

**Review Article**

Copper Oxide Nanoparticles: Reactive Oxygen Species Generation and Biomedical Applications

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Abstract: Copper oxide is a p-type semiconductor which has many applications in a different field. Copper oxide has excellent applications as an antioxidant, antibacterial, and antitumor or anticancer. Copper oxide nanoparticle combines with the cell membrane and enters into a cell; generate reactive oxygen specie (ROS), which causes oxidative stress in the cell. Oxidative stress leads to metastasis, cancer proliferation, apoptosis, DNA damage, cytotoxicity, and unregulated cell signaling. Hydroxyl free radical generated by Nanoparticles, combined with DNA and yield 8-hydroxyl-2-deoxyguanosine (8-OHdG), resultantly DNA is damaged. CuO nanoparticle shows antibacterial activity on different bacterial strains such as staphylococcus aureus, bacillus circulens BP2, Escherichia coli, and P. aeruginosa. Recently, CuO nanoparticles have applications in the detection of Cholesterol, lactate biosensor, DNA sequencing of microbe, and anti-HIV drug analysis. There is specialized CuO nanoparticle such as Glucose sensor, Hydrogen peroxide sensor, Immunosensor, Dopamine sensor for the detection of the different biomolecule. ROS generated by CuO nanoparticle causes toxicity, which leads to cell death. There is a fascinating area of research against tumors by nanoparticle use because of its antitumor nature. Metal nanoparticle exhibits anticancer activity due to physicochemical properties as antioxidant action or use of external stimuli. Free radical which are produced by the metal nanoparticle, kill cancer cells.

Keywords: Copper Oxide Nanoparticle, Reactive Oxygen Specie (ROS), Sensors, Cancer Therapy, Biomedical Applications, Cytotoxicity and Toxicity

1. Introduction

Nanotechnology has several applications in biosensors, cosmetics, imaging and diagnosis, and target drug delivery. Nanoparticles have excellent physiological properties due to which they got great attraction [1]. As the size of metal reduced from bulk to nanometer, properties like active surface area, hardness, electrical conductivities, biological activities, and chemical reactivity, are also changed. Due to the high surface to volume ratio of metal nanoparticle, they have significant applications in antibacterial activities. Metal nanoparticles can also be coated onto the polymer surface or

combined with a polymer to achieve antimicrobial applications [2]. Copper oxide is in semiconductor nature and possesses a monoclinic structure. Copper oxide has wide

application due to physical properties [3]. It is a p-type semiconductor, so used in many applications like field emission emitters, high-temperature superconductors, catalysis, sensors, and batteries [4]. In recent times, CuO nanoparticle was observed for microbial activity and reach to the respective organ by crossing the biological membrane [5]. There has been a utility of copper for many years as a

fungicidal agent. Free form or complex form of copper has germicidal activity. In recent Studies, Copper nanoparticles are used in the paint industry as the antifouling coating. Copper has also been used as a copper nanocomposite to control fungi [6]. There is a fascinating area of research against tumors by nanoparticle use because of its antitumor nature [59]. Metal nanoparticle exhibits anticancer activity due to physicochemical properties as antioxidant action or use of external stimuli. Free radical which are produced by the metal nanoparticle, kill cancer cells and also reduce tumor environment. Metal nanoparticles reduced tumor development due to their antioxidant abilities [7].

Active or Passive processes can employ against cancer. There are merits of the passive process like Retention effect and permeability. The nanoparticle can quickly pass into the cancerous cell and kill them [17]. In the Active process, the nanoparticle is synthesized to target the cancerous cell. A ligand used as a receptor to the nanoparticle to specify target cancerous cells [8]. Apart from biomedical applications, CuO nanoparticles are toxic for animal cells, including mammalian cells, due to more production of reactive oxygen species (ROS). Thus oxidative stress is induced and increases the toxic effect, which leads to damage of mitochondria and DNA [5, 9, 11].

In this review article, our aim to represent CuO nanoparticle's mode of action i.e generation of Reactive oxygen species (ROS) and biomedical applications along with its toxicity toward environment and animals, including humans.

