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COPPER NANOPARTICLES AS THERAPEUTIC ANTI-CANCER AGENTS

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ABSTRACT

Bio-nanotechnology uses physicochemical approaches and biological principles to produce specifically functionalized nanosized particles. Nanoparticles can be very effective molecules for developing new therapies against several diseases, including cancer. Therefore, the synthesis of metal nanoparticles to improve the therapeutic index and drug delivery applications becomes an effective strategy in conventional therapeutic anticancer research. In recent years, the favorable anticancer potential of gold, silver, and copper metal forms made of nanoparticles has been gradually strengthened. As a result, the development of copper-derived nanotherapy is difficult due to the cost-effectiveness of copper and the already well-studied anticancer potential of copper-based nanoparticles such as copper oxide nanocomposites. However, there is limited research on the anticancer effects of metallic copper nanoparticles. Here we present an analytical review of the therapeutic applications of copper nanoparticles as potent anticancer agents.

INTRODUCTION

Copper (Cu) is a common metallic element and also a transition element characterized by redox activity. Under physical conditions, copper (Cu) can be reduced to oxidized copper by a normal chemical reaction. Copper ions participate in several metabolic functions as cofactors or structural components that provide or accept electrons to regulate several physiological processes, including energy metabolism, mitochondrial respiration, and antioxidants. The presence of copper ions maintains a balance that, if disturbed, can lead to oxidative stress and abnormal autophagy. Tumor tissue and blood copper concentrations have been shown to be much higher in individuals with various malignancies, and abnormal

copper binding in patients with Wilson's disease may promote malignant transformation of liver cells. Therefore, copper homeostasis plays an important role in the survival and development of tumor cells. Copper is generally known to be important for embryogenesis and cell reproduction. Copper levels are elevated in cancer patients and tumors compared to healthy controls, and copper levels correlate with tumorigenesis, angiogenesis, tumor metastasis, and recurrence in various human cancers. Thiele and colleagues found that copper transporter-1 (CTR1) inhibited tumor cell proliferation by copper depletion by activating the mitogen-activated protein kinase (MAPK) pathway, which can broadly control proliferation and proliferation, and that the chelating agent tetrathiomolybdate (TTM) inhibited. tumorigenesis. cell proliferation by significantly inhibiting its downstream kinase-mitogen-activated protein kinase kinase 1 (MEK1) signaling. As the demand for copper gradually increases during the abnormal spread of cancer, copper depletion therapy can provide effective anticancer and antimetastatic effects. Cui et al. showed that a mitochondrially targeted copper-depleting nanoparticle inhibited tumor growth and significantly improved survival in triple-negative breast cancer. In addition, the delivery can induce anticancer effects due to the cytotoxicity of copper. Tsvetkov recently proposed for the first time a form of copper-dependent cell death called "coppertosis" in which copper homeostasis depends on the tricarboxylic acid (TCA) cycle during mitochondrial respiration, causing protein-toxic stress and cell death. In tumor cells dominated by mitochondrial respiration, enhancing antitumor therapy by increasing the copper death pathway is also a promising anticancer approach. Copper-based nanoparticles have attracted great interest in tumor therapy in recent decades, and their unique physicochemical properties and remarkable biocompatibility make them suitable for biomedical applications, especially tumor imaging and antitumor therapy. Copper-based nanoparticles have strong infrared absorption and remarkable photothermal properties and have been widely used in photothermal therapy and cancer imaging. In addition, copper-based nanomaterials provide a large specific surface area that can be used to load multiple antitumor drugs.

In addition, they also produce large amounts of reactive oxygen species (ROS) when exposed to light, which could be used in photodynamic therapy. As a common metal, copper offers significant advantages in cancer therapy and transformative potential by exploiting its unique bioactivity, convenient synthetic methods, low reaction conditions and high yields. Therefore, this review focuses on copper-related cell death and the biological applications and potential of copper-based nanomaterials.

