Copper Nanoparticles as Therapeutic Anticancer Agents

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ABSTRACT

Bio-nanotechnology exploits physicochemical approaches and biological principles in order to produce specifically functionalized nano-sized particles. Nanoparticles may be very effective molecules in the development of novel therapeutic approaches against several diseases including cancer. Consequently, synthesis of metallic nanoparticles for the improvement of therapeutic index and drug delivery applications is coming up as an effective strategy in the conventional therapeutic anticancer research. In recent years, the auspicious anticancer potential of nano-particulate metallic forms of gold, silver, and copper is progressively being established. As a result, the development of copper-derived nano-therapeutics is challenging due to the cost-effectiveness of copper, and the already extensively studied anticancer potential of copper-based nanoparticles such as copper oxide nano-formulations. However, limited investigations have been conducted on the anticancer efficacy of metallic copper nanoparticles. Herein, we present an analytical overview of the therapeutic applications of copper nanoparticles as efficient anticancer agents.

Introduction

Nano-biotechnology, and more specifically the synthetic procedures of metallic nanoparticles (NPs) along with their biological applications have attracted increasing scientific interest due to their distinctive chemical and physical properties [1], synthetic feasibility, low production cost [1] and, additionally, because of their significantly valuable applicability in the fields of medicine and pharmaceutical science [3-6]. Until today, several physicochemical approaches have been developed on the synthesis of diversified types of metallic NPs. Various research fields such as experimental medicine, drug design, drug delivery, electrical and electronics engineering, electrochemical sensor and biosensor development, agricultural science and biochemistry are delving into the development and further implementation of novel metallic NPs [7-9].

Worldwide, cancer constitutes one of the most common health issues. Nowadays, novel cancer treatment approaches include utilization of nanomaterials along with their unique biochemical properties. The therapeutic efficacy of nano-scale molecules relies heavily on their enhanced reactive surface area, compared to conventional small-molecule drugs and pharmaceuticals. Until recently, the anticancer potential of nano-particulate
forms of metals including gold, silver, and copper has progressively being documented [10-13]. However, the low cost of copper compared to that of gold and silver results in the enhanced development of copper-based NPs as therapeutics. Copper NPs constitute a particular group of metallic NPs produced via contemporary technologies and with advantageous physicochemical, biological and mechanical properties [14,15]. Moreover, it has been proved that copper and copper-based NPs exhibit enhanced toxicity against various microorganisms, thus becoming an effective antimicrobial and antifungal agent [16-22]. Synthesis of copper NPs involves short and simple experimental procedures and cost-effective reaction conditions with high production yields compared to other types of metal NPs [23,24]. Their wide range of nano-dimensions and surface-to-volume ratios abet their potency as medicinal, pharmaceutical, and therapeutic agents [25,26]. Moreover, copper NPs can be utilized as DNA-cleavage agents and potent anticancer therapeutics due to their binding capacity and modifiable surface properties through conjugation with various bio-molecules including enzymes and proteins [27-30]. Copper NPs can also function as effective drug delivery nano-formulations and molecular doping systems operating as controllers of cancer cell growth [31-33]. However, the toxic profile of the implemented chemicals and the environmentally harmful physicochemical methods applied during the production of copper NPs [34,35] urges the need for the development of eco-friendlier methods of synthesis [36], maintaining the desired morphology, size, and stability combined with improved pharmaceutical and medicinal properties [37-40]. Eco-friendly synthesized bio-mediated nanoparticles [41] can be used as an effective nano-drug against several types of cancer disease [42,43]. Although there are several scientific reports on the anticancer potential of copper oxide NPs [44-48], limited research results have been disseminated on the antitumor potency of metallic copper NPs [49,50], mainly due to their unstable and easily oxidized form at ambient temperature conditions. Herein, we present a concise overview of the therapeutic applications of copper NPs as anticancer agents.

