties

The optical and catalytic properties of the localized surface plasmon resonance (LSPR) behavior and the surface reactivity of the CuNPs are primarily governed by nanoscale structural parameters and highly influenced by the nanoscale structure. CuNPs exhibit a typical optical absorption at the visible regime that can be explained by the collective oscillation of the conduction electrons and that the collection of this process strictly depends on the size, morphology and the dielectric environment of the particles that allow applying them in sensing, photothermal gadgets [71]. At the same time the active surface sites and facilitation of electron transfer are correlated with and high in smaller particles and individual crystallographic facets manifested in shapes (i.e. spherical, rod-like, and polyhedral).



The properties may also be utilized in redox reactions and make redox reactions more catalytic both in organic reactions and in the environmental remediation process [72]. Furthermore, surface engineering with capping agents or support materials can also be used to control optical response and catalytic activity by stabilizing reactive sites and controlling charge transfer pathways [73]. Plasmonic behaviour and surface-driven catalysis are connected in this manner and this defines the versatile work of CuNPs in the realm of the contemporary technology and biomedical clinical application.

4. Cellular Uptake and Biological Interactions

Biologic behaviour of CuNPs has emerged to be one of the most significant problems of biomedical efficacy and biomedical safety of nanoparticles. Exposed to the biological systems the CuNPs undergo complicated biological behavior, including those which involve cellular recognition, internalization, and intracellular transportation, and rely on the physicochemical properties (size, shape, surface chemistry, etc.) of the nanoparticles [74]. These interactions are able not only to control the extent of cellular uptake, but also the subsequent biological processes including oxidative stress and protein binding, and signaling pathway regulation [75]. Moreover, a biomolecular envelop sheathing around the CuNPs also further regulates its identity and action in the physiological conditions that regulate its distribution and localization in cells [76]. This dynamic interaction would be fundamental in exploring the design of CuNPs optimization to employ in delivery of drugs, antimicrobial treatment and cancer treatment and minimize unexpected cytotoxic effects as schematically presented in Fig. 7.

4.1. Internalization pathways (endocytosis mechanisms)

Cellular internalization of CuNPs is primarily due to the capabilities of energy dependent endocytic routes, which strongly affect in intracellular fate and biologic action of nanoparticles. Among them, the clathrin-mediated endocytosis can be mentioned as one of the key mechanisms of internalizing the small-sized nanoparticles, where the inside-out process of the CuNPs into receptor-mediated vesicles occurs, which offers a

possibility to carry out controlled transport to the early endosomes [77]. On the other hand, the bigger or coalesced nanoparticles more frequently are possibly engulfed via the caveolae-mediated endocytosis or macropinocytosis, the latter that is the uptake of the particles that are not uniquely linked to the ligand-receptor interaction and allows a deeper cytoplasmic penetration, respectively [78,79]. Moreover, a route of phagocytosis is also the most common pathway, which is primarily associated with immunologic cells such as macrophages, in which the CuNPs are consumed by phagosomes, and then degraded in lysosomes [80]. Once ingested, the CuNPs may travel through endosomal-lysosomal routes and undergo partial dissolution in the acidic endosomal-lysosomal environment triggers the release of copper ions ($\text{Cu}^+/\text{Cu}^{+2}$), and enhanced the intracellular reactivity and potential cytotoxicity of the nanoparticles [81,82]. Most interestingly, the size, shape and surface functionalization of nanoparticles are key determinants of their efficiency and preference of these uptake mechanisms, which dictate the cellular recognition, membrane interaction and internalization of these mechanisms in total [27,83]. Hence, an in-depth knowledge of such endocytic processes is therefore essential in a more favorable planning of the CuNPs in a particular application in the therapeutic and antimicrobial applications and the minimal undesirable biological side effects. The scheme of the first contact of CuNPs with the cell membrane such as adsorption, penetration and membrane destabilization are shown schematically in Fig. 7.

4.2. Interaction with cellular membranes

As depicted in Fig. 7, the interaction between CuNPs and the cellular membrane represents the initial and critical step governing subsequent internalization processes. CuNPs are known to attach directly to the lipid bilayer by electrostatic, van der Waals and hydrophobic forces due to its nanoscale dimensions and high levels of surface reactivity resulting in membrane damage and permeability [79,84]. Surface-functionalized or positively charged CuNPs have also been shown to have a high affinity to negatively charged phospholipid membranes and have been shown to induce membrane adhesion and local structural disruption [85,86].

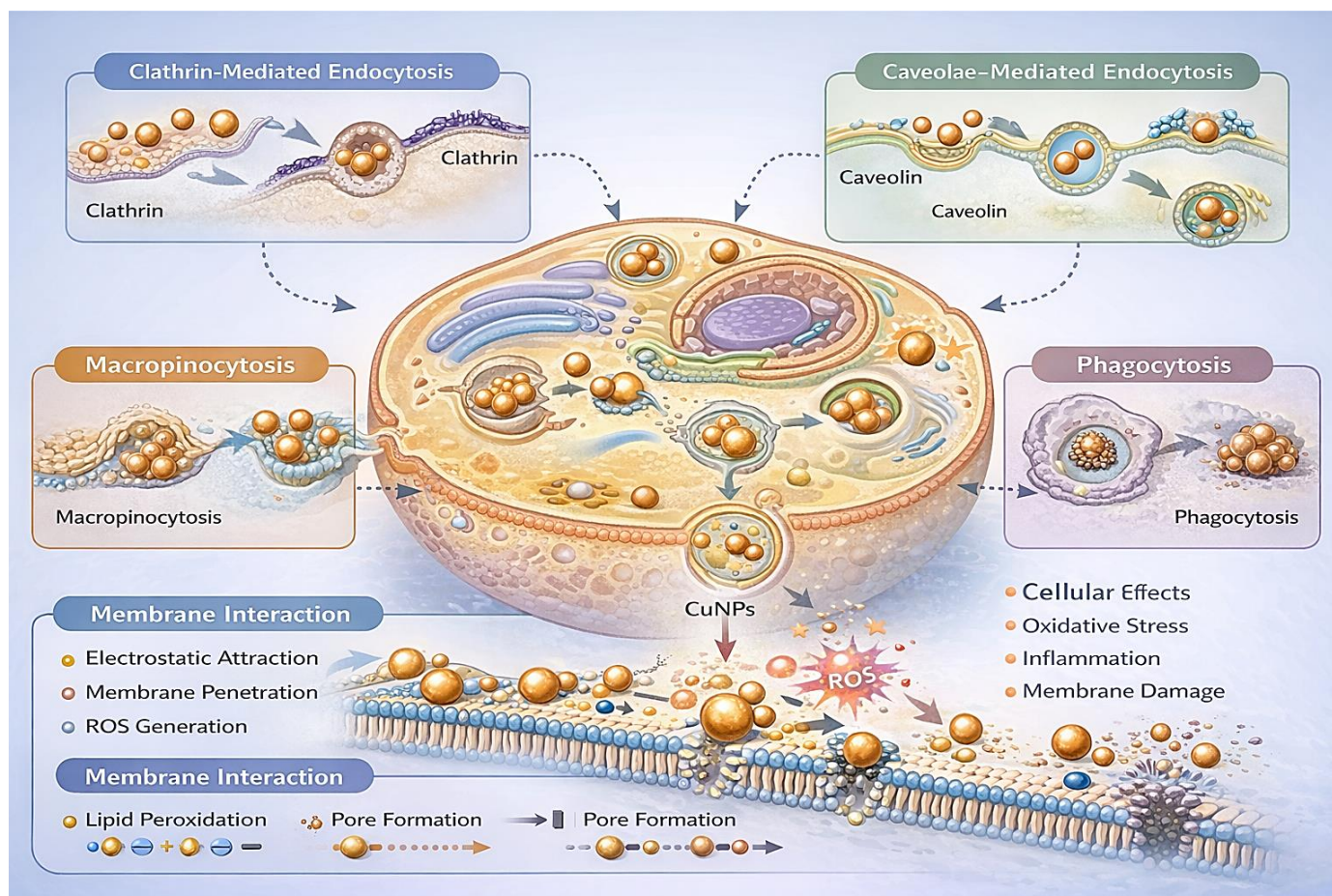


Figure 7. Schematic representation of the interaction, cellular uptake, and intracellular disposition of CuNPs, updated from [87–90]. First, the CuNPs connect with cell membrane via an electrostatic attraction resulting in adhesion to membrane, penetration and possible destruction of the cell structure. Then nanoparticles are endocytosed through several endocytic routes, among them clathrin-mediated endocytosis, caveolae-mediated endocytosis, macropinocytosis, and phagocytosis. After internalization, CuNPs are trafficked via endosomal-lysosomal pathways, in which the acidic environment of the endosome facilitates the dissolution of the nanoparticles and the subsequent release of copper ions ($\text{Cu}^+/\text{Cu}^{2+}$). The process causes the generation of reactive oxygen species (ROS), oxidative stress, membrane damage, and possible mitochondrial dysfunction, which eventually interferes with cell viability.