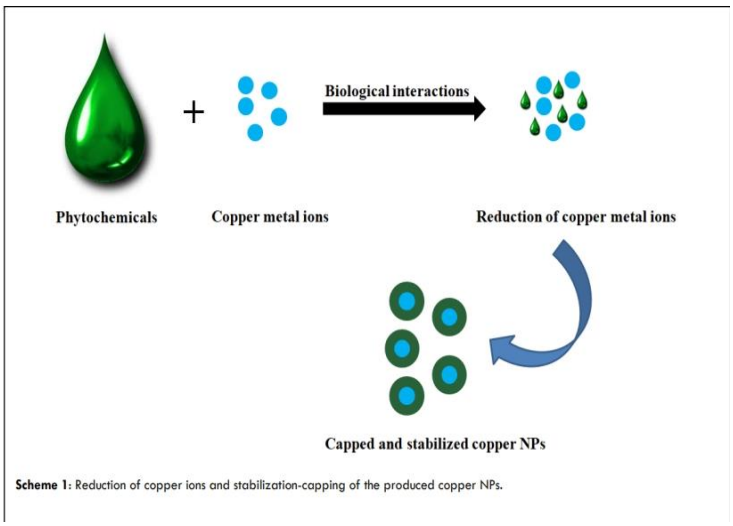
Synthesis and characterization techniques of metallic NPs

1. Physical and chemical synthetic methods of copper NPs

Various physical and chemical methods have been used to synthesize metal NPs, including copper NPs, such as: microwave processes and sol-gel methods, coprecipitation, pulse wire discharge, laser ablation, high energy irradiation, vacuum evaporation deposition, mechanical milling, lithography, electrochemical and photochemical reduction, hydrothermal reaction, microemulsion, electrospray synthesis and chemical reduction.

2. Biosynthetic methods or "green"-synthesized copper NPs

Biosynthesis methods for NPs are based on green chemical methods using a variety of biological systems, including plants, fungi, actinomycetes, yeast, bacteria and viruses. "Green" synthesized copper NPs are produced using phytochemicals to achieve more flexible shapes and sizes of nanopreparations by controlling either the reaction temperature, time, and pH, as well as the concentration and/or metal salt of the plant extract used. The successful reduction of copper ions and the subsequent formation of targeted NPs, which is an immediate phenomenon, is confirmed by the observed color change of the reaction mixture. Phytochemicals also act as stabilizers for the produced NPs, as shown in Scheme 1.



Scheme 1: Reduction of copper ions and stabilization-capping of the produced copper NPs.

Synthesis of CuS nanoparticles

CuS NPs were synthesized according to a previously described method (Zhou et al., 2010). To a 1000 mL aqueous solution of sodium citrate (0.2 g, 0.68 mmol) and CuCl2 (0.1345 g, 1 mmol) was added 1 mL of sodium sulfide solution (Na2S, 1 M) at room temperature under magnetic stirring. Ten minutes later, the mixture was heated to 90 °C and stirred until a dark

green color appeared, and then the mixture was transferred to ice-cold water. Cit-CuS NPs were obtained and stored at 4 °C for further use.

3. Characterization techniques

The most commonly used analytical methods for the determination of copper in aqueous media and/or biological fluids are atomic absorption spectrometry (AAS) with flame detection or a graphite furnace operating on a stabilized temperature platform, inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma atomic emission spectroscopy and X-ray fluorescence spectrometry. One of the main applications of special analytical techniques is the determination of concentrations of important and trace elements in various human fluids and tissues. Elevated serum copper concentrations have been shown to stimulate tumorigenesis, malignant proliferation, and recurrence in several types of human cancer.

A scanning electron microscope (JEM-2010F; Japan) was used to determine the size, microstructure, and morphological characteristics of CuS NPs. X-ray diffractometer analysis was performed using a BT beam diffractometer (Olympus, Tokyo, Japan). UV-vis absorption spectra and diffuse reflectance spectra were recorded on a TP720 UV-vis-NIR spectrophotometer (Olympus, Tokyo, Japan) between 400 and 1000 nm. Fourier transform infrared spectra were measured by KBr pellet methods using an infrared spectrometer (IRPrestige-21; Japan). The concentrations of ions released from the synthesized CuS NPs were determined by inductively coupled plasma atomic emission spectroscopy (Thermo Fisher, New York, USA). The 915 nm solid-state laser (Thorlabs, USA) was externally adjustable (0–2 W). The output power calibration of the lasers was performed using a portable optical power meter (OLP-35, VIAVI, USA). To measure the photothermal property, 100 µL of CuS NPs with different concentrations were irradiated with a 915 nm semiconductor laser device at a power density of 0.5 W cm-2 for 5 minutes. To evaluate the photostability of CuS NPs, the solution was irradiated with a 915 nm laser for 5 minutes, followed by natural cooling without irradiation for 5 minutes. The procedure was repeated five times. The temperature was recorded and photographed simultaneously with a thermal camera (FLIR A300, USA).[61]

Table 1: Determined characteristics and attributes of each morphological and physico-chemical characterization technique [67].

