Genotoxicity of Graphene Family Nanomaterials on DNA

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Graphene family nanomaterials (GFNs), including graphene, graphene oxide (GO), reduced graphene oxide (rGO), and graphene quantum dots (GQDs), have manifold potential applications, leading to the possibility of their release into environments and the exposure to humans and other organisms. However, the genotoxicity of GFNs on DNA remains largely unknown. In this review, we highlight the interactions between DNA and GFNs and summarize the mechanisms of genotoxicity induced by GFNs.

graphene family nanomaterials genotoxicity DNA damage safety toxicity

1. Introduction

Currently, GFNs, as promising nanomaterials, have attracted increasing attention in the scientific community and are in commercial production for many applications, such as energy storage [1][2][3][4][5][6][7][8], medicine [9][10][11][12][13][14][15][16], environmental protection [17][18][19][20][21][22], and industrial manufacturing [23][24][25]. For example, the market for graphene-based products is forecast to reach $675 million by 2020 [26]. With rapid developments in application and production of GFNs, their potential for release into the environment and the environmental risks of GFNs have become emerging issues [27][28][29]. Consequently, many studies have shown that adverse effects can be induced by GFNs in vivo and in vitro, such as organ (e.g., lung, liver, and spleen) toxicity, cytotoxicity, immunotoxicity, neurotoxicity, and reproductive and developmental toxicity [30][31]. Moreover, the toxicity mechanisms of GFNs to organisms, including physical destruction, oxidative stress, inflammatory response, apoptosis, autophagy, and necrosis, are summarized in **Table 1** . However, the genotoxicity of GFNs on DNA (e.g., DNA damage) remains largely unknown.

Table 1. The toxicity of GFNs in vivo and in vitro.

Products	Supplier or Synthesis Methods	Dose	Animal or Cell Models	Toxicological Mechanisms	Adverse Effects	Ref.
graphene nanoplatelets	cheaptubes.com (Brattleboro, VT, USA)	0.3, 1 mg/rat	rat	oxidative stress, inflammation	lung inflammation	[32]
commercial GO and rGO	Nanjing XFNANO Materials Tech Co., Ltd., (China)	2.0 mg/kg	rat	transcriptional and epigenetic	liver zonated accumulation	[33]

Products	Supplier or Synthesis Methods	Dose	Animal or Cell Models	Toxicological Mechanisms	Adverse Effects	Ref.
		body weight				
amination GQDs carboxylated GQDs hydroxylated GQDs	Nanjing XFNANO Materials Tech Co., Ltd., (China)	100, 200 μg/mL	A549 cells	autophagy	cytotoxicity	[34]
GO and rGO oxidated from carbon nanofibers	Grupo Antolin (Spain)	0.1, 1.0, 10, 50 mg/L	erythrocyte cell	oxidative stress	genotoxicity	[35]
GO nanosheets	Sigma-Aldrich (St. Louis, MO, USA)	40, 60, 80 mg/L	Human SH-SY5Y neuroblastoma cell	oxidative stress, autophagy–lysosomal network dysfunction	cytotoxicity	[36]
pristine rGO	Chengdu Organic Chemicals Co., Ltd., the Chinese Academy of Sciences	1–100 mg/L	Earthworm coelomocytes	oxidative stress	immunotoxicity	[37]
single layer GO (product no. GNOP10A5)	ACS Materials LLC (Medford, MA, USA)	1, 10, 50, 150, 250, 500 mg/L	*Escherichia coli*	physical destruction	toxicity against bacteria	[38]
GO	modified Hummers method	25 mg/L	THP-1 and BEAS-2B cells	lipid peroxidation, membrane adsorption, membrane damage	cytotoxicity	[39]
GO	modified Hummers method	2 mg/kg	rat	lipid peroxidation, membrane adsorption, membrane damage	acute lung inflammation	[39]

Products	Supplier or Synthesis Methods	Dose	Animal or Cell Models	Toxicological Mechanisms	Adverse Effects	Ref.
GO	Nanjing XFNANO Materials Tech Co., Ltd., (China)	0–100 mg/L	zebrafish embryos	oxidative stress	developmental toxicity	[40]
GO	modified Hummers method	10 mg/L	*Caenorhabditis elegans*	oxidative stress	toxicity	[41]
graphene, GO	modified Hummers method	3.125–200 mg/L	human erythrocytes and skin fibroblasts	oxidative stress	cytotoxicity	[42]
graphene exfoliated form graphite, GO oxidated from carbon fibers	Grupo Antolin Ingeniería (Burgos, Spain)	1, 10 mg/L	primary neurons	inhibition of synaptic transmission, altered calcium homeostasis	neurotoxicity	[43]