This interaction can lead to either more fluidity in the membrane, pore development or even to lipid peroxidation through reactive oxygen species (ROS) undermining cellular homeostasis [91,92]. Moreover, the interactions can be biomolecular coated or corona of protein can regulate the interactions by either stabilizing the nanoparticle surface or minimizing direct membrane damage based on the nature of the adsorbed molecules [93,94]. Notably, these cellular interactions at the membrane level not only affect nanoparticle uptake efficiency, but in addition the subsequent cellular response, such as oxidative stress, inflammation, and cytotoxicity, which in turn enable downstream cellular uptake through various endocytic routes as seen in Fig. 7. The elements which control the responses between the CuNPs and the membrane, thus, should be comprehended in order to produce less hazardous and more productive nanomaterials to be utilized in a biomedical application.

4.3. Intracellular fate of CuNPs

The intracellular fate of CuNPs is a decisive determinant of their biological activity and potential toxicity, in connection to the discovery of its biological action and possible harmfulness following its absorption to the cell. When the energy source is internalized into cells via some endocytic pathways, the CuNPs will typically be transported to late endosomes, lysosomes, where the acidic compartment of the cell promotes partial dissolution and release of copper ions ($\text{Cu}^+/\text{Cu}^{2+}$) [95]. This intracellular release of ions increases intracellular redox reactions and helps to produce reactive oxygen species (ROS) that can lead to oxidative damage to proteins, lipids, and nucleic acids [91]. Moreover, CuNPs can be removed out of endosomal vesicles into the cytoplasm where they can interact with organelles, including the mitochondria, resulting in mitochondrial membrane potential disruption and apoptotic activation [96]. Nanoparticle physicochemical characteristics, especially size and surface functionalization, have a strong effect on the extent of these processes and dictate the stability of nanoparticles intracellularly, the rate of dissolution, and localization within the subcellular

space [83]. Thus, understanding how CuNPs are processed and transformed intracellularly is essential for optimizing their biomedical performance and minimizing cytotoxic effects. The overall sequence of interaction, uptake, and intracellular fate is summarized in Fig. 7.

5. Anticancer Mechanisms of CuNPs

CuNPs have an anticancer effect acting via oxidative stress-dependent processes that disturb the homeostasis of cells and trigger the different processes that accompany death. The CuNPs can release copper ions after internalization and augment intracellular redox cycling that promotes the buildup of reactive oxygen species (ROS), mitochondrial impairment, DNA damage and programmed cell death [97]. These events are not isolated, as described in Fig. 8, but in a cascade mechanism which is the foundation to the selectivity of CuNPs to malignant cells. Additional studies also indicate the fact that this oxidative pressure could also interfere with cell-cycle control, and may enhance tumor-cell sensitivity to combination therapies [98].

5.1. Reactive oxygen species (ROS) generation

The overproduction of ROS is among the first and most reliable responses ever recorded upon exposure of cancer cells to CuNPs. Since copper can undergo repeated oxidation and reduction, CuNPs and released copper ions mediate redox reactions that cause intracellular oxidants to surpass the buffering ability of tumor cells. This redox imbalance increases oxidative stress and may cause damage to lipids, proteins, and nucleic acids, thus changing cancer cells to death signaling. In bladder cancer models, cuprous oxide nanoparticles triggered ROS-dependent cytotoxicity and activated downstream ERK signaling associated with apoptosis and autophagy [99]. In a broader sense, most recent reviews focus on the fact that ROS-focused approaches are especially appealing to the field of oncology, as cancer cells already are close to the maximum level of oxidative tolerance, and they are more susceptible to further intensification of the ROS effects than normal cells are [98,100]. The oxidative stress-centered anticancer cascade of CuNPs, including ROS accumulation, mitochondrial dysfunction, DNA damage, and apoptotic signaling, is schematically summarized in Fig. 8.

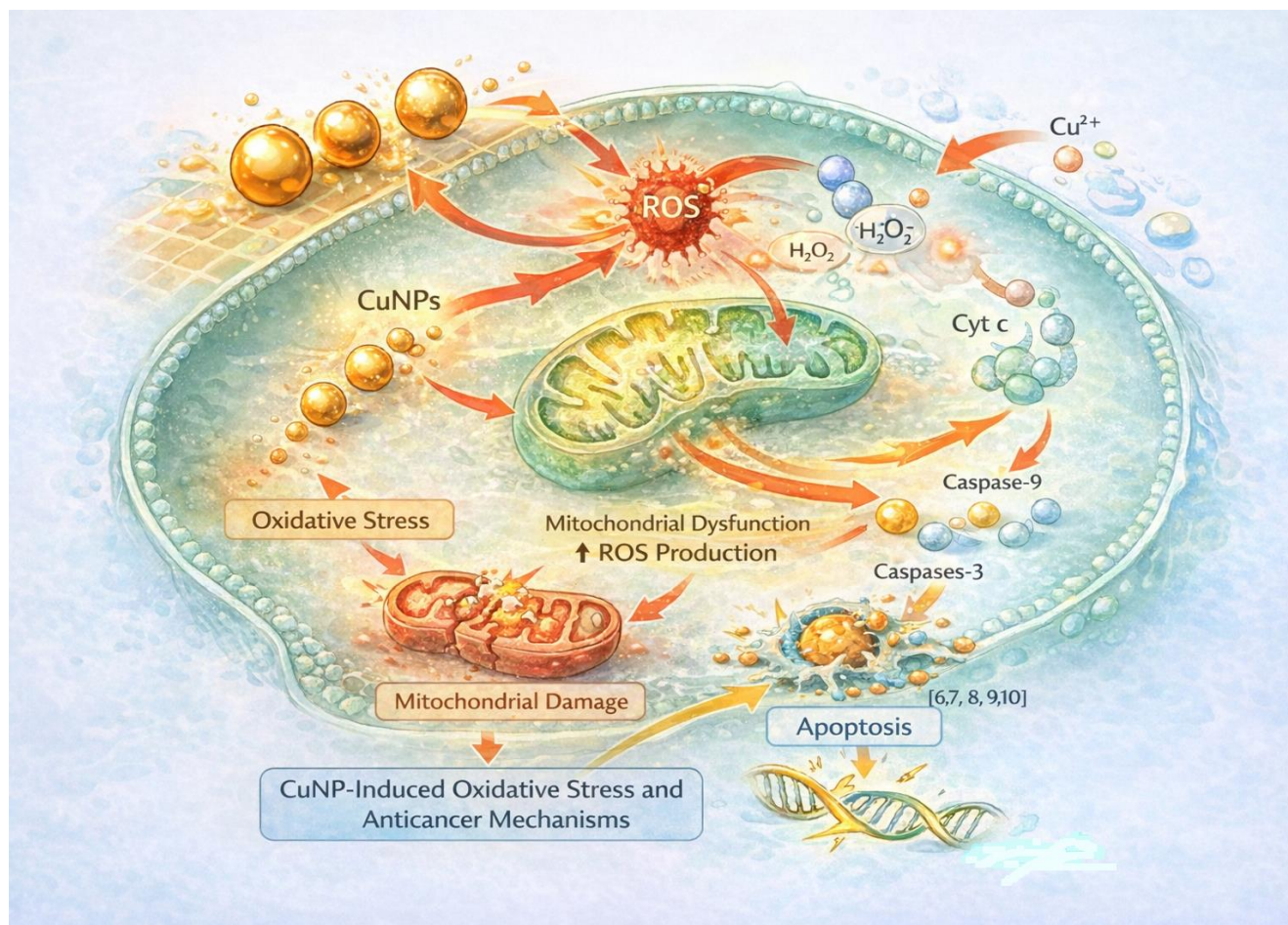


Figure 8. Schematic figure of the key anticancer activity of CuNPs, based on relevant literature [89,101]. After the cellular internalization, CuNPs and released copper ions trigger the formation of ROS, which enhances the occurrence of oxidative stress, mitochondrial dysfunction, DNA damage, cell-cycle disruption, and apoptosis via primarily intrinsic mitochondrial signaling.



5.2. Mitochondrial dysfunction

Following ROS elevation, mitochondria act as a primary intracellular target, where oxidative stress disrupts membrane potential and triggers cytochrome c release.