Synthesis and characterization techniques of metallic NPs

1. Physical and chemical synthetic methods of copper NPs

Various physical and chemical methods have been applied for the synthesis of metallic NPs including copper NPs such as: microwave-assisted processes and sol-gel procedures [51], co-precipitation [52], pulsed wire discharge [53], laser ablation [54], high-energy irradiation [55], vacuum-vapor deposition [56], mechanical milling [57], lithography [58], electrochemistry and photochemical reduction, [59-63], hydrothermal reaction [64], micro-emulsion [65], electrospray synthesis [66], and chemical reduction [67].

2. Biosynthetic methods or “green”-synthesized copper NPs

The biosynthetic procedures of NPs are based on green chemistry methods employing different biological systems including plants [68,69], fungus [40,70-72], actinomycetes [73,74], yeast [75-77], bacteria [78-82], and viruses [83,84]. “Green”-synthesized copper NPs are produced through the utilization of phytochemicals in order to generate nano-formulations of more flexible shapes and sizes by controlling either the reaction temperature, time, and pH and, additionally, the concentrations of the employed plant extract and/or metal salt [67]. The successful reduction of the copper ion and the subsequent formation of the pursued NPs, which is an instant phenomenon, are confirmed through the observed color change of the reaction mixture. Phytochemicals also operate as the stabilizing agents of the produced NPs, as depicted in Scheme 1 [67].

“Bionanofactories”, such as macroalgae, can synthesize highly stable and functional metallic NPs capable of eliminating cell maintenance [85]. Recently, gold NPs were synthesized through the utilization of algae extracts including Sargassum wightii [86], Laminaria japonica [87], Turbinaria conoides [88], and Stoechospermum marginatum [89]. In general,
Scheme 1: Reduction of copper ions and stabilization-capping of the produced copper NPs.

Scheme 2: Morphological and physicochemical characterization techniques of metallic NPs.
Macrocyanine (seaweeds) constitute a significant source of biomedical substances and compounds with exceptional antibacterial [90], antifouling [91], and anticoagulant activity [92].

3. Characterization techniques

Several techniques are employed for the physicochemical and morphological characterization of the produced NPs as depicted in Scheme 2. A detailed overview of the determined characteristics and attributes of each morphological and physicochemical characterization technique is presented in Table 1. The most widely used analytical methods for the detection of copper in aqueous media and/or biological fluids are Atomic Absorption Spectrometry (AAS) with flame detection or stabilized temperature platform graphite furnace, inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma atomic emission spectroscopy, and X-ray fluorescence spectrometry [93-97]. One of the main applications of the specific analytical techniques is the determination of main and trace element concentration in different human fluids and tissues [98,99]. It has been proved that increased copper concentrations in serum results in tumor genesis, malignant proliferation and recurrence in several types of human cancers [98-100].

4. Dietary copper intake and therapeutic copper dosing

Copper is considered an essential nutrient. In the majority of foods, copper is presently bound to macromolecules rather than as a free metal ion [101]. Recent recommended dietary allowance (RDA) reports established a daily copper intake of 900 μg for adults, 340 μg/day for children for the first 3 years of age, 440 μg/day for ages between 4-8 years, 700 μg/day for ages between 9-13 years, and 890 μg/day for ages between 14 and 18 years [102]. For 19-year-old men and women the copper RDA is 900 mcg/day, and for pregnancy and breastfeeding age the copper RDA is 1000 mcg/day and 1300 mcg/day for women of all ages, respectively [104]. The therapeutic copper dose levels that have been scientifically studied are [105-109]:

**Orally:**
- For copper deficiency: daily doses up to 0.1 mg·kg⁻¹ of CuSO₄.
- For osteoporosis: daily mixed doses of 2.5 mg copper in combination with 1000 mg calcium, 15 mg zinc, and 5 mg manganese.

**Intravenously:**
- For severe copper deficiency: intravenous provision of copper (2-4 mg/day). For mild to moderate copper deficiency: intravenous provision of copper (3-8 mg/day).