Morphological and physico-chemical characterization techniques	Determined characteristics and attributes
Ultraviolet-visible spectroscopy (UV-vis)	Concentration and shape of NPs
Fourier transform infrared spectroscopy (FTIR)	Nature of bonds and functional groups
X-ray diffraction (XRD)	Size and crystallinity of NPs
Scanning electron microscopy (SEM)	Shape, size and structure of nano-formulations
Field emission scanning electron microscopy (FESEM)	Structural and morphological characteristics
Transmission electron microscopy (TEM)	Shape, size and structure of nano-formulations
Particle size analysis (PSA)	Size distribution of solid or liquid particulate materials
Malvern Zetasizer (MZS)	Size, zeta potential, and protein mobility
Energy-dispersive X-ray spectroscopy (EDX/EDS)	Composition of NPs
Nanoparticle tracking analysis (NTA)	Particle size, concentration, and fluorescent properties
Small-angle X-ray scattering (SAXS)	Shape and size conformation
X-ray reflectometry (XRR)	Thickness, density, and roughness
X-ray fluorescence spectroscopy (XRF)	Chemical composition and concentration
X-ray photoelectron spectroscopy (XPS)	Elemental composition
Brunauer-Emmett-Teller analysis (BET)	Specific surface area
Selected area electron diffraction (SAED)	Shape, size and structure of nano-formulations
Atomic force microscopy (AFM)	Particle size and surface characterization
Atomic absorption spectroscopy (AAS)	Amount of metal present in metallic nano-formulations
Inductively coupled plasma mass spectrometry (ICP-MS)	Amount of metal present in metallic nano-formulations

Anticancer Activity of Copper NPs

1. Anticancer activity of "green" synthesized copper NPs Recent studies on the anticancer activity of "green" synthesized copper NPs from the seaweed Sargassum polycystum using copper sulfate (CuSO4) as a precursor showed that NP concentrations of 100 µg. ml-1 could effectively inhibit the growth of MCF-7 breast cancer cells by more than 93% with an IC50 value of 61.25 µg. ml-1 Studies on the biosynthesis of copper NPs using plants including Nerium oleander and Magnolia Kobus have been previously reported. Results of recent studies on a copper NP biosynthesis method using E. Aqueous soil extract as a reducing agent suggested a promising copper-based nanomaterial with increased 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 cancer cell line HepG2, as assessed by the MTT assay, showed even cytotoxicity values 54.5%.

Prosopis cineraria is a well-known medicinal tree species with excellent analgesic, blood sugar, antipyretic, blood cholesterol, antioxidant and antitumor properties. An investigation of the anticancer potential of Prosopis cineraria leaf extracts showed inhibition of the growth of human HeLa and MCF-7 cancer cells.

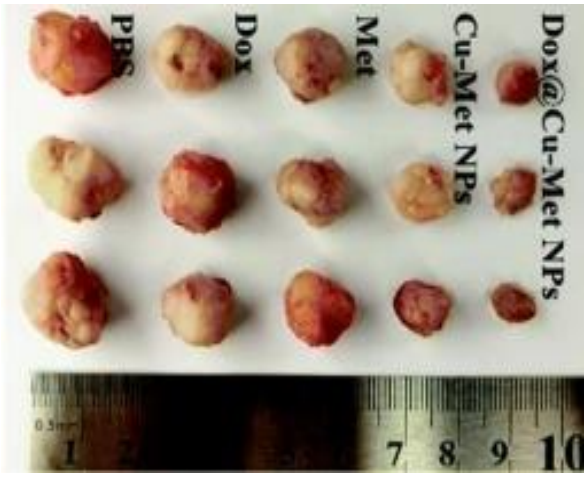
2. Antitumor Bioactivity of Copper Nanomaterials