Too much oxidative stress may paralyze the mitochondrial membrane potential, disrupt respiration and release cytochrome c, therefore transforming mitochondrial damage into an apoptotic signal. This mechanism has been seen on nanoparticle based and copper-nanomedicine systems. Cuprous oxide nanoparticles were also shown to implicate the mitochondria and release cytochrome c in connection with apoptosis in the case of bladder cancer [99,102]. Similarly, in recent copper-cysteamine nanoparticle research, which was summarized in a 2025 systematic review, mitochondrial damage was mentioned as a common phenomenon of anticancer activity, including in endometrial cancer models [103]. Therefore, the mitochondrial compartment can not only serve as a source of further amplification of ROS, but also act as a decision-making center whether to continue with oxidative damage to cell death. As illustrated in Fig. 8, mitochondrial dysfunction represents a central amplification step linking oxidative stress to apoptotic signaling.

5.3. DNA damage and cell cycle disruption

Another major consequence of copper-induced oxidative stress is DNA damage. ROS produced following CuNPs exposures have the potential to oxidize nucleic acids and indirectly stimulate the pathways involved in DNA damage response, and sustained redox damage can modify checkpoint signaling and to stop cell-cycle progression. In a 2024 article involving walnut shell-derived CuO nanoparticles, MCF-7 and HCT-116 cell treatment was linked with an increase in p53 and Bax expression, which justified a relationship between ROS/DNA damage signaling and apoptosis [104]. Cuprous oxide nanoparticles have been found to drastically increase the G2/M-phase cells and decrease the cyclin B1 expression, thereby signaling cell-cycle arrest in combination with cytotoxic stress in bladder cancer cells [2]. On the same note, *Bacillus coagulans*-derived CuO nanoparticles of biogenic origin arrested breast cancer cells in the sub-G1 and down-regulated CCND1 and CDK4, which is yet another indication in support of disruption of the proliferative control [105]. As such, Fig. 8 provides a mechanistic framework whereby oxidative stress advances to subcellular damage followed by deregulation cell-cycle and genomic cell-damage.

5.4. Apoptosis and programmed cell death pathways

The most characterized terminal cell event of CuNPs-induced stress in the cancer cells is apoptosis. Most available evidence supports a predominantly intrinsic, mitochondria-mediated apoptotic pathway that is largely mitochondria-linked, whereby the endogenous oxidative damage, mitochondrial rupture, cytochrome c leakage and caspase activation results in apoptotic death. Indicatively, biogenic CuO nanoparticles enhanced BAX, CASP3 and CASP9 expression or reduced the expression of BCL2 in breast cancer cells, which is in line with the activation of intrinsic pathway [105]. The other copper-based nanostructures have also been linked to enhanced Bax/Bcl-2 ratio and p53 dependent signaling [15]. There is also evidence of more generalized programmed cell death signaling: other than apoptosis, copper nanomaterials can interact with either autophagy programs or other controlled death programs depending on their composition and context in the cell [98,104]. Though the intrinsic pathway seems to dominate in the majority of CuNPs research, extrinsic signaling can also play a role when the oxidative stress overlaps with the signaling of death receptors or inflammatory stress mediators [106]. Consequently, the apoptotic module in Fig. 8 integrates ROS generation, mitochondrial dysfunction, and DNA damage into a unified cell death pathway.

5.4.1. Intrinsic vs extrinsic pathways

The intrinsic pathway is more reliably reported in the study of CuNPs due to its close relationship with mitochondrial depolarization, cytochrome c release and caspase-9/caspase-3 activation [107]. By contrast, direct evidence for extrinsic apoptosis is comparatively limited and appears to be more context-dependent, often secondary to broader oxidative and inflammatory signaling rather than the primary initiating route. The distinction is significant in the interpretation of mechanistic studies where not all reports identifying apoptosis line the distinction between rigorously identifying mitochondrial and receptor-mediated activation [99].

5.4.2. Selectivity toward cancer cells.

A notable feature of the anticancer action of CuNPs is that they are more cytotoxic to cancerous cells than to normal ones. Such selectivity is also much contributed by the difference in redox between the cancer and non-cancer cells. The basal concentrations of reactive oxygen species (ROS) and the oxidative stress state in tumor cells are generally elevated and are maintained at a steady level, which makes tumor cells more susceptible to the additional accumulation of ROS promoted by CuNPs [98,108]. Conversely, in normal cells, the antioxidant defense mechanisms tend to be healthier thus, being able to resist a modest oxidative attack. As a result, ROS amplification induced by CuNPs preferentially crosses the oxidative stress threshold in cancer cells resulting in the impairment of mitochondria, DNA

damage, and apoptosis, but not normal cells. This selectivity is one of the main advantages of copper-based nanomaterials in cancer treatment because it is redox-dependent.

5.5. Emerging Mechanisms Beyond ROS-Mediated Apoptosis

The anti-cancer effect of CuNPs has been shown to be more than just the ROS dependent apoptotic pathway in recent studies. Since the introduction of cancer nanomedicine, several other mechanisms have been uncovered which could significantly increase the therapeutic efficacy of CuNPs and extend their biological reach. These emerging pathways provide new

opportunities for designing multifunctional copper-based nanotherapeutics with improved selectivity and clinical potential. The principal emerging mechanisms through which CuNPs may exert anticancer effects beyond conventional ROS-mediated apoptosis are conceptually summarized in Fig. 9.

One of these mechanisms, known as cuproptosis, is recently recognized as a distinct copper-dependent form of regulated cell death. Cuproptosis is a form of cell death that involves the accumulation of copper ions within the cell, which then bind to lipoylated components of the tricarboxylic acid (TCA) cycle, as opposed to apoptosis or ferroptosis [109].

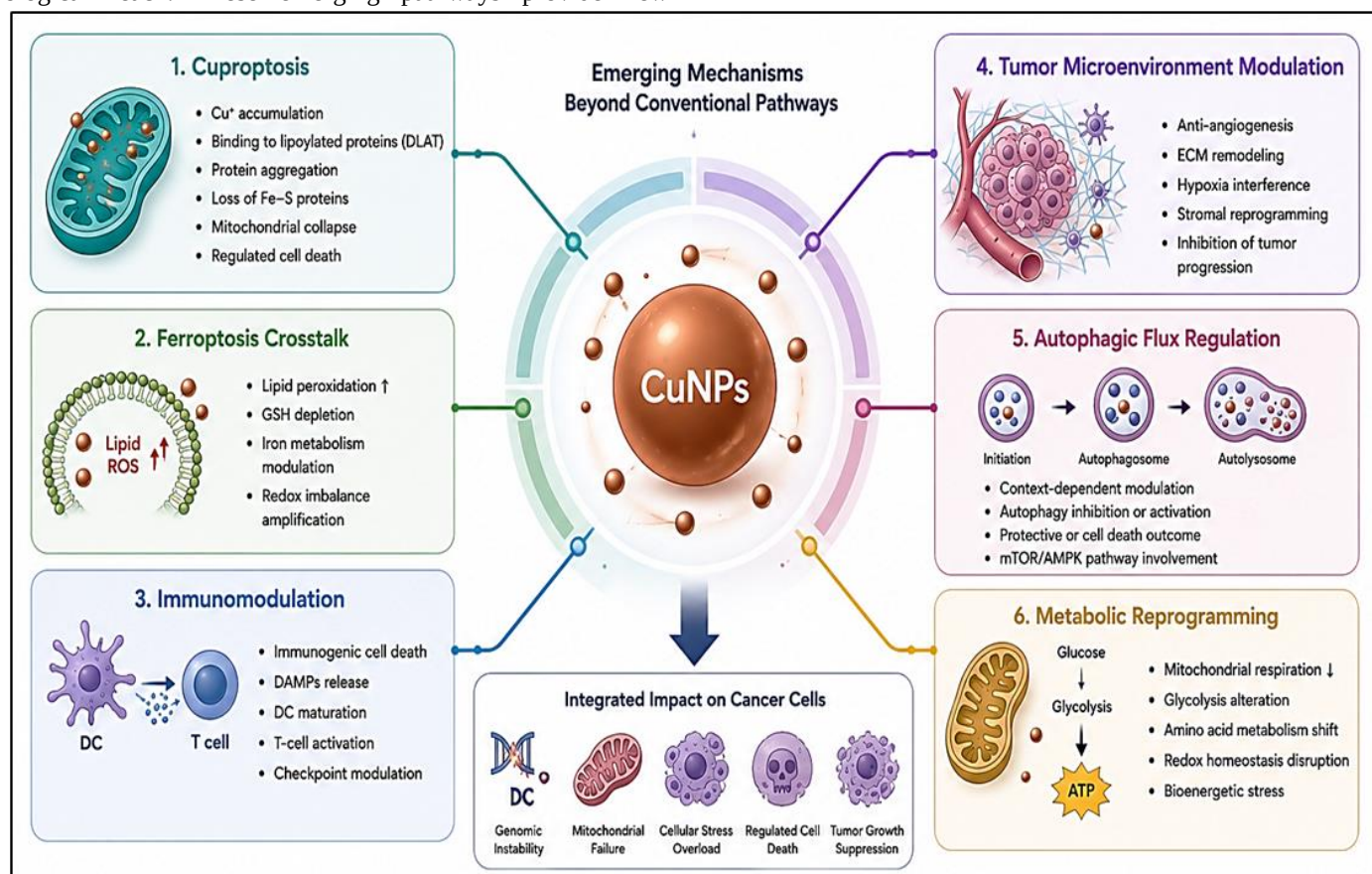


Figure 9. Conceptual overview of the emerging anticancer mechanisms of CuNPs beyond ROS-mediated apoptosis, including cuproptosis, ferroptosis crosstalk, immunomodulation, tumor microenvironment modulation, autophagic flux regulation, and metabolic reprogramming.