Anticancer Activity of Copper NPs

1. Anticancer activity of “green”-synthesized copper NPs

Recent studies on the anticancer potential and cytotoxic activity of “green” synthesized copper NPs from Sargassum polycystum brown seaweed (Figure 1), utilizing copper sulfate (CuSO₄) as the precursor, indicated that NP concentrations of 100 μg·ml⁻¹ can efficiently provoke inhibition of the growth of MCF-7 breast cancer cells at percentages higher than 93% and with an IC₅₀ value of 61.25 μg·ml⁻¹ [110]. Studies on the biosynthesis of copper NPs utilizing plants including Nerium oleander [111], and Magnolia Kobus [112], have previously been reported. The findings of recent studies on the biosynthetic procedure of copper NPs utilizing the E. prostrate aqueous leaf extract as a...
reducing reagent, suggested a promising copper-based nanomaterial with improved activity against cell proliferation. The in vitro cytotoxic potential of increasing concentrations of the corresponding copper NPs (1-500 μg.ml⁻¹) on the growth and morphological characteristics of the human HepG2 cancer cell line, estimated via the MTT assay, indicated cellular toxicity values of up to 54.5% [113].

*Prosopis cineraria* are a well-known therapeutic tree species with excellent analgesic, antihyperglycemic, antipyretic, antihypercholesterolemic, antioxidant, and antitumor properties. Examination of the anticancer potential of *Prosopis cineraria* leaf extracts indicated growth inhibition of human HeLa and MCF-7 cancer cells [114,115].

<table>
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<tr>
<th>Table 1: Determined characteristics and attributes of each morphological and physico-chemical characterization technique [67].</th>
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<tr>
<td><strong>Morphological and physico-chemical characterization techniques</strong></td>
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<tr>
<td>Ultraviolet-visible spectroscopy (UV-vis)</td>
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<td>Fourier transform infrared spectroscopy (FTIR)</td>
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<td>X-ray diffraction (XRD)</td>
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<td>Malvern Zetasizer (MZS)</td>
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<td>Energy-dispersive X-ray spectroscopy (EDX/EDS)</td>
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<td>Nanoparticle tracking analysis (NTA)</td>
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<td>Small-angle X-ray scattering (SAXS)</td>
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<td>X-ray reflectometry (XRR)</td>
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<td>Brunauer-Emmet-Teller analysis (BET)</td>
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<td>Selected area electron diffraction (SAED)</td>
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<td>Atomic force microscopy (AFM)</td>
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<td>Atomic absorption spectroscopy (AAS)</td>
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<td>Inductively coupled plasma mass spectrometry (ICP-MS)</td>
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*Figure 1: Sargassum polycystum brown seaweed.*
Studies on the microwave-assisted synthesis of “eco-engineered” copper NPs using P. cineraria leaf extracts and their cytotoxic and antiproliferative effects against human MCF-7 cancer cell line, estimated by the MTT assay, indicated that stabilized copper NPs improved the cytotoxic activity strongly associated with the DNA-fragmentation procedure, significantly more effectively compared to that of stabilized silver NPs and plant leaf extracts [116].

Quisqualis indica Linn is an evergreen plant species with therapeutic phytoconstituents exhibiting anti-inflammatory, antipyretic, immuno-modulatory, and antibacterial properties [117]. Efforts on the development of a green synthetic procedure of copper NPs utilizing the Quisqualis indica Linn floral extract resulted in the production of mono-disperse nano-formulations with a dose-dependent (40-120 μg/ml) cytotoxic activity, as evidenced through extracellular Lactate Dehydrogenase (LDH) release, reactive oxygen species (ROS) generation, and intracellular reduced glutathione (GSH) content depletion in B16F10 melanoma cells and with an IC50 value lower compared to that of the floral extract [118].

In recent studies, the eco-friendly synthetic approach of highly crystalline and small-sized copper NPs using Broccoli Green Extract (BGE) resulted, initially, in rapid reduction of CuSO4 precursor and, subsequently, in the production of relatively small and spherical shaped copper nano-formulations. Cytotoxicity studies of the corresponding surface modified copper NPs via cellular density measurements presented no effect on prostate cancer (PC-3) cells in concentrations ranging between 0.5-1.5 μM and after 2 h of exposure time. The collective anti-proliferative outcome is comparable to reported results on the observed 44-times more intense inhibitory effect of silver NPs against HepG2 cells compared to normal cells [119,120].