2. Reactive Oxygen Specie (ROS)

It is a natural byproduct resulting from cellular oxidative metabolism and a vital role in cell homeostasis [12]. ROS is produced by intracellular organelles like mitochondria, endoplasmic reticulum, etc. as shown in figure 2 [21, 22]. By contrast, extracellular ROS are produced by ROS-inducing agents like nanoparticle, radiation, and pollutants when exposed to cell [23] Figure 1. It includes free radical and non-radical such as superoxide and hydrogen peroxide, respectively [13]. It is oxygen free radical and short-lived specie. It is converted into hydrogen peroxide with the help of superoxide dismutase (SODs) [14]. Superoxide is produced by incomplete electron reduction of oxygen and then converted into hydrogen peroxide by SOD, which acts as an antioxidant in cells for oxygen exposure. ROS are intermediate in cellular levels, which are controlled by several enzymes like SOD, Catalase (CAT), and Glutathione (GPS) or by several antioxidants like Glutathione, Vitamin E, ascorbic acids and flavonoids [9]. It imbalances between ROS generation and its neutralization, which leads to harmful effects on cell signaling mechanisms or oxidative harm for biomolecules like nucleic acid, lipid, and protein [15]. ROS generation promotes signaling molecule activation that leads to cell death [16-19].

2.1. Mechanism of ROS

CuO nanoparticles act as a catalyst and enhance ROS

formation through Fenton reactions or Haber- Weiss reaction, which produces hydroxyl radical [20, 21]. While hydroxyl free radical is formed by the reaction of oxidized metal ion and hydrogen peroxide in Haber-Weiss reaction [22, 23]. Nanoparticle interacts with the membrane and invades the cell. They are ultimately transported into the cell by endocytosis. Nanoparticle starts intracellular ROS generation by catalysis of free radical reaction in mitochondria. The mitochondrial membrane is depolarized and NADPH enzyme activation due to interference in the electron transport chain by nanoparticles [24]. CuO nanoparticles block the electron transport chain and increase oxygen-free radicals and lead to oxidative stress in the cell [29].

Moreover, in the ROS-dependent mechanism, immune cells are activated by nanoparticle exposure via NADPH oxidase (NOX) activation [25]. Free radicals produced by nanoparticles reduced the Glutathione (GSH) into Glutathione disulfide, leading to oxidative stress in cells [26, 27]. Cell experience showed antioxidant defense when exposed to a low level of CuO nanoparticle, and oxidative stress is overcome. In contrast, the antioxidant system is overwhelmed by exposure to a high level of CuO nanoparticle, which leads to inflammation and cytotoxicity. Oxidative stress leads to metastasis, cancer proliferation, apoptosis, DNA damage, cytotoxicity, and unregulated cell signaling [28-30].

2.2. Cytotoxicity and DNA Damage

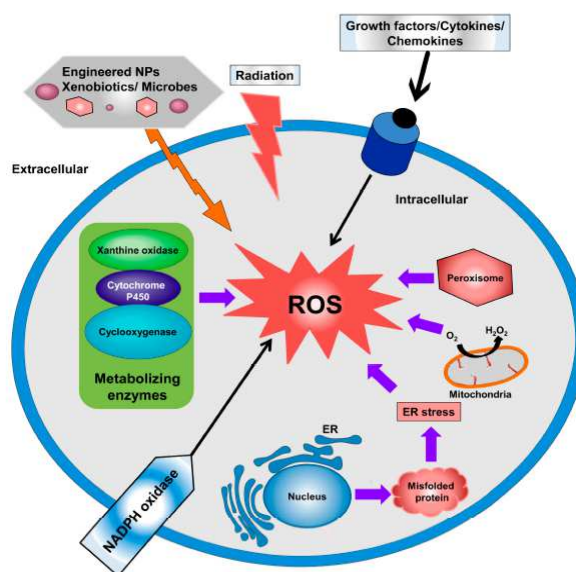


Figure 1. Reactive oxygen species (ROS) generation [35].

Hydroxyl free radical generated by nanoparticles, combined with DNA and yield 8-hydroxyl-2-deoxyguanosine (8-OHdG), resultantly DNA is damaged [31]. Polyunsaturated fatty acids also oxidize, and lipid peroxide is produced at the start of ROS generation. Nanoparticle genotoxicity caused mutation by lipid peroxidation [32, 33]. Nanoparticle induced ROS cause toxicity in several biological systems like tumor cell, human erythrocytes, and skin fibroblasts [34].