This interaction triggers mitochondrial proteins to aggregate, destabilizes iron-sulfur cluster proteins, creates proteotoxic stress and leads to irreversible mitochondrial dysfunction. Some cancerous cells have been shown to have high mitochondrial metabolism and a high demand for copper, which means that CuNPs could be a useful intracellular provider of copper to initiate this recently discovered pathway of cell death, going beyond the stress from ROS, and thus have a broader therapeutic application than just ROS-induced cell death [110,111]. An emerging mechanism that is gaining more and more interest is the cross-talk between CuNPs and ferroptosis, which is an iron-dependent regulated necrotic cell death,

associated with excessive lipid peroxidation. Ferroptosis is classically known to be associated with iron metabolism; however, recent studies indicate that Cu^{2+} may exacerbate lipid oxidative damage by redox cycling, glutathione depletion, and disruption of the antioxidant defense systems. The multiple activation of ROS production, lipid peroxidation, and mitochondrial dysfunction indicate that these three pathways may interact synergistically through crosstalk among cuproptosis, ferroptosis, and apoptosis, which is currently being investigated [112,113].



The emerging evidence also suggests that CuNPs have direct effect on the tumor immune microenvironment. In addition to direct cytotoxicity, CuNPs can trigger immunogenic cell death by the release of damage-associated molecular patterns (DAMPs), which can lead to increased maturation of dendritic cells and activation of cytotoxic T-cells. Moreover, CuNPs may affect macrophage polarization, inflammatory cytokines release and immune checkpoints regulation, which would allow them to enhance the effectiveness of cancer immunotherapy in combination with other immune-modulating drugs [114,115]. Additionally, the tumor microenvironment (TME) can influence the biological activity of CuNPs. Copper ions are involved in various mechanisms that control angiogenesis, ECM remodeling, hypoxia adaptation, and stromal cell communication. Thus, engineered CuNPs have been tested both as direct agents for cytotoxic activity and as modulators, which can alter the microenvironmental conditions that promote tumor growth, leading to increased drug penetration and therapeutic responsiveness [116,117].

Moreover, several studies have now shown that CuNPs are involved in the autophagic intracellular pathways. Autophagy can either be protective for cancer cells against oxidative injury or be a component of the autophagy associated cell death, depending on the characteristics of the nanoparticles and the cellular environment. This dual action implies that the process of autophagic flux might be a key parameter for the efficacy of CuNP action and offers possibilities of combination strategies with drugs targeting autophagy [118,119]. More recent metabolomic studies also indicate that CuNP exposure causes a significant reprogramming of the metabolism of cancer cells. After the treatment with copper nanoparticles, changes in mitochondrial respiration, glycolytic activity, amino acids metabolism and cell redox balance have been found. The metabolic changes seem to be linked to the accumulation of copper in mitochondria, and could increase the sensitivity of cancer cells to various programmed cell death mechanisms, such as apoptosis, cuproptosis, and ferroptosis [96,120].

All of these new mechanisms indicate that the biological activity of CuNPs is more complex than ROS-induced apoptosis. The introduction of these recently identified pathways into the classical oxidative stress pathway could aid in the rational design of novel nanomedicines based on copper ions with increased therapeutic specificity, multimodal anticancer activity, and increased translational potential.

5.6. Structure-Activity Relationship (SAR) of CuNPs

The anticancer activity of CuNPs depends not only on their intrinsic redox activity but also on the complex interplay between their physicochemical properties and biological

interactions. The structure-activity relationship (SAR) is a phenomenon that highlights how the size, shape, surface chemistry, oxidation state, and synthesis strategy all contribute to the properties of nanoparticles that affect their cellular uptake, trafficking, generation of reactive oxygen species (ROS), cytotoxicity, and therapeutic selectivity [121]. However, knowledge of these relationships is critical for rational design of CuNPs with enhanced anticancer activity and minimized off-target toxicity. Optimizing these structural parameters is becoming more and more crucial to improve the clinical application of CuNP-based nanomedicines [122].

One of the most important factors affecting the biological activity of CuNPs is the size of the particles. The smaller the particle size, the higher the surface-area-to-volume ratio, which leads to a higher catalytic activity, copper ion release and ROS production [32]. The cellular internalization of nanoparticles is typically more efficient for smaller-sized particles, which also tend to have a higher affinity for interactions with the intracellular organelles, resulting in greater mitochondrial dysfunction, DNA damage, and apoptosis. The problem is that overly fine particles can also cause nonselective oxidative damage in healthy tissues, so there is an optimal range between the two extremes that should be considered to achieve the greatest therapeutic benefit with the least systemic side effects [122,123].

Apart from size, the morphology and crystalline phase of nanoparticles also significantly affect the biological response. Spherical nanoparticles typically show more uniform uptake and distribution of cells while anisotropic nanoparticles like rods or wires could have different interactions with the cell membranes and intracellular trafficking [124]. Similarly, various copper oxidation states ranging from metallic Cu to Cu₂O and CuO nanoparticles have different redox characteristics, which influence the process of ion dissolution and ROS formation. The nanoparticles of CuO are usually more active oxidants due to their higher ion release capacity, while the metallic Cu nanoparticles tend to lose their physicochemical integrity, depending on the physiological environment, in the absence of proper surface stabilization [125,126]. All of these structural differences will eventually result in the differences in cytotoxic potency, therapeutic efficacy, and biosafety [122].

Surface chemistry is another important factor in the determination of SAR. Functionalization with polymers, proteins, polysaccharides, peptides or phytochemicals derived from plants enhances colloidal stability, limits aggregation, increases circulation time and influences cellular recognition [127]. Naturally occurring phytochemicals are also reducing and capping agents, making green synthesized CuNPs more biocompatible, and still retaining their oxidative anti-cancer effect, which makes them very appealing [128]. Furthermore,

surface engineering can be used to improve the selectivity of the tumor by making it easier to be taken up by the receptors and reducing the nonspecific interaction with the healthy tissues [35]. The overall structure–activity relationship (SAR) concept discussed in this section is summarized in Fig. 10.

The synthesis procedure itself plays a significant role in the biological property of CuNPs. While, green synthesis can add

biologically active surface molecules that can synergistically improve therapeutic performance, chemical synthesis provides excellent control over particle size, morphology and reproducibility [129]. It is therefore not surprising that the biological responses to nanoparticles fabricated by varying routes of production can be very different despite the same chemical composition [130].