A relatively rapid and simple synthetic procedure of peptide capped copper NPs in the latex of the therapeutic Euphorbia nivulia plant species [121] has also been reported [122]. Further studies on the cytotoxic potential of the resulting biocompatible latex-capped nano-formulations in human A549 lung carcinoma cells indicated the dose-dependent anti-proliferative activity of the corresponding nano-formulations in concentrations ranging between 1-100 μg/ml. These observations confirm the ability of copper NPs to induce structural damages of the cellular milieu, mitochondrial dysfunction, and high indices of oxidative stress [104].

Research reports on the “green” and cost-effective production of copper NPs utilizing green tea aqueous extract as reducing, stabilizing and capping agent indicated the synthesis of nano-sized copper formulations (≈ 20 nm) with a concentration-dependent (2-1024 μg/ml) cytotoxic activity against animal cells as evidenced via the MTT assay [123].

2. Anticancer activity of stabilized copper NPs

2.1. Anticancer activity of chemically stabilized copper NPs: In an effort to produce bioactive metallic NPs of variable sizes and shapes, with the desired stability and resistance against atmospheric parameters, researchers developed several synthetic approaches such as the Brust-Schiffrin technique [124], as a two-step phase transfer synthetic procedure with a mediated phase transfer agent (PTA). The unstable nature of the as-produced NPs can be controlled through the utilization of a capping stabilizing ligand including the biologically effective Schiff base ligands [125]. Increasing concentrations (0-250 μg/ml) of newly synthesized Schiff base stabilized copper NPs with pyrimidine derivatives of 2-(4,6-dimethoxy/4-nitrophenyl)-6-methoxyphenol (DPMM) showed enhanced CT-DNA binding affinity through hydrophobic interactions and increased anti-proliferative activity against MCF-7 cancer cells as evidenced via the MTT assay after 24 h of post-exposure [126].

Benzimidazole (BMZ) is considered an effective antibacterial agent and its derivatives have been proved to possess anticancer properties [127,128]. Recent studies on nanoparticulate copper based benzimidazole complexes (≈ 100 nm), produced via the re-precipitation method, demonstrated the improved therapeutic potential of the corresponding nano-
formulations against malignant cells [129], such as NIH/3T3 and A549 cell lines. The novel nanomaterials operated as adequate photothermal transducers, during the application of the Photothermal Therapeutic method (PTT), presenting enhanced binding ability to the target cells, energy absorbance, cytotoxic efficacy, and limited interaction with the healthy cells [130].

2.2. Anticancer activity of physically stabilized copper NPs

Efforts on the production of nano-formulations with enhanced bactericidal activity resulted in the development of gelatin stabilized copper NPs by reduction of copper chloride (CuCl₂) [131]. The corresponding NPs presented exceptional stability and sizes of around 50-60 nm and were further attested against three cancer cell lines such as human skin melanoma A-375, human lung cancer A-549, and rat glioblastoma C6-G. The estimated IC₅₀ values of the novel copper NPs were 1.71 μg.ml⁻¹ for the first cancer cell line, 1.81 μg.ml⁻¹ for the second, and 1.88 μg.ml⁻¹ for the third, respectively. These results implied a similar cytotoxic profile of the produced copper NPS to all the attested cancer cell lines, indicated the effectiveness of the corresponding nanomaterials compared to other alternatively synthesized types of copper NPs [132,133], and stressed the ability of these copper nano-formulations to selectively target only the cancer cells leaving the peripheral healthy cells unaffected [134].

Naturally occurring active biomolecules have gained great attention due to their utilization in the synthesis of metallic NPs [135]. Among various functional and therapeutic food ingredients, polyphenols, and more specifically curcumin, have been proved effective anticancer agents [136]. Efforts on the development of adequate curcumin diverse nanocarriers, in order to improve the limited aqueous solubility and bioavailability of the corresponding polyphenol, led to the synthesis of curcumin-capped copper NPs. The novel copper nano-formulations were synthesized according to the Creighton method [137], and were further evaluated for their anticancer potential via the MTT and cell migration assays against human breast cancer cells (MDA-MB 231), treated with concentrations ranging between 5-25 μM. Furthermore, their antiangiogenic potential was further attested using Human Angiogenesis ELISA Strip I Kit for profiling eight cytokines and in vivo chorioallantoic membrane model (CAM assay). The overall results pointed out that the anticancer and antiangiogenic activity of the corresponding copper NPs did not exceed that of native curcumin [138].