The essence of oxidative stress (OS) is an imbalance of the oxidant-antioxidant system, which manifests itself as an immediate or chronic increase in the concentration of ROS. Most cancer cells showed increased aerobic glycolysis and high oxidative stress. However, such amounts were less toxic than in normal cells. Specifically, persistently low levels of OS can promote cell proliferation and tumor migration, while high concentrations of OS can trigger cancer cell death. As a therapeutic transition metal in cancer, increased Cu ions exert antitumor effects in cancer tissue, mostly involving OS, triggering the Fenton reaction, which can generate ROS. Cu2+ is sensitive to reduction to Cu+, where Cu causes the Fenton reaction and generates hydroxyl radicals (·OH). In addition, the Cu-based Fenton reaction can react with a higher reaction rate (k = 1.0 × 104 M-1s-1) over a wider pH range compared to other metals (iron, chromium, cobalt, and nickel). The Cu-based catalytic reaction can be represented by Equations (1)–(3).

$$Cu{+}{+}H2O2 \rightarrow Cu2{+}{+}OH{-}{+}OH \quad (1)$$

$$Cu2{+}{+}H2O2 \rightarrow Cu{+}{+}OH{-}{+}OH2. \quad (2)$$

$$Cu2{+}{+}GSH \rightarrow Cu{+}{+}GSSH.$$



Photothermal Therapy (PTT)

Photothermal therapy (PTT) refers to a type of antitumor treatment based on.

Near-infrared (NIR) laser for hyperthermic ablation in response to cancer radiation. It was mechanically propagated in a photothermal substance that absorbs light energy and also converts it into heat energy that comes into contact with the irradiated area, killing the cancer cells.

Exceeding the cytotoxicity threshold (42.5 °C) with high tumor selectivity and is minimally invasive without systemic effects.[97] Li's group developed nuclear spacer-derived PTT based on copper sulfide NPs (CuS NPs) to overcome the clinical challenge of cancer recurrence caused by residual cancer cells after surgery or chemotherapy/radiotherapy.

Therapy CuS NPs had the ability to directly target cancer cells and then induce in the nucleus modifying RGD and TAT peptides, thus heating the cancer cell to complete apoptosis with 980 nm NIR.[98] Chen et al. generated NIR-activated CuS anoplatform (CuS-RNP/DOX@PEI) that directly delivers Cas9 RNP and DOX for synergistic antitumor therapy. This nanoplatform can be absorbed into cancer cells through the endocytic process and NIR-triggered CuS (41 °C) to cleave double-stranded DNA, inducing the accelerated release of Cas9 RNP and DOX. Targeting Cas9 during this process HSP90α reduced the heat tolerance of the cancer, which could therefore improve mild PTT effect. The team of Zhouand #039; developed a Cu-based nanoplatform coated with polyethylene glycol (PEG-[(64)Cu]CuS) for anaplastic thyroid.

carcinoma PEG-[(64)Cu]CuS-mediated combination can inhibit tumor growth RT/PTT, which can significantly prolong the survival of compared tumor-bearing mice with RT alone.[100] Yan et al. developed a PTT synergized immunotherapy strategy based on CuS-RNP@PEI with an engineered Cas9 targeting PTPN2 that could not only triggers NIR-mediated PTT but also stimulates CD8 T-cell accumulation.

Upregulation of IFN-γ and TNF-α.[101] In addition, copper-based NPs can synergize the PDT effect. Zhang et al. developed transferrin-labeled Cu@Gd2O3, which triggered the PTT effect under 808 nm laser irradiation, increased mock peroxidase activity and promoted intracellular ROS accumulation by PDT, which significantly inhibits the growth of cancer. In addition, it also showed an improved MRI contrast, which could obtain more pathological

information.[102] Metal-organic frameworks (MOFs) have recently attracted considerable attention due to their large specific surface areas and tunable porosity.[103] Weng et al. prepared the Cu-BTC metal-organic framework Cu@CPP-800 with the highest photothermal conversion efficiency (48.5%) in 808 nm laser irradiation three times higher than indocyanine green (15.1%).[104] The above-mentioned studies have shown that combination therapy can increase the anticancer effect compared to monotherapy. Therapeutic outcomes of chemotherapy not usually due to tumor heterogeneity and dose-limited anticancer activity. Combination with another treatment model can effectively kill cancer cells through different mechanisms of the activity. Hyperthermia released by PTT can not only kill cancer cells, but also help releases heat-sensitive drugs and improves absorption of cancer drugs into cells.