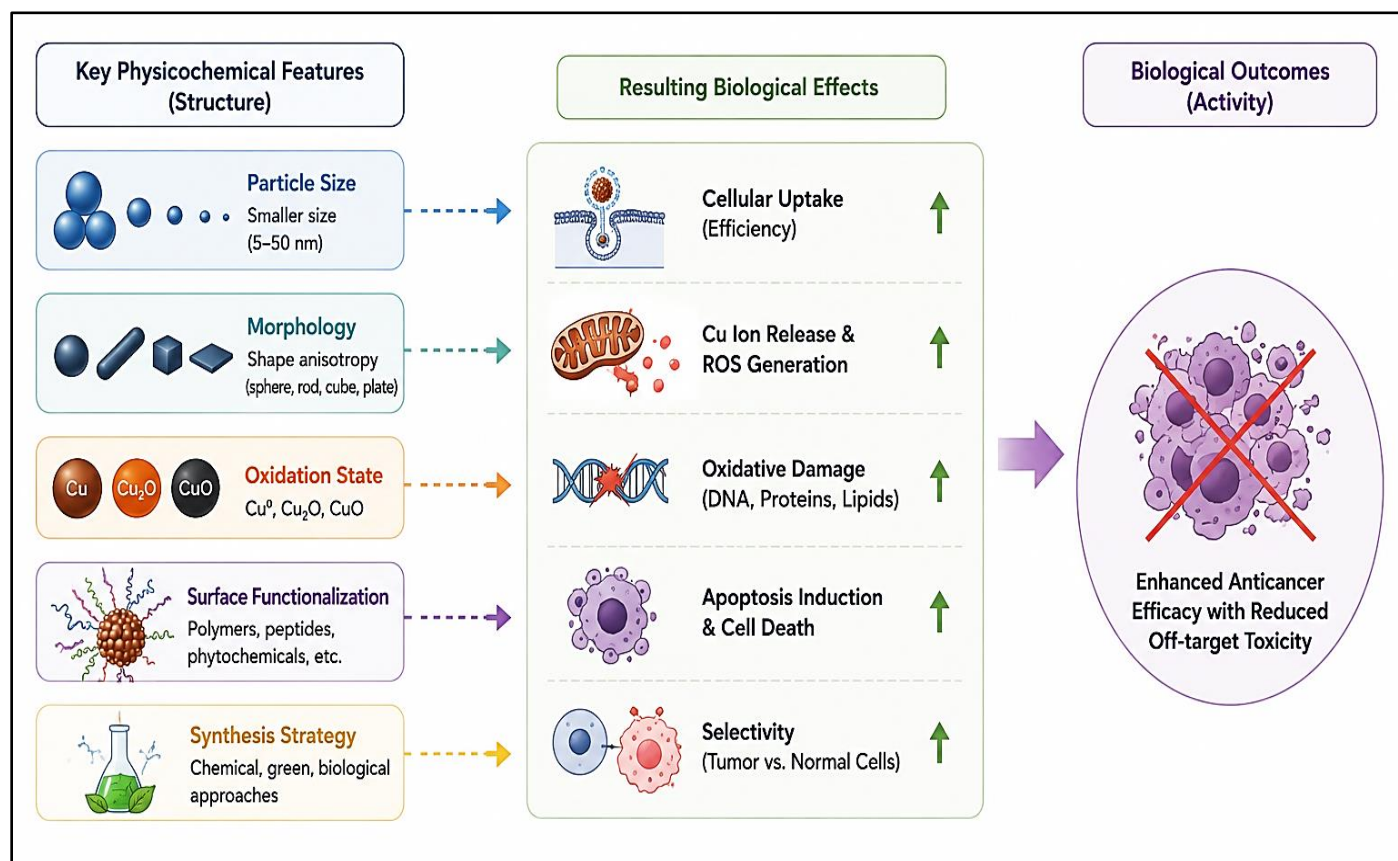


Figure 10. Schematic representation of the structure–activity relationship (SAR) of CuNPs, illustrating the integrated influence of particle size, morphology, oxidation state, surface functionalization, and synthesis strategy on biological responses that collectively determine therapeutic efficacy, cancer selectivity, and biosafety.

The observations suggest that the therapeutic activity of CuNPs is not only attributed to the elemental composition of CuNPs but also to the combined effect of structural and surface properties of CuNPs [14]. Therefore, the development of comprehensive SAR models, correlating nanoparticle physicochemical parameters with biological outcomes, is deemed an important prerequisite for the development of safer and more effective clinically translatable copper-based nanotherapeutics.

6. In Vitro and In Vivo Anticancer Studies

6.1. Overview of studied cancer cell lines

Preclinical anticancer studies on CuNPs have examined a broad spectrum of tumor models, although the strongest evidence remains clustered around a few recurrent cell systems.

In vitro work has prominently involved bladder cancer cell lines such as T24, J82, 5637, and UMUC3, where cuprous oxide nanoparticles showed marked antiproliferative activity with comparatively weaker effects in non-cancerous urothelial cells (SVHUCs) [99]. Breast cancer models are also well represented, including MCF-7 and SKBR3 in studies using biogenic CuO nanoparticles, as well as MCF-7 and AMJ-13 in plant-mediated CuO nanoparticle systems; in both settings, the nanoparticles preferentially suppressed malignant rather than normal control cells [105,131]. More recent reviews additionally indicate that copper-cysteamine nanoparticles have been investigated across multiple tumor types, including colon, esophageal, endometrial, liver, melanoma, osteosarcoma, and breast cancer models, particularly in combination with external activation modalities [103]. Collectively, these studies show that the in vitro literature



is broad but still dominated by a limited number of highly reused cancer cell lines.

6.2. Dose-dependent cytotoxic effects

A consistent feature across the available literature is the dose- and time-dependent nature of cytotoxicity. In bladder cancer, cuprous oxide nanoparticles significantly inhibited proliferation, migration, and invasion in a dose-dependent manner, while also increasing apoptosis and inducing G2/M cell-cycle arrest [99]. In breast cancer, *Bacillus coagulans*-derived CuO nanoparticles showed concentration-dependent cytotoxicity against MCF-7 and SKBR3 cells, with lower apoptotic injury in normal HFF cells than in tumor cells [105]. Likewise, *Annona muricata*-mediated CuO nanoparticles reduced proliferation and colony formation in MCF-7 and AMJ-13 cells, while showing little or no comparable cytotoxicity toward HBL-100 normal breast cells [131]. These studies collectively support the conclusion that CuNPs exert stronger anticancer effects as concentration and exposure time increase, and that this response is often accompanied by oxidative stress, cell-cycle perturbation, and apoptosis-related signaling [99,105,131].

6.3. Animal model findings

Compared with the in vitro literature, in vivo evidence is more limited, but the available studies are encouraging. The most direct example comes from bladder cancer, where cuprous oxide nanoparticles suppressed tumor growth in both orthotopic xenograft and subcutaneous nude mouse models, confirming that the antitumor activity observed in vitro can translate into animal systems [99]. The same study further showed that combining these nanoparticles with gemcitabine potentiated antitumor efficacy in vivo without worsening adverse effects, supporting their potential as an intravesical combinational nanomedicine [1]. Beyond single-particle systems, a recent systematic review of copper-cysteamine nanoparticles concluded that tumor reduction in animal models is repeatedly enhanced when these nanomaterials are paired with external stimuli such as X-rays, UV light, microwaves, or ultrasound, indicating that stimulus-responsive copper-based nanoplatforms may offer the strongest current route toward in vivo efficacy [103]. Overall, the animal data remain promising but still comparatively scarce, highlighting the need for more standardized tumor models, dosing schedules, and toxicity evaluations before translation can be considered robust [98,103,122]. Representative in vitro and in vivo anticancer studies of CuNPs are summarized in Table 2.

Table 2. Summary of representative preclinical anticancer studies involving CuNPs, highlighting the most frequently used cancer cell lines, dose-dependent cytotoxic responses, and currently available in vivo findings.

Nanoparticle system	Cancer model / cell lines	Main in vitro findings	In vivo evidence	Ref.
Cuprous oxide nanoparticles (CONPs)	Bladder cancer: T24, J82, 5637, UMUC3 ; normal SVHUCs	Dose- and time-dependent inhibition of proliferation; reduced migration/invasion; apoptosis; G2/M arrest	Significant tumor suppression in orthotopic and subcutaneous nude mouse bladder cancer models; enhanced efficacy with gemcitabine	[99]
Biogenic CuO nanoparticles from <i>Bacillus coagulans</i>	Breast cancer: MCF-7, SKBR3 ; normal HFF	Concentration-dependent cytotoxicity; apoptosis; anti-metastatic activity; increased BAX/CASP3/CASP9 and reduced BCL2	Not reported in the primary study	[105]
<i>Annona muricata</i> -mediated CuO nanoparticles	Breast cancer: MCF-7, AMJ-13 ; normal HBL-100	Reduced proliferation and colony formation; apoptosis induction; selective toxicity toward cancer cells	Not reported in the primary study	[131]
Copper-cysteamine nanoparticles (Cu-Cy NPs)	Multiple models: colon, esophageal, endometrial, liver, melanoma, osteosarcoma, breast	Broad anticancer activity, especially under external activation; lower IC ₅₀ in some cancer lines than matched normal cells	In multiple reviewed tumor models, especially when combined with X-rays/UV/microwave/ultrasound or drugs	[103]



7. Toxicological Considerations and Biosafety

Although these nanoparticles have a promising anticancer activity, the toxicological aspects of using CuNPs need to be critically considered to warrant safe biomedical use of the nanoparticles. CuNPs have a biomedical use that requires meticulous consideration of the potential effects of toxicology, as well as the therapeutic potentials of the nanoparticles. Although their capacity to cause oxidative stress is the primary cause of the anticancer effect, the same effect has concerned unintended injury to normal tissues. Thus, cytotoxicity profiles, risks associated with oxidative stress, and ways to enhance biosafety should be understood to advance the use of CuNPs to clinical translation.