Recently, studies on the synthesis of colloidal copper nanomaterials led to the production of glycerin stabilized copper NPs generated via the reduction of CuSO₄ by utilizing oxalic acid. The emerged nano-formulations were further capped with Poly vinyl alcohol (PVA). The anticancer activity of the synthesized colloidal suspension of copper NPs was determined against MCF-7 cancer cells (breast carcinomas) via the MTT assay. The results at hand showed that 50% cell inhibition was observed after the addition of 250 μg.ml⁻¹ of colloidal copper NPs [139].

Anticancer Nanocomposites of Copper NPs

Polymer/metal nanocomposites represent a category of hybrid materials deriving from the synthesis of an organic polymer matrix bearing dispersed metallic NPs. They are widely applied in drug delivery, molecular imaging, cell labeling, diagnosis/treatment, cancer therapy, material chemistry and bio-sensing [140-147]. Chitin is a non-toxic, renewable, biodegradable, non-immunogenic, and biocompatible natural polymer that can be easily modified into membranes, hydrogels, beads, nanofibers, sponges, scaffolds, and micro/nanoparticles [148-154].

Recently, chitin nanocomposites with several metallic NPs have attracted extensive scientific interest for their electronic, optical, catalytic, and mechanical properties. In an experimental study on the synthesis of chitin-copper nanocomposites, the novel nano-formulations were further attested for their anticancer activity against MCF-7 cancer cells and normal HEK-293T cells. The corresponding nanomaterials exhibited enhanced anticancer effect, induction of apoptosis, and significant
Cytotoxicity only to the cancer cells, implying extended selectivity and targeted anticancer potential [155]. Chitosan-copper nanocomposites are a specific type of nanomaterials widely used in the fields of biological application and environmental remediation [156-158]. Studies on newly synthesized spherical chitosan-copper nano-formulations proved the ability of the novel nanomaterials to induce blebbing and shrinkage of A549 cancer cells, reduce cell density and the cell-to-cell contact in comparison to the corresponding behavior of untreated cells. The observed cytotoxic effect increased from 0.2% to 10.5%, with increasing nanocomposite concentrations from 10 μg.mL⁻¹ to 100 μg.mL⁻¹, and with the highest IC₅₀ value estimated around 434.6 μg.mL⁻¹ [159].

In general, the basic characteristic of a potent chemotherapeutic drug is its ability to destroy selectively only the targeted cancer cells without altering the cellular integrity of the peripheral healthy tissues. Albumin NPs, as nano-carriers for targeted chemotherapeutic drug delivery applications, have attracted the scientific attention due to their main ability to enhance the endocytic drug uptake by cancer cells [160]. This attribute relies heavily on: a) the strong permeability and retention effect (EPR) of albumin NPs mediated by passive albumin uptake in cancer cells, and b) the enhanced active drug absorption by cancer cells, initiated by albumin NPs via the albumin receptor. As a result, novel drug delivery nano-formulations have been designed based on albumin such as prodrugs, drug-albumin conjugates, albumin-binding drug derivatives, and albumin NPs [161]. Another important advantage of albumin NPs is their ability to eliminate ethanol, cremophor and emulsifiers due to the enhanced drug solubility [162]. Serum albumin is considered a very functional blood protein due to its ability to maintain osmotic pressure and blood pH, transport various types of exogenous and endogenous molecules [163], bear several binding sites, and possess high half-life in blood circulation, great stability and solubility [164,165]. Recent efforts on the synthesis of a potent chemotherapeutic nano-formulation based on albumin led to the production of albumin nano-carriers of copper NPs. The novel nanomaterials were further attested for their anticancer potential against invasive MDA-MB 231 cancer cells and normal MCF-10A counterparts. The overall findings suggested that the synthesized spherical nanomaterials (≤ 100 nm) induced cell death via a dose-dependent manner through apoptosis. Their cytotoxic activity was estimated 5.7 times more acute compared to that on healthy cells as evidenced through the observed cyto-morphological changes, cell permeability, and the created DNA ladder pattern [166].