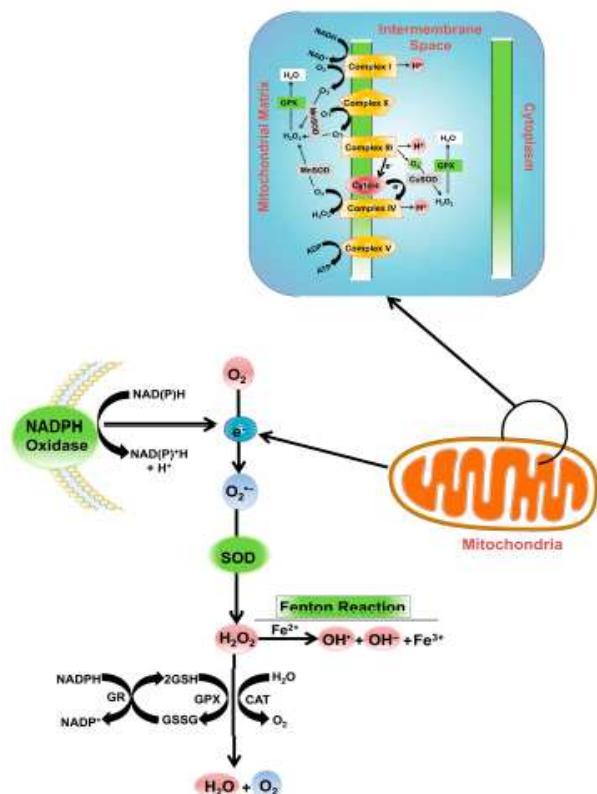


Figure 2. ROS generation by mitochondria and NADPH oxidase [13].

3. Biomedical Application of CuO

3.1. Antibacterial Activity

CuO Antibacterial activity is investigated on different bacterial strains such as staphylococcus aureus, bacillus circulens BP2, Escherichia coli, and P. aeruginosa. The growth curve of bacterial culture was measured in antibacterial activity test in the presence of CuO nanoparticles. Spectrophotometer (600nm) was used to measure the optical density (OD) of bacterial culture [60]. The result showed prominent bacterial growth inhibition in culture concerning control [18]. It is supposed that bacterial growth is inhibited/killed by the use of CuO nanoparticles, although a high concentration of copper oxide nanoparticles shows more bactericidal effect [36]. Copper shows antibacterial activity because it damages protein, nucleic acid, and cell membranes, respectively [37, 38]. Copper attach to guanine of DNA molecule activation of oxidative stress occur, which leads to dislodging of DNA strands and resulting from 8-hydroxy-2-deoxyguanosine [39]. It is observed that copper has biocidal activity because it is effective against bacteria and viruses such as staphylococcus aureus strain, influenza viruses, bronchitis, human immunodeficiency virus, and bacteriophage [40, 41]. Reactive oxygen species produced by CuO nanoparticle penetrate cell by interacting with cell membranes, which lead to disruption of cell enzyme [42].

3.2. Sensors for Biomolecules Detection

Ultrafine mono-dispersed CuO nanoparticle deposited on

Indium tin oxide glass substrate electrophoretically, used as a sensor for the detection of cholesterol [43]. CuO-graphene nanospheres used as a cholesterol sensor. CuO nanoparticle consisting of copper oxide-CeO₂ used as a lactate biosensor [44]. Glassy carbon electrode having CuO nanoparticle with single-walled carbon nanotube, used in DNA sequence detection of bacteria and virus for identification of disease [45, 46]. CuO nanoparticles used for the assay of anti-HIV drugs [47].

3.3. Sensor for Glucose Detection

CuO nanoparticles are used in a glassy carbon electrode to detect glucose in the basic medium [48]. CuO/graphene nanocomposite modified glassy carbon electrode highly selective and sensitive for the detection of glucose [49].

3.4. Sensor for Hydrogen Peroxide Detection

Hydrogen peroxide is an intermediate in the biological system. So its detection is essential in hypoxic conditions and oxidative stress in all tissue and cell [50]. CuO nanoparticles can oxidize many chemical compounds. Hydrogen peroxide determined by the nanostructure of CuO [51]. CuO graphene nanocomposite can be used to monitor hydrogen peroxide in vivo methods.

3.5. Immunosensor for Immune Complex

Copper nanostructure such as CeO₂-CuO [52], Ag-CuO [53], platinum CuO [54], Palladium CuO [51], ferrocene CuO [55] used as tumor biomarker like alpha-fetoprotein [56, 57]. It has noted that CuO nanoparticles give synergetic effects and accelerate signal transduction.

3.6. Sensors for Dopamine Detection

Dopamine is a neurotransmitter that is an integral part of the central nervous system. It causes low-level neurological disorders like senile dementia, epilepsy, Parkinson's Huntington's schizophrenia, and Alzheimer's disease [58-61].

Modified glassy carbon electrode CuO nanoparticle is used for dopamine sensors [62]. CuO/CNTs/Nafion composite used to enhance dopamine oxidation along with better peak current signals [63].