7.1. Cytotoxicity toward normal cells

Even though nanoparticles made of copper have a selective toxicity to cancer cells, the impact they have on the normal cells cannot be overlooked. A number of studies have showed that at higher concentrations or longer exposure time, CuNPs and copper oxide nanoparticles have the ability to cause cytotoxicity in non-cancerous cells. As an example, cuprous oxide nanoparticles appeared less toxic in normal urothelial cells than in the bladder cancer cells, but cytotoxic effects were observed at high doses [99]. Similarly, biogenic CuO nanoparticles were also found to have lower levels of toxicity in human fibroblast cells (HFF), which confirmed selectivity as relative, as opposed to being absolute [105]. This implies that although there is a therapeutic window with regard to the differential redox balance, there is a need to optimize the dosage to prevent off-target effects. In addition, other physicochemical characteristics including particle size, surface charge, and aggregation state play a major role in the interaction of nanoparticles with healthy tissues and can regulate cytotoxic reactions [132].

7.2. Oxidative damage concerns

Excessive production of reactive oxygen species (ROS) is highly related to the rudimentary mechanism behind the toxicity of CuNPs. Although ROS overproduction is good in cancer cells targeting, uncontrolled oxidative stress can cause impairment in normal cells, such as lipid peroxidation, protein denaturation, and DNA fragmentation [100]. Also, copper ions emitted by nanoparticles may be involved in Fenton-like reactions, which further propagate oxidative damage and upsets

cellular redox homeostasis [133]. This oxidative damage has been not only associated with cytotoxicity but in addition to this has been associated with inflammation and even long-term genotoxic effects. Notably, healthy cells that have low antioxidant defense can be highly vulnerable when exposed in high conditions. Thus, a compromise between therapeutic efficacy mediated by ROS and regulated oxidative production is one of the main issues in the safe usage of copper-based nanomaterials [100,133].

7.3. Strategies to reduce toxicity

To improve biosafety of the CuNPs, several measures have been suggested to reduce the negative effects and maintain anticancer properties. One of the most useful methods is surface modification, in which coating nanoparticles with biocompatible polymers, proteins or polysaccharides can reduce the nonspecific interactions, enhance stability, and restrict excessive ROS formation [41,132]. Moreover, the green synthesis technique, where plant extracts or microbial metabolites are used to form nanoparticles, has become the focus of much interest due to its capacity to form nanoparticles that are both more biocompatible and less toxic than chemically synthesized analogs [105,134]. These biologically obtained capping agents also have the capability of promoting dispersion and minimizing aggregation hence reducing unintended cellular damages. Moreover, stimulus-reactive systems and targeted delivery plans have been considered in order to restrict the functions of nanoparticles to tumor tissues, thus reducing the exposure of systems and toxicity [41]. All these measures are important milestones toward ensuring the optimization of the safety profile of copper-based nanomaterials in biomedical use. The selectivity and biosafety issues are highlighted in Table 3, where there is a comparative summary of cytotoxic effects of CuNPs on cancerous and normal cells depending on dose.

As seen in Table 3, the comparative data clearly show that CuNPs have dose-dependent cytotoxic effect with a concise pattern of increased sensitivity among cancer cells in comparison to normal cells. It is worth noting that the reduced values of IC₅₀ in cancerous cells reveal increased vulnerability to the effects of oxidative stress, which could be explained by the increased basal level of ROS and impaired antioxidant capacities. Conversely, regular cells are normally more tolerant to high levels of nanoparticles and this idea underlines the principle of redox-based selectivity.



Table 3. Summary of representative studies assessing the cytotoxic effects of CuNPs on cancer and normal cell models. The table demonstrates dose-dependent reactions, mechanisms that are mediated by oxidative stress, and the relative specificity of nanoparticles to the malignant cells in relation to their non-cancer counterparts.

Nanoparticle type	Cancer cell lines	Normal cell lines	Reported cytotoxic range	Key findings	Selectivity	Ref.
Cu ₂ O NPs (CONPs)	T24, J82, 5637	SVHUC	~1–5 µg/mL (cancer)	Strong ROS-mediated apoptosis; inhibits proliferation & invasion	Higher toxicity in cancer vs normal	[99]
CuO NPs (<i>Bacillus coagulans</i>)	MCF-7, SKBR3	HFF	~10–20 µg/mL	Dose-dependent cytotoxicity; caspase activation	Moderate selectivity	[105]
CuO NPs (<i>Annona muricata</i>)	MCF-7, AMJ-13	HBL-100	~15–30 µg/mL	Reduced proliferation; apoptosis induction	Clear selectivity	[131]
CuO NPs (walnut shell)	MCF-7, HCT-116	Not reported	~20–40 µg/mL	p53, Bax upregulation; DNA damage	Not fully evaluated	[104]
Cu-Cysteamine NPs	Multiple cancers	Various	Variable (stimulus-dependent)	ROS enhanced under X-ray/UV	High (targeted activation)	[103]

The dispersion in the IC₅₀ values of various nanoparticle systems however, underscores the effect of method of synthesis, surface characteristics and experimental conditions on the toxicity of nanoparticles. All the above findings highlight the need to design nanoparticles with maximum therapeutic selectivity and minimum off-target effects, which is achieved by the differences in intracellular redox balance. Being under increased basal

oxidative stress, as depicted in Fig. 11, cancer cells are susceptible to additional ROS production caused by CuNPs. On the other hand, normal cells have more effective antioxidant systems, and therefore are more resistant to moderate levels of oxidative damage. This oxidation-reduction imbalance is the cause of selective anticancer action of copper-based nanomaterials.

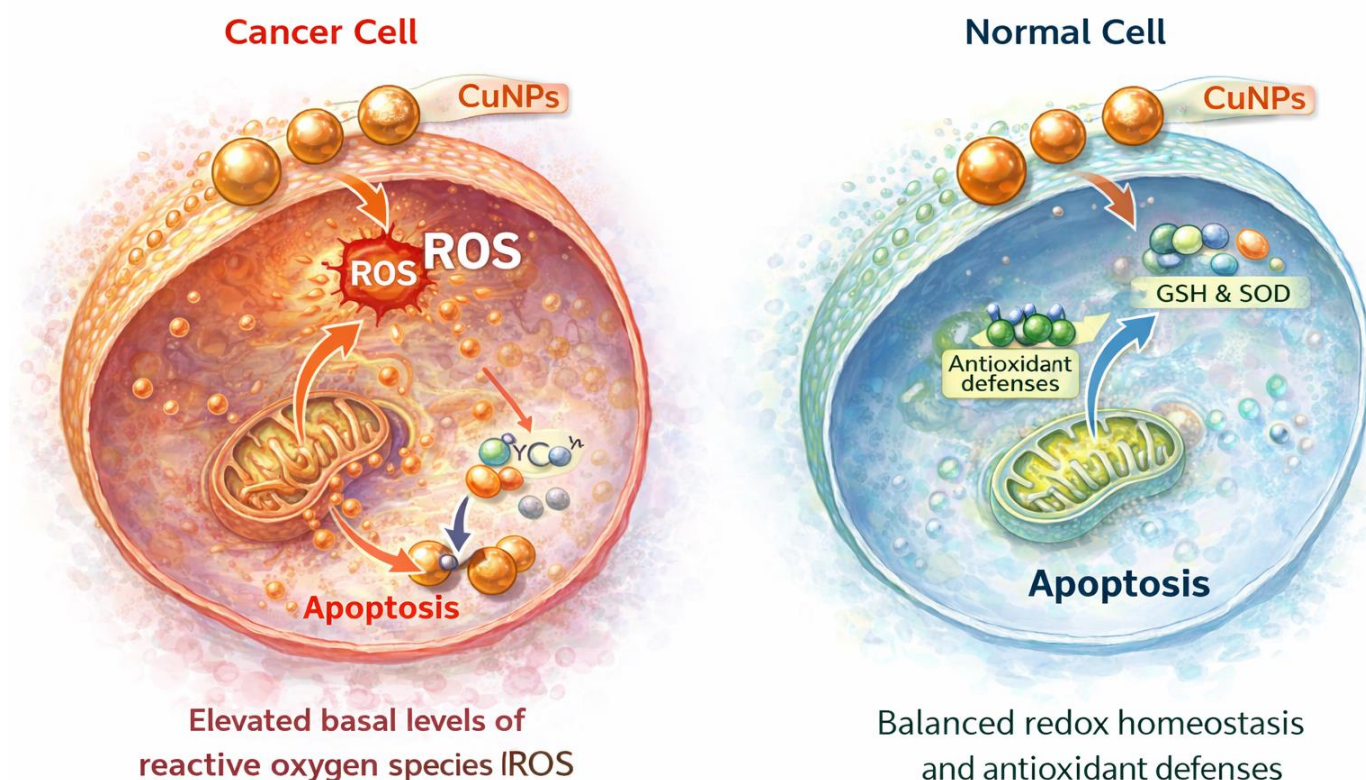


Figure 11. Schematic illustration of the redox-based selectivity of CuNPs, updated according to [135,136]. Cancer cells exhibit elevated basal levels of reactive oxygen species (ROS), rendering them highly sensitive to further oxidative stress induced by CuNPs, leading to mitochondrial dysfunction and apoptosis. In contrast, normal cells maintain balanced redox homeostasis and stronger antioxidant defenses, allowing them to better tolerate nanoparticle-induced oxidative stress.