Comparison of the Anticancer Activity between Copper NPs and other Types of Metallic NPs

Various types of metallic NPs have been extensively studied for their anticancer properties such as gold [167], cobalt [168], silver [169], cerium [170], and others. However, copper NPs have become increasingly applicable due to their cost effectiveness, enhanced cytotoxic potency against cancer cells at low doses and longer stability period compared to Au and Ag NPs [171-176]. As an alternative to silver or gold NPs, CuS NPs and generally copper based chalcogenide semiconductor NPs represent a novel group of photothermal conducting agents that can be easily and cost-effectively synthesized and possess a remarkable translational potential. Moreover, in contrast to gold NPs, they possess a tunable absorption, based on the particle size, which can be formulated below 20 nm ensuring near-infrared absorption, improved clearance and pharmacokinetic properties, high tumor accumulation, and efficient elimination from the body when their size is below 6 nm [177]. Compared to silver and gold, the cytotoxic activity of copper has been less studied [178]. However, recent investigations on the toxicity of several types of metallic NPs (Au, Ag, Cu and Co NPs) through the laser ablation method that generates uncapped NPs in solution [179], indicated that all types of NPs possess moderate cytotoxic behavior to PC3 and HeLa human cell lines.
with copper NPs presenting the lowest cytotoxicity [180].

Recent studies on the synthetic procedure of water soluble starch stabilized copper NPs produced via the utilization of ascorbic acid as the reducing agent resulted in the generation of 10 nm copper NPs with relatively low cytotoxic levels and enhanced bactericidal effect [181]. Evaluation studies of the cytotoxic activity of DPMM-stabilized copper and nickel NPs revealed that the newly synthesized DPMM-Cu NPs are more biologically active compared to the corresponding nickel NPs due to their lower 50% cell viability, better binding affinity with CT-DNA, increased interactions with DNA and enhanced cytotoxicity against MCF-7 cancer cells [126]. Comparative studies through MTT assay on the cytotoxic and antiproliferative activity of Prosopis cineraria biofabricated copper and silver NPs against MCF-7 cancer cells proved that the bioengineered copper NPs presented better cytotoxic effect (lower IC50) compared to that of silver NPs [116].

Comparative Analysis of the Anticancer Activity of Copper-Derived NPs

There are several types of copper-derived NPs with proved anticancer activity such as CuI, CuO, CuCO3, Cu(PO4)2, Cu2O, CuS [171-176]. Recent reports on the anticancer potential of CuI and Cu(PO4)2 NPs, with sizes ranging from 35 nm to 67 nm, respectively, showed that low doses of CuI NPs were more effective in comparison to Cu(PO4)2 NPs with 2.5 μg.ml-1 LD50 value for CuI and 10 μg.ml-1 for the corresponding Cu(PO4)2 NPs. Both types of NPs cause apoptotic mediated cell death by inducing ROS-mediated DNA damage [171].

Newly synthesized crystalline and amorphous CuS NPs of similar sizes [50-60nm] presented enhanced cytotoxicity on tumor cells. The LD50 values on HL-60 cell lines were 29 μg.ml-1 for CuS nanocrystals and 18.5 μg.ml-1 for amorphous CuS NPs. Moreover, both types of NPs mediated targeted apoptosis in tumor cells unaffected peripheral healthy cells [176].

