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Selma Nacak, declare that I am the sole author of this review article titled "Toxicity Assessments of Carbon-Based Nanomaterials: A mini review." I confirm that I have contributed to all aspects of the manuscript, including the conceptualization, data collection, analysis, and writing of the article. Additionally, I assure that this manuscript is an original work and has not been previously published, nor is it under consideration for publication elsewhere.

Toxicity Assessments of Carbon-Based Nanomaterials: A mini review

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Abstract
Carbon-based nanomaterials (CNMs) are materials with exceptional properties that play an important role in the development of new technologies. Their widespread use, however, has raised concerns about their possible harmful effects on the environment and human health. Safe use of CNMs can be possible by performing toxicity tests and determining an attitude based on the test results. To date, researchers have conducted toxicology tests with carbon-based nanomaterials such as carbon nanotubes, fullerene, graphene, carbon dot and nanodiamond. According to the results of the researches, it has been revealed that these materials can cause toxic effects such as DNA damage, inflammation, protein stress and oxidative stress, depending on factors such as concentration, surface charge and material size. There are different types of toxicity tests currently used. However, a uniform international protocol is still a requirement. This study will present various research on the toxic effects of CNMs and provide an overarching perspective.

Keywords: Carbon-based nanomaterial, Toxicity, Biomedical

INTRODUCTION
In recent years, research on carbon-based nanomaterials has been increasing exponentially. These nanomaterials offer a wide range of application due to their large surface areas, exceptional optical properties, high electrical and thermal conductivities, and outstanding mechanical properties. These properties enable the successful use of carbon-based nanomaterials in solar energy systems, flexible electronics production, molecular recognition applications, as well as in areas such as bio-imaging, biosensing, super-resolution imaging and nanoscale temperature sensing.(1)

Carbon, with an atomic number of six, has an average atomic mass of 12 amu (2). As one of the most abundant elements on Earth, carbon is a key component in many macromolecules vital for life, including sugars, proteins, and DNA (3). Pure carbon exists in several forms, such as allotropes including diamonds and graphite, which come from variations in the arrangement of carbon atoms (2)(3). Amorphous allotropes of carbon include coal, lampblack, and charcoal (3)(4). CNMs encompass a variety of carbon forms as shown in Figure 1. These include sp^2 carbon nanomaterials (like graphene, carbon nanotubes, fullerene), amorphous carbon nanoparticles (like carbon dots, ultrafine carbon particles and carbon nanoparticles), and nanodiamonds (3).

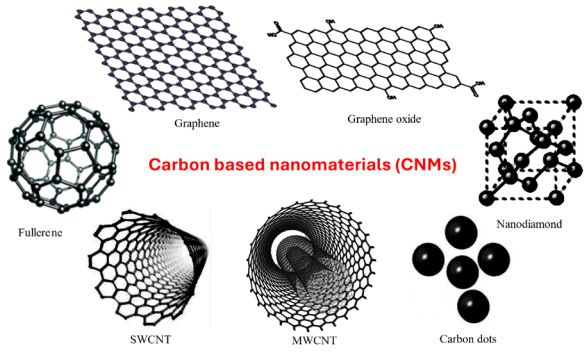

Figure 1. Structures of various types of carbon-based nanomaterials.

Carbon nanotubes (CNTs) possess cylindrical tubular structures with a nanometer diameter, formed by rolling graphene sheets (5). These are classified into two basic types. One of them is single-walled carbon nanotubes (SWCNTs) and the other is multi-walled carbon nanotubes (MWCNTs). SWCNTs are formed from a single layer of a graphene sheet, whereas MWCNTs comprise several concentric layers of graphene (6).

Fullerene is named after architect Buckminster Fuller, who in the 1960s constructed a cagelike lightweight dome made of carbon atoms. These molecules consist only of carbon atoms arranged in various shapes like hollow, tube, sphere, or ellipsoid, in which carbon atoms interconnect in pentagonal and hexagonal rings (2).

Graphene, the main structure of graphite, is one of the most researched CNMs (7)(8)(9). This material consists of two-dimensional, single, or few sheets of sp^2 arranged carbon atoms (7). Graphene serves as the structural precursor to various carbon allotropes including carbon nanorings, carbon nanotubes, carbon fibers, graphite, and graphyne (8)(9).

Carbon dots (CDs) are carbon nanoparticles, which are found in spherical-like shape and in a size less than 10 nm. CDs show tunable and efficient photoluminescence properties. Furthermore, they are cost-effective and environmentally-friendly type of nanomaterials (10).