8. Comparison Between Copper and Other Metal Nanoparticles

In order to enhance the context in which CuNPs can be appropriately placed in the greater context of nanomedicine, it is necessary to put their characteristics in comparison with other metal nanoparticles that have been the focus of numerous studies, including silver and gold. CuNPs have become an attractive substitute to other noble metal nanoparticles that include silver (AgNPs) and gold nanoparticles (AuNPs), in biomedical and anticancer studies. Although similar physicochemical properties at the nanoscale are common between all three classes there is a great deal of variation in their mechanism of action, cost-effectiveness, and therapeutic performance. These differences are necessary in understanding to be able to use the most suitable nanomaterial in particular biomedical applications.

8.1. CuNPs vs AgNPs

Silver nanoparticles (AgNPs) are considered one of the most widely researched nanomaterials that possess good antimicrobial and anticancer activity. AgNPs, like CuNPs,

produce cytotoxic effects, and this mode of action is mainly due to the production of reactive oxygen species (ROS), which causes oxidative stress, mitochondrial dysfunction, and apoptosis [95]. AgNPs can, however, be more intrinsically toxic to the cancer cells, as well as healthy cells, thereby restricting their therapeutic window [137].

Conversely, the redox behavior of CuNPs is more manageable because copper is capable of undergoing reversible redox cycles ($\text{Cu}^+/\text{Cu}^{2+}$) and therefore can generate continuous ROS at relatively lower concentrations [41]. This feature leads to better specificity between cancerous and healthy cells as it was already mentioned in Section 5. Moreover, nanoparticles made of copper have shown similar anticancer activity as AgNPs with a possible reduction of systemic toxicity in case of an adequate design [98]. AgNPs, however, can have better immediate cytotoxicity, but CuNPs has a better balance on efficacy and biosafety. AgNPs have better ROS generation and cytotoxic potency as shown in Fig. 12, but at the cost of selectivity relative to CuNPs.

8.2. CuNPs vs AuNPs

Gold nanoparticles (AuNPs) are best known to be biocompatible, stable in chemistry and can be functionalized with ease to the surface. Compared to CuNPs and AgNPs, the AuNPs can be regarded as biologically inert in general and do not inherently produce significant amounts of ROS [138]. This means that they tend to rely on external functionalization, like drug loading, targeting ligands or photothermal therapy to increase their anticancer activity [139].

In comparison, CuNPs have inherent cytotoxicity effects based on oxidative stress, which does not necessitate the high levels of modification to attain therapeutic effects [41]. Nonetheless, this benefit is accompanied by some more serious concerns about oxidative injury and biosafety. Although AuNPs would be the best choice in circumstances where minimal toxicity and high stability are needed, CuNPs are less toxic and more applicable in circumstances whereby direct anticancer activity and ROS-mediated approaches are required [139,140]. In such a way, the decision between CuNPs and AuNPs will rather rely on the ratio between intrinsic demands in terms of activity and safety. Fig. 12 also indicates that in comparison to CuNPs, AuNPs depend more on surface functionalization than on inherent ROS generation in order to be anticancer agents.

8.3. Cost, availability, and therapeutic efficiency

Cost-efficiency and availability of CuNPs is one of the greatest benefits of the nanoparticles compared to the noble-metal nanoparticles. Copper is less expensive and more abundant than gold and silver and therefore, CuNPs would be more applicable in mass production and clinical translation [140]. By contrast, AuNPs and AgNPs are based on rather limited and expensive raw materials, possibly preventing their widespread application in resource-constrained environments.

As a therapeutic agent, CuNPs have inherent anticancer characteristics, redox reactivity, and cost-effectiveness, which qualify them as promising compounds in the next-generation nanomedicine. Whereas AuNPs are indeed superior in biocompatibility and AgNPs in antimicrobial activity, CuNPs provide an unparalleled efficiency, selectivity and scalability [41,140]. Still, it is necessary to optimize their safety profile further to achieve the maximum of their clinical potential. The interplay of therapeutic effectiveness and cost of CuNPs, AgNPs and AuNPs is relatively summarized in Fig. 12.

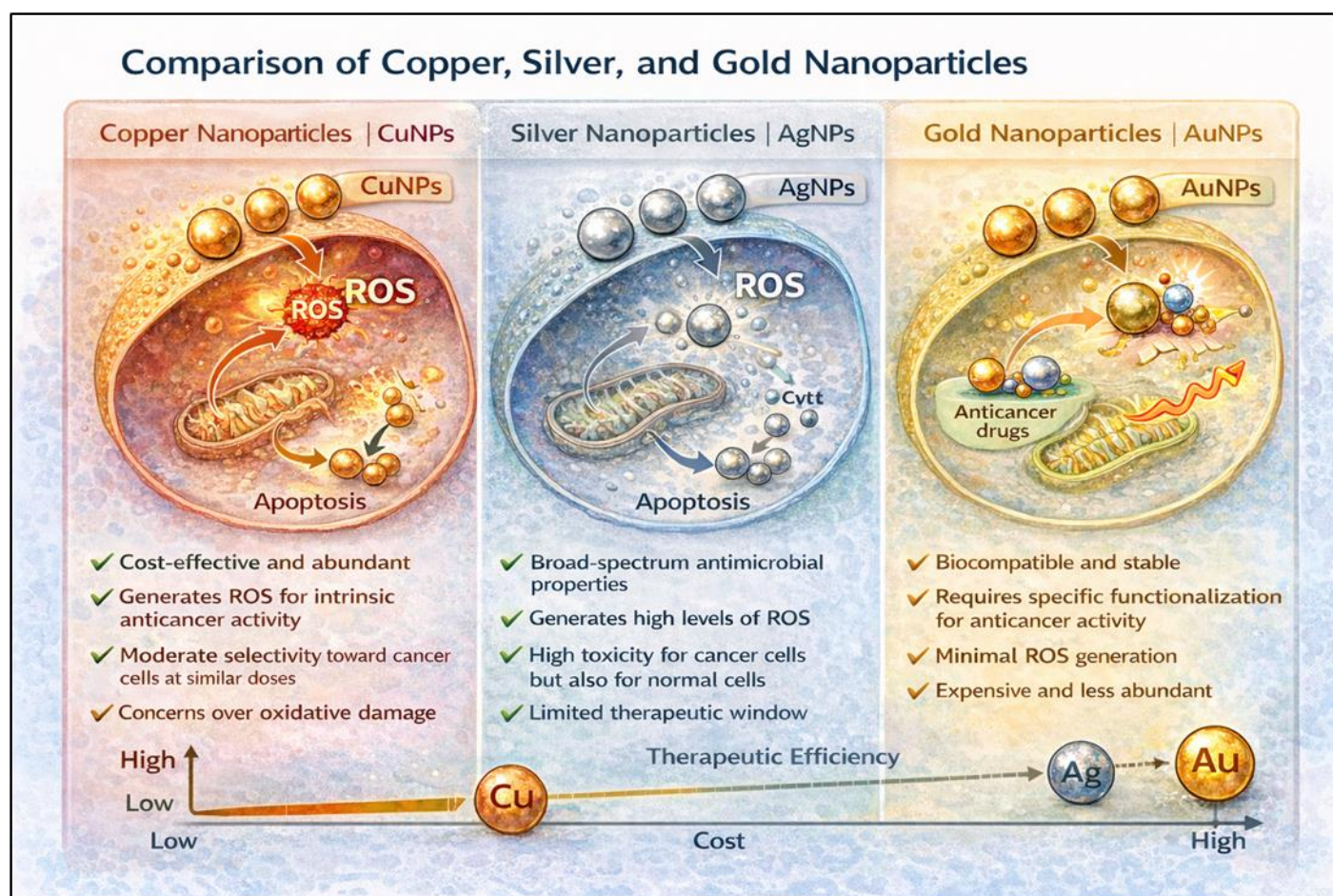


Figure 12. Comparative overview of CuNPs, AgNPs, and AuNPs in anticancer therapy, highlighting differences in intrinsic ROS generation, therapeutic selectivity, biocompatibility, surface-functionalization requirements, and cost-effectiveness, this schematic is informed by published



data [141–143]. CuNPs have inherent ROS-generating and cost-effectiveness and moderate specificity to cancer cells. AgNPs are highly ROS-mediated cytotoxic with a reduced therapeutic window because of greater toxicity to normal cells. Conversely, AuNPs are the ones that are highly biocompatible and stable, need to be functionalized on the surface to acquire anticancer properties, and cost more. There is also an example of the trade-off between therapeutic effectiveness and cost-effectiveness.