Research studies on the anticancer potential of Cu2O NPs indicated the specific and selective anticancer activity of the corresponding type of copper NPs only against cancer cells, even at low concentrations, without affecting the normal cells [175]. Novel synthetic approaches resulted in the production of CuCO3 NPs of 20 nm average size that can induce ROS-mediated DNA and mitochondria damage, resulting in apoptosis in tumor cells. The corresponding CuCO3 NPs were modified with folic acid in order to obtain targeted action against cancer cells. Folic acid is used as a precursor for DNA synthesis and its high effectiveness depends on its demand for fast reproducibility. Furthermore, the corresponding folate receptor is over-expressed in the majority of cancer cells leading to the internalization of folic acid. Consequently, upon treatment of mice cancer models with folic acid modified CuCO3 NPs, toxicity decreased to the lowest level and survivability increased significantly in comparison to the control group. Additionally, a reduction of the tumor volume was also observed [173].

Newly synthesized CuO NPs of spherical shape from “green” synthetic approaches and chemical reduction processes, resulted in the production of NPs with sizes ranging from 5-100 nm depending on the employed plant species and the type of the applied chemical reduction process. The corresponding group of CuO NPs showed enhanced effectiveness against cervical, HeLa and MCF-7 breast cancer cells. The proposed anticancer mechanism includes autophagy which is provoked by the intracellular stress induced by the ROS mediated DNA damage leading to apoptosis. Furthermore, conjugation of folic acid to the corresponding CuO NPs utilizing APTS enhanced their targeted activity and selectivity towards cancer cells when attested in vivo [173-176,182].

Several novel types of copper-containing NIR-absorbing nano-formulations have been synthesized and further attested for PTT such as copper selenide (Cu2-xSe) nanocrystals, monodispersed CuTe nanorods, nanoplates, and nanocubes, copper bismuth sulfide (Cu3BiS3) nanostructures. All of the above-mentioned copper-derived nano-molecules present enhanced photothermal heating efficiency and significant anticancer potential [177].
Developing Approaches of Targeted Anticancer Copper and Copper-Derived Nano-Formulations

The development of lipid and polymer nanocarriers has eased the delivery of several therapeutic molecules to diseased tissues and cells. Copper NPs, copper-derived NPs or copper complexes with organic substrates can be easily encapsulated into bioavailable, biodegradable, and non-toxic nano-vehicles for targeted administration to specific cellular targets.

Recent scientific results on the encapsulation of anticancer copper complexes indicated the development of a hydrophobically stearic acid modified chitosan nano-formulation bearing hydrophobic copper complexes [183,184]. The resulting nanomaterials targeted effectively the folate and HER 2 receptors of tumor cells, resulting in tumor regression when attested in vivo [183].

Recently, a novel liposomal nanocarrier bearing the antialcoholic drug disulfiram (lipo-DS) combined with copper gluconate, was tested in vivo against CSCs (tumor initiating cells) in order to avoid pan-chemoresistance. The corresponding nano-formulations targeted the NFκB pathway, presenting an enhanced anti-CSC efficacy towards hypoxia-induced CSCs [185].

Furthermore, newly synthesized soft copper oleate NPs were proved to possess effective photoacoustic (PA) contrast, equivalent to gold-based nano-formulations but more cost-effective and with higher availability of the starting materials. The corresponding type of copper NPs presented a strong PA contrast, similar to that of gold nanobeacons and relative to blood. Additional in vivo studies in the Matrigel angiogenesis model showed that α,β,γ-CuNPs significantly increased the neovessel sprout formation levels and operated as efficient anti-angiogenic fumagillin-prodrug nanocarriers providing the first applied targeted approach for the development of a drug delivery treatment combined with a PA contrast agent [186].

Research studies on the development of double-strand DNA-hosted copper nanoclusters by utilizing random dsDNA (double-stranded DNA) or poly(thymine) ssDNA (single-stranded DNA) as the template, indicated that the fluorescent properties of the corresponding nanomaterials could be applied in the identification processes of SNPs (Single nucleotide polymorphisms), providing a tool for the fluorimetric diagnosis of DNA mismatches [187].

Experimental studies on the development of targeted drug delivery and bioimaging molecules resulted in the formation of transferrin (Tf) templated copper nanoclusters (Tf-CuNCs) with enhanced luminescence. The corresponding nano-molecules were further formulated into spherical transferrin copper nanocluster-doxorubicin (Dox) NPs (Tf-CuNC-Dox-NPs) of spherical shape, based on the electrostatic interactions with doxorubicin. The newly synthesized nanomaterials were further assessed in vivo on TfR (Transferrin Receptor) positive DLA (Daltons Lymphoma Ascites) bearing mice revealing enhanced inhibition of tumor growth and prolongation of the survival of the animals [188].