Diamond is a metastable allotrope of carbon with an unstable face-centered cubic crystal structure. It is known for its exceptional hardness and thermal conductivity. Nanodiamonds (ND) were first made in 1963 by detonating an oxygen-deficient trinitrotoluene and hexogen composition. They consist of a diamond core covered an amorphous carbon shell. The average size of NDs is 4-5 nm, which allows for their existence in colloidal suspensions (2)(11)(12).

BIOMEDICAL APPLICATION OF CARBON-BASED NANOMATERIALS
Biosensors identify disease biomarkers, enabling diagnosis and monitoring. Biomarkers are key molecules like proteins, hormones, glucose, and others, found in body (3). CNMs are widely employed in biosensing due to their conductivity, catalytic activity, and biocompatibility. Various carbon-based nanomaterials, including CNTs, graphene oxide (GO), and fullerene, are utilized for optical and electrochemical biosensor development (13).

The rising prevalence of cancer worldwide imposes significant

emotional, physical and financial burdens on individuals and families. Therefore, it's crucial to develop new technologies that effectively treat cancer (14). Barahuie et. al. synthesized GO to investigate its potential use as a nanocarrier for chlorogenic acid (CA) known as one of the active anticancer agents. The study confirmed the successful conjugation of CA onto GO through π–π interaction and hydrogen bonding. The CA loading in the nanohybrid was around 13.1%. The release profiles exhibited favorable, sustained, and pH-dependent release of CA from the CA-GO nanocomposite. This aligned well with the pseudo-second order kinetic model. Additionally, the designed anticancer nanohybrid proved to be thermally more stable than its counterpart (15). Recent research has highlighted the potential of quasi-freestanding bilayer epitaxial graphene for detecting SARS-CoV-2 in body fluids or exhaled breath, offering rapid, cost-effective, and efficient alternatives to conventional detection methods (16). Gene therapy holds significant promise as a therapeutic approach for treating a wide range of diseases. Wu et. al. have synthesized a new multifunctional theranostic folate conjugated-reducible polyethyleneimine-carbon nanodots/small interference RNA (fc-rPEI-Cdots/siRNA) nanoagent. The fc-rPEI-Cdots act as a siRNA carrier, releasing siRNA in a reducing environment, with enhanced accumulation in lung cancer cells. Viability of H460 treated with the fc-rPEI-Cdots/ pooled siRNA complex for three days is reduced to nearly 30%. Furthermore, clear inhibition of cyclin B1 and epidermal growth factor receptor (EGPR) expression was determined. Hence, this novel nanoagent has potential for targeted lung cancer treatment (17). Monitoring cholesterol levels is clinically significant, and both enzymatic and nonenzymatic methods are employed for this purpose. Multiwalled carbon nanoparticle electrodes in a metal-carbon-polymer nanocomposite functionalized with cholesterol oxidase enzymes were utilized as an enzymatic method with good selectivity, sensitivity and reproducibility (18). Glucose monitoring is integral in diabetes diagnosis and management. CNMs, including nanotubes, graphene, and graphene dots, modified with glucose oxidase exhibit high sensitivity and selectivity in glucose detection. These nanosensors have been evaluated for interference from substances like acetaminophen, uric acid, and ascorbic acid (19).

TOXICITY ASSESSMENTS

CNMs have gained significant importance in various fields, including biomedicine, due to their unique properties such as high conductivity, structural diversity, and ease of functionalization. However, the increasing use of CNMs has also raised concerns about their potential toxicity and impact on human health and the environment. The toxicity assessment of CNMs is crucial for their safe application in biomedicine. Key findings from toxicity studies suggest that the toxicity of CNMs depends on their physicochemical properties like size, shape, surface area, and metal impurities (20)(21). The most common methods used to assess the toxicity of carbon-based nanomaterials in biomedicine include in-vitro cell culture assays, physicochemical characterization, flow cytometry, comprehensive toxicological studies (20) (21)(22) (23) (24).

Garriga et. al. studied the in-vitro toxicity of carbon nanotubes (CNT), graphene oxide (GO), carbon nanoplatelets (CNP), carbon nanohorns (CNH), nanodiamonds (ND) and reduced graphene oxide (RGO) on human breast adenocarcinoma (MCF-7) cells and human epithelial colorectal adenocarcinoma (Caco-2) cells, after 24 h and 72 h incubation. After the CNMs treatment, the cell viability shown by toxicity assessments is in the order: CNP < CNH < RGO < CNT < GO < ND. The fast-dividing Caco-2 cells were more effected from the CNMs treatment. The lowest toxicity was exhibited by ND and GO because of the functional groups with oxygen on the surface of nanomaterials. Researchers of the study emphasized that the long-term toxicity assessments remain an important requirement (23).