9. Current Limitations and Challenges

The key to the future of copper-based nanotherapeutics lies in the overcoming of these limitations and subsequent development and clinical translation. Although there is a strong potential to use CuNPs in anticancer, some important drawbacks have been identified that act as obstacles to the transfer of the experimental studies to clinical practice. These difficulties are mainly related to physicochemical unsteadiness, inconsistency in synthesis results, lack of clinical validation and regulatory uncertainties. These are critical concerns that have to be confronted to ensure the safe and effective development of nanotherapeutics based on CuNPs. Among the key drawbacks of CuNPs is the susceptibility of the former to oxidation. The CuNPs possess high surface reactivity which makes them highly susceptible to conversion to copper oxides (CuO/Cu₂O) when they come into contact with the air or biology [28,144]. The results of this oxidative instability can drastically modify their physicochemical characteristics, such as particle size, surface charge, and reactivity and hence their biological performance, and reproducibility [145]. In addition, the uncontrolled oxidation could increase the over-release of copper ions, which could increase the non-selectivity of cytotoxicity and decrease therapeutic selectivity. Thus, the stability of the CuNPs can still be enhanced by coating the surfaces or properly designed synthesis conditions, which is a major concern in nanomedicine [41].

9.1. Reproducibility issues

The other major issue in the production and use of CuNPs is reproducibility. The change in the synthesis techniques especially the green synthesis process may result in differently dispersed particle sizes, morphology, and surface functionalization [134]. These differences have a direct effect on the biological activity such as cellular uptake, ROS production and cell death. Moreover, the experimental conditions of studies, including cell lines, exposure times, and methods of the assays, also contribute to making the respective results more difficult to compare [83]. This has resulted in the unreliability and scalability of the CuNPs-based systems due to the absence of standardized protocols. It is therefore necessary to develop reproducible and well characterized strategies of synthesis to develop these materials to potentially be used in clinical practice.

9.2. Lack of clinical trials

Even though the anticancer efficacy of CuNPs has been demonstrated in many in vitro and in vivo studies, their clinical

translation has been limited. Up until now, the majority of research is limited to preclinical models with very minimal studies advanced to clinical trials [98]. The reason behind this gap can be explained by the issues of toxicity in the long term, biodistribution, and the possibility of copper ions accumulation in the key organs. The multiplicity of the interactions between nanoparticles and biological systems also provides a challenge in forecasting their behavior in human beings. Therefore, there is a need to conduct more extensive in vivo experiments and properly designed clinical trials to confirm the safety and therapeutic effectiveness of CuNPs in the real-world.

9.3. Regulatory considerations

The control of nanoparticles, including CuNPs, is inadequately developed and unevenly distributed in different locations. Regulatory authorities like FDA and EMA have not yet come up with guidelines specific to nanoparticle-based therapeutics, especially those that have inherent cytotoxic nature [146]. These challenges involve: standardization of methodology, long-term toxicity and environmental impact assessment. Besides, the dual nature of CuNPs, therapeutic agents and potential toxicants, complicates their regulatory safety. In this regard, there is need to establish clear and consolidated regulatory frameworks so as to facilitate safe delivery of copper-based nanomaterials to clinical practice.

10. Future Perspectives and Research Directions

Their future use in the cancer treatment field requires that the existing drawbacks should be surmounted and that the distinctive redox characteristics of CuNPs should be exploited. Further research is guided towards improvement of selectivity and biosafety as well as clinical translation with new engineering design and multidisciplinary solutions. The advancing trends in nanotechnology and precision medicine have offered a good direction on how to maximize the therapeutic potential of CuNPs.

10.1. Surface engineering and hybrid systems

Surface engineering is one of the best approaches towards improving the functionality and safety of CuNPs. Nanoparticle stability can be enhanced dramatically by functionalization with biocompatible polymers, ligand targeting, or targeting with biomolecules; nonspecific interactions can be avoided, and specific targeting of tumors can be enhanced [147]. Specifically, hybrid nanostructures of copper other materials-such as polymers, liposomes, or other metals-have demonstrated the prospects to regulate the ROS generation as well as regulate



the drug release profiles [148]. These hybrid systems can also incorporate other forms of therapeutic modalities including chemotherapy, photothermal therapy and catalytic therapy thereby increasing the efficacy of treatment. As a result, the rational design of surface-modified and hybrid CuNPs should be the key point of future-generation nanotherapeutic.

10.2. Combination therapies

Combining CuNPs with conventional cancer treatments is a promising approach to enhance therapeutic outcomes and overcome drug resistance. CuNPs have demonstrated the ability to sensitize tumor cells to chemotherapy and radiotherapy by amplifying oxidative stress and disrupting cellular repair mechanisms [41]. Indicatively, anticancer drugs could be utilized with the CuNPs to obtain synergies, which would bring decreased drug dosage in the body and less systemic toxicity. Also, stimulus-responsive copper-based systems, e.g. those responding to light, ultrasound or X-rays, have demonstrated existence of better anticancer efficacy when used as combination therapy [103]. These multimodal therapies can significantly impact accuracy and efficacy of treatment (refractory/aggressive malignancies).

10.3. Personalized nanomedicine

Introduction of CuNPs into personalized medicine is one of the new frontiers of cancer treatment. Since different tumors vary and they experience different redox conditions, the design of nanoparticles according to specific patient profiles can be highly desirable to enhance the selectivity and effectiveness in their treatment [149]. The recent advances in genomics, proteomics, and tumor microenvironmental studies will allow defining patient-specific biomarkers that could be utilized to create particular CuNPs systems. Also, tailor-made nanoparticle preparations could enable the ability to specify the generation of ROS, the delivery of drugs, and targeting of cells, suitable to reduce side effects. Such personalization will change the generalized therapies of CuNPs into specific therapeutic platforms.

10.4. Translational and clinical research outlook

Although the preclinical results are promising, the effective clinical translation of CuNPs has to be carefully assessed in terms of safety and pharmacokinetics and other long-term impacts of their use. The future studies should give priority on in vivo models that are standardized, the toxicity analyses in low and high numbers, and the studies of reproducibility [98]. Secondly, cooperation among scientists, practitioners, and authorities will be needed in order to develop clear principles on nanoparticle-based treatments. The feasibility of CuNPs in practice will, ultimately, be determined by the development of

clinically relevant delivery systems, as well as properly designed clinical trials. Since interdisciplinary research persists, copper-based nanomaterials have an excellent future due to the potential of becoming a significant component of the future cancer treatment protocol. In general, CuNPs are a potentially promising but currently developing concept in cancer nanomedicine, the success of which in the future depends on how much more sophisticated material design, biological knowledge, and clinical tests can be integrated.

11. Conclusion

CuNPs represent a promising and rapidly developing platform in cancer nanomedicine because of their intrinsic redox activity, tunable physicochemical properties, and relatively low production cost. Their ability to selectively intensify oxidative stress in cancer cells underlies coordinated anticancer effects involving mitochondrial dysfunction, DNA damage, cell-cycle disruption, and apoptotic signaling. This redox-based selectivity supports the therapeutic potential of CuNPs, although it remains dependent on nanoparticle design, dose, and biological context.

However, the clinical translation of CuNPs is still limited by oxidative instability, synthesis variability, insufficient standardization, and limited translational evidence. Addressing these challenges through rational surface engineering, hybrid nanostructure development, standardized evaluation protocols, and rigorous in vivo studies will be essential for improving their safety and therapeutic performance. Future integration of CuNPs with combination therapies and personalized nanomedicine approaches may provide more effective and selective cancer treatment strategies. Ultimately, their successful translation will depend on bridging the gap between fundamental nanoscience and clinically relevant validation while maintaining an appropriate balance between therapeutic efficacy and biosafety.

Data availability statement

No primary datasets were generated or analyzed during this study. The review is based exclusively on previously published literature, which has been appropriately cited.

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Declaration Statement



During the preparation of this manuscript, the author used ChatGPT (OpenAI) to assist with language refinement and improving the academic style of the manuscript. In addition, ChatGPT was used to generate schematic illustrations based on information and reference figures provided from published scientific literature. All generated figures were critically reviewed, modified, and verified by the author to ensure their scientific accuracy, consistency with the cited literature, and suitability for the objectives of this review. The author takes full responsibility for the content of the manuscript.

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