3.7. Cancer Nano Medicine

CuO nanoparticles selectively induce apoptosis in tumor cells and inhibit growth, which leads to metastasis of melanoma [64, 65]. CuO nanoparticle used against tumor of prostate, eye [66], breast [67], liver [68], brain [69], kidney [70] and lungs [71]. Upon entering of nanoparticle into cell or nucleus, it causes mitochondrial localization, DNA damage, mutation, and alteration of gene expression. CuO nanoparticle targets mitochondria and starts apoptosis due to oxidative stress in cells [70].

4. Toxic Effects

CuO nanoparticles have many applications in-vitro and

in-vivo experiments. CuO nanoparticles pose toxicity due to small size and positive surface charge, which facilitate interactions between nanoparticles and cells. CuO nanoparticles pose a more toxic effect on algae and protozoa *Tetrahymena Thermophila* as compared to bulk form [4]. A study revealed that CuO nanoparticles enhance oxidative DNA damage, mitochondrial, and DNA damage [72]. CuO nanoparticles showed a more toxic effect on human skin organ culture and lungs culture cells [73]. A study reports that hemolysis increase when cell membrane is damaged by CuO nanoparticles [10]. A study reports that hemolysis increase when the cell membrane is damaged by CuO nanoparticles [10]. It has stated that CuO nanoparticles affect human hepatoma (HEPG2) cells, which stop melanoma cell growth [74]. CuO nanoparticles pose neuronal toxicity by the loss of neurons membrane. CuO nanoparticles induce cell death in lymphocyte and hence weaken the human immune system [75].

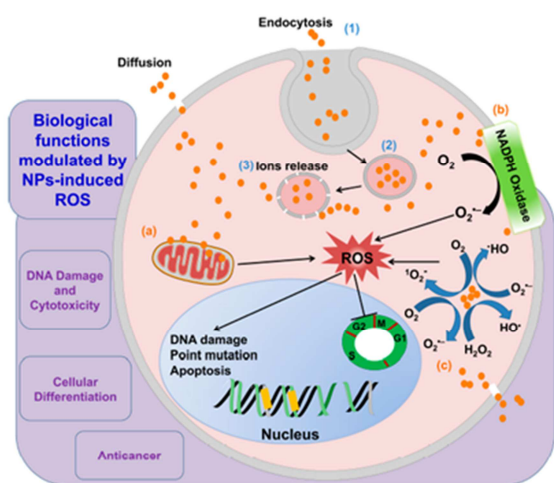


Figure 3. ROS generation and its consequence, including DNA damage, cell cycle arrest, alteration in apoptosis, and damage to the cell membrane [23].

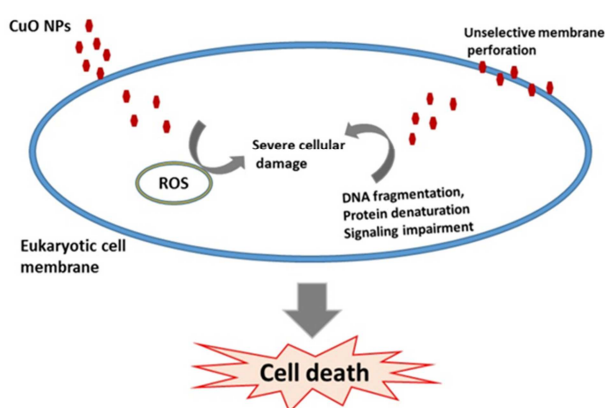


Figure 4. CuO nanoparticles Toxicity mechanism [76].

5. Conclusions

CuO nanoparticles possess fascinating physiological properties that can be used to get desirable properties. CuO is an emerging horizon for in-vitro and in-vivo therapy and application in the biomedical field.

CuO nanoparticle is an excellent antibacterial, antifungal, and antioxidant material which control microbes effectively because CuO penetrates membranes due to its high surface area. CuO nanoparticles are used in food packaging because they inhibit bacterial growth. Cu nanocomposites have many applications in biomedical detection due to their excellent chemical and thermal stability and electrical conductivity. CuO acts as a drug carrier in nanomedicine. Nanomaterial-based drugs have been developed to selectively kill cancerous cells in target cancer therapy in the field of nanomedicine. Modified CuO nanocrystals with specific ligands interact with specific receptors on the tumor cell. CuO nanoparticles can be used to detect many diseases like cardiac syndrome, neurological disorder, tumors, stress, and diabetes. CuO acts as a biosensor for pollutants and metabolites. CuO nanoparticles can be used to sense different compounds such as the detection of cholesterol, lactate biosensor, DNA sequencing of microbes, and analysis of the anti-HIV drug. It was found that excess quantity of CuO nanoparticles promotes toxicity to humans, other living beings, and the environment as well.

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