When MWCNTs are inhaled, alveolar macrophages and pulmonary alveolar epithelium are activated. This may result in a pro-inflammatory response or even chronic pathology. Sweeney et. al investigated the bioreactivity of MWCNT length by utilizing primary human alveolar type-II epithelial cells (ATII) and alveolar macrophages (AMs) as well as a human alveolar type-I-like epithelial cell line (TT1) to find the role that the length of MWCNTs plays in pulmonary toxicity. Bioreactivity caused by MWCNTs of different lengths (MWCNT 0.6 µm, MWCNT-3 µm and MWCNT-20 µm) resulted in negative effects. TT1 and ATII epithelial cells exhibited higher reactivity when exposed to shorter MWCNTs. This phenomenon was observed even at very low concentrations. Long MWCNTs exhibited high reactivity with alveolar macrophages. It also caused a high rate of cell death. For this reason, it has been reported that inhalation of MWCNTs will cause serious health problems (25).

Montes-Fonseca et. al studied the cytotoxicity of functionalized carbon nanotubes dependent on the functionalization grade. They functionalized CNTs with different concentration of 46 kDa surface protein, P46, (6 mg/L, 0.6 mg/L, 0,006 mg/L). Then they investigated toxic effect CNTs with various functionalization grade on J774A macrophages. The study revealed that CNTs functionalized with high concentration of P46 were more toxic to J774 macrophages than CNTs functionalized with low concentration of P46 (26).

Hiraku et al exposed RAW 264.7 macrophages and A549 lung epithelial cells to carbon black (CB) with primary diameters of 56 nm (CB56) and 95 nm (CB95). They comparatively investigated whether these nanomaterials could form 8-nitroguanine on DNA. Both nanomaterials induced the formation of 8-nitroG in the nucleus of the cells examined. Flow cytometry showed that CBs with a diameter of 95 nm generated higher amounts of reactive oxygen species in RAW 264.7 cells and caused more 8-nitroguanine formation than CBs with a diameter of 56 nm. As a result of the research, it was revealed that DNA damage may occur in lung epithelial cells exposed to CBs and that these CNMs may contribute to carcinogenesis (27).

Jiang at. al. revealed in their article published in 2020 the results of the study on the toxic effects of 6 SWCNT samples with different lengths, functional groups and electronic structures. Quantitative toxicogenomic assay endpoint protein expression level index (PELI) examination revealed that short SWCNTs (0.5–2 µm) caused a higher toxicity and

oxidative stress than long SWCNT (5–30 µm). Carboxylated SWCNTs caused higher genotoxicity, protein damage, chemical stress, and overall toxicity than hydroxylated SWCNTs. While semiconductor SWCNTs exhibited almost no toxicity, metallic SWCNTs showed more toxic behavior. In conclusion, these materials exhibited molecular toxicity dependent on their physicochemical properties (28).

Adamson et. al. investigated cellular uptake, cell viability, mitochondrial membrane potential, and macrophage responses in graphene nanoplates-exposed mice. Different exposure times (1, 3 and 6 hours) and different graphene nanoplate (GNP) concentrations (0, 25, 50 or 100 µg/ml) were used in the study. They also evaluated the effect of CD36 on responses to GNPs. This study revealed that GNPs increased mitochondrial potential and were easily internalized by macrophages. However, by blocking CD36 using an antibody, internalization of GNPs by macrophages was reduced. The study revealed that exposure time and GNP concentration affected macrophage responses in different ways. Additionally, data explaining the metabolic pathways disrupted due to exposure and the role of CD36 in GNP-macrophage interaction were obtained (29).

CONCLUSION

Carbon-based nanomaterials and their hybrid nanocomposites exhibit excellent properties, making them useful across various fields. New products containing carbon-based nanomaterials emerge every year. Therefore, the society is increasingly interested in their reliability. Scientists use various in vivo and in vitro methods to investigate the toxic effects of carbon-based nanomaterials and try to reveal their toxic effects related to their various properties. However, these studies lack of a standard methodology which leads to confusion the scientific community. Toxicity tests developed in accordance with internationally accepted proficiency standards should be used as soon as possible. These tests should consider factors like the physicochemical properties of the CNMs, environmental interferences, nano-bio interactions, and the type and concentration of the solution for easy evaluation.

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