Nanoparticles can convert incident light into heat and increase the temperature of cancer cells, eventually killing them. Anticancer drugs administered in bloodstreams tumor containing nanoparticles usually appear very bright in CT, MRI, ultrasound image helping surgeon treat the right area. Once the tumor is located, much more intense beam of light or magnetic field can be applied. The energy absorbed by the nanoparticles heat up and kill cancer cells. Copper-based nanoparticles (NPs) are used in photothermal therapy (PTT) because they have strong near-infrared absorption and significant photothermal capabilities. They are also used in cancer photoimaging.

Copper-based NPs are used in PTT because they have:

1. Effective near-infrared absorption
2. Significant photothermal capabilities.
3. Unique optical properties
4. Small size
5. Low cost of production
6. Low cytotoxicity

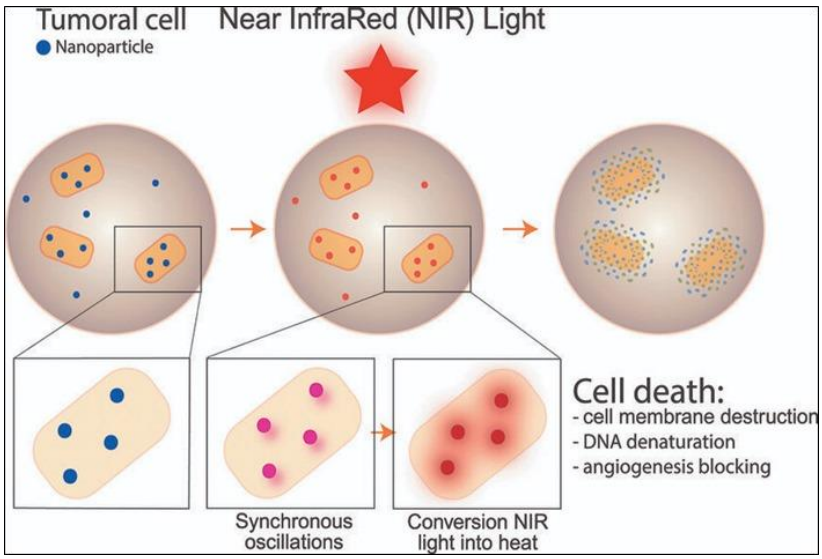
- **Cu NPs are used in photothermal therapy for the following reasons**
- They can effectively convert NIR laser irradiation into localized heat.
- They have a high NIR absorption capacity.
- They interact with surrounding oxygen molecules during NIR irradiation, leading to an elevated energy level and the formation of 1O2 through oxygen energy transfer.
- They can be used to eliminate cancer cells.

- They can be used to elevate the temperature of aqueous solutions of CuS nanoparticles as a function of exposure time and nanoparticle concentration.
- They show a dose-dependent photothermal property.

Some copper-based NPs used in PTT include CuS nanoparticles: These are effective near-infrared absorption agents.

Copper selenide nanocrystals: These have a higher photothermal transduction efficiency than gold nanorods and nanoshells.

Copper-palladium alloy tetrapod nanoparticles: These were used in a 15-day treatment regimen.



CONCLUSION

NPs can act like a conventional anticancer drug, reducing both the side effects and the required dose. Despite increased research on the anticancer potential of metal NPs, limitations due to the heterogeneity of cells used in each tumor environment remain, preventing comparative studies. Another limitation concerns the formation of a protein corona after interactions of NPs with blood and plasma proteins, which affects in vivo clearance and distribution. New researches allow the development of new types of metal NPs, such as copper NP, with enhanced and selective anticancer activity, better biocompatibility and biodistribution, and low toxicity to normal tissues.

In this review, we have comprehensively discussed the anticancer therapeutic applications of copper and copper-derived NPs (PTTs) and the development of approaches to selectively target and mitigate potential toxicity, characterization techniques and synthesis of copper nanoparticles.

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