Additionally, integration of 64Cu radionuclide into CuS NPs without the introduction of radiometal chelators, led to the production of the radioactive $[^{64}\text{Cu}]\text{CuS NPs}$, adequate for quantitative tissue analysis and PET (positron emission tomography) imaging. Quantitative tissue analysis plays a significant role in the design of targeted approaches for NP-based therapy [177].

In Vivo Effects of Copper and Copper-Derived NPs

Studies on the systematic comparison of renal clearance rates of luminescent degradable glutathione-coated copper NPs (GS-CuNPs) and their dissociated products, Cu(II)-glutathione disulfide (GSSG) complexes (Cu(II)-GSSG), in vivo, indicated that GS-CuNPs can be accumulated in the liver less than their dissociation counterparts and eliminated through the urinary system significantly faster. This behavior can be attributed to the increased resistance of GS-CuNPs against serum protein adsorption compared to that of Cu(II)-GSSG. Additionally, the biodistribution and renal clearance of GS CuNPs depend on the type of the dissociation process (zero-order chemical kinetics) and the primary injection doses [189].

Physicochemical studies on copper NPs indicate their tendency to form aggregates of low solubility in vehicle...
or simulated intestinal environment. It has also been proved that copper NPs present a satisfactory dissolution level in acidic environment but lower than that of copper ions. These observations indicate the existing interdependence of the biological system and the physicochemical characteristics of copper NPs that further affect their in vivo toxic behavior. Copper NPs, via kinetic studies, have been proved to present delayed and lower concentrations of accumulated or absorbed copper levels in tested organs and blood compared to copper ions. Additionally, the in vivo toxic behavior of copper NPs is more profound at high doses compared to that of copper ions, eliciting similar response with distinct sex-related differentiations.

Furthermore, copper NPs can induce morphological and functional changes in the spleen, kidneys, and liver due to in vivo systemic toxic effects associated with their biodistribution rate and solubility [190]. Further in vivo studies have proved that the decreased particle size of copper NPs along with their morphological characteristics and the species-specific vulnerability of cells can affect their toxicity [191].

Conclusion

Several diversified strategies utilize metallic NPs against cancer disease. It is proved that NPs can present either direct antitumor effects or indirect hyper thermic anticancer activity, in vitro and in vivo. Moreover, NPs can operate as a conventional anticancer drug, reducing both the side effects and the required dose. Despite the fact that research on the anticancer potential of metallic NPs has been increased, there are still limitations due to the cell heterogeneity utilized for each tumor environment which hinders the comparative studies. Another limitation regards the formation of protein corona after the interactions of NPs with the blood and the plasma proteins, affecting the in vivo clearance and distribution. New research efforts will enable the development of novel types of metallic NPs, such as copper NPs, with improved and selective anticancer activity, enhanced biocompatibility and bio-distribution, and low toxicity for normal tissues.

In this review, we have thoroughly discussed the anticancer therapeutic applications of copper and copper-derived NPs in vitro and in vivo including drug delivery, cancer imaging, image-guided therapy, PTT, and developing approaches on selective targeting and mitigation of potential toxicities. In comparison to other inorganic nanomaterials, such as carbon nanotubes, gold nanoshells or nanorods, and other types of metallic NPs, copper NPs have not been extensively studied, especially in vivo. Their multifunctional features and characteristics render copper NPs ideal nanomaterials for theranostic applications as contrast agents or nuclear tracers for various diagnostic and imaging techniques, photothermal cancer cell destruction, and controlled drug release applications. Despite many experimental reports in vitro, more effort should be focalized on the in vivo efficiency, disposition, and properties of copper NPs, including their potential cytotoxicity, pharmacokinetics, and pharmacodynamics in order to evaluate their limitations and advantages along with their controllable long-term cytotoxicity, favorable in vivo pharmacology, and photothermal conversion capacity.

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