Genotoxicity is broadly defined as ‘damage to the genome’ and also a distinct and important type of toxicity, as specific genotoxic events are considered hallmarks of cancer [44]. Generally, the genotoxicity can be sub-classified into direct genotoxicity and indirect genotoxicity in cells or the nucleus [45][46][47]. Nanoparticles (NPs) can be uptaken by the nucleus and induce DNA damage, leading to direct genotoxicity on organisms [46]. While many studies have shown that most NPs cannot enter the nucleus, they still indirectly affect genotoxicity by oxidative stress, epigenetic changes, inflammation, and autophagy [46]. Moreover, genotoxicity plays a key role in assessing the safety of NPs on human health and the environment [48][49][50][51]. Although there has been many researches about the genotoxicity of NPs in recent years, it is mainly focused on traditional artificial nanomaterials, such as TiO 2, carbon nanotubes, and silver and gold NPs [52][53][54]. However, the existing literature on genotoxicity of GFNs remains limited and conflicting. A few studies showed that GFNs had no adverse effects on genotoxicity [55]. In contrast, many researchers have reported that the small size and sharp edges of GFNs (e.g., GO and GQDs) can induce genotoxicity on aquatic organisms (e.g., fish and algae) [56][57][58]. However, the direct and indirect genotoxicity mechanisms of GFNs remain unclear, despite genotoxic phenomena being widely reported.

2. Factors Influencing Genotoxicity of GFNs

As is known to all, there is a strong correlation between cytotoxicity and the physicochemical properties of NPs, such as particle size and shape, surface characteristics, and surface functionalization. Similarly, the genotoxicity of GFNs can be affected by these factors [59]. The genotoxicity of GFNs is greatly varied in the literature, which can be attributed to numerous factors including physicochemical properties (morphology, surface chemistry, size, shape, and purity), dose, test species, exposure time, and exposure assay [60][61].

2.1. Surface Properties

The oxygen-containing functional groups play a key role in the genotoxicity of GFNs [35][62][63][64][65]. For example, the rGO with lower oxygen content can induce stronger genotoxicity on ARPE-19 cells than these GO with higher oxygen content, suggesting that GO has a better biocompatibility owing to more saturated C–O bonds [62]. The remove of epoxy groups from the GO surface mitigates GO in vivo genotoxicity toward *Xenopus laevis* tadpoles [35]. Compared with GO, graphene, rGO, and graphite all induce higher levels of genotoxicity in glioblastoma multiforme cells, and the difference was attributed to the hydrophilic and hydrophobic surface and edge structure of GFNs [65]. GO has hydrophilic properties and smooth and regular edges, while rGO and graphene have hydrophobic properties and sharp and irregular edges, which can damage the integrity of cell membranes greatly. The carboxyl groups in the surface of carboxyl-FLG may scavenge oxidative radical on bronchial epithelial cells to alleviate the genotoxicity of FLG [64]. Moreover, different immunological mechanisms triggered by GFNs can be attributed to the proportion of hydroxyl groups [63]. Cells produce a stronger inflammatory response after being exposed to GO than rGO by detecting transcriptomic changes, and the reason is attributed to the large number of hydroxyl groups on the surface of GO [63]. The surface functionalization also can significantly modulate the toxicity of GFNs [57][66][67][68]. For example, amino functionalized GQDs induced lower ferroptosis effects than nitrogen-doped GQDs [66]. Similarly, the DNA methylation of various tissues induced by GQDs was depend on their different surface chemical modifications [57]. Increased cytotoxicity and genotoxicity of the aminated GO were detected by following 24 h exposure on Colon 26 cells [68]. A study on the genotoxicity reduced by GO and rGO showed that the GTPs-rGO reduced by green tea polyphenols (GTPs) yielded more biocompatible and reduced sheets with lower genotoxic effects, as compared to the N_2H_4–rGO, which were reduced by hydrazine (N_2H_4) [69]. The acid-polyethylene glycol (LA-PEG) and PEG modified GO induced gentle DNA damage and decreased the genotoxicity of GO to HLF cells [67]. Surface charge also influences significantly the genotoxicity of GFNs [67][70]. The genotoxic effect of GO on cells is proportional to the amount of positive charge on the surface [67]. The surface charge density of graphene in aqueous solution can transform to chemically-converted graphene, leading to the capture of large amounts of DNA [70]. The different hydrophilic and hydrophobic properties of GO/rGO regulated by differential surface chemistry (especially the O/C ratio) determine the potential of graphene to interact with organisms [71][72][73]. Despite hydrophilic and hydrophobic rGO exhibiting similar toxic responses (e.g., cytotoxicity, DNA damage, and oxidative stress) to cells, their biological and molecular mechanisms are different [71]. The hydrophilic GO and hydrophobic rGO induce both kinds of DNA damage, namely single stranded and double stranded breaks, but the dose dependency was very significant and evident in GO exposure in DNA damage but not in rGO exposure [71]. Hydrophilicity, also an important factor in determining the biocompatibility and colloidal stability of GFNs, leads to different interactions with cells and bio-distribution of GFNs [72][73]. For example, simple accumulation of hydrophobic pristine graphene on the surface of monkey kidney cells without any cellular internalization led to severe metabolic toxicity, whereas hydrophilic GO was internalized by the cells and concentrated near the perinuclear region without causing any toxicity under lower concentrations [72]. Therefore, the surface properties play an important role in understanding the genotoxicity manifestations and biological and molecular mechanisms of GFNs.

2.2. Size and Structure

The genotoxicity of GFNs within organisms is size-dependent. Compared with large GFNs, small GFNs have bigger surface areas and provide more sites to interact with cells, leading to greater cellular uptake of GFNs [74]. The size effect plays a key role in the genotoxicity of GFNs. For example, small rGO (average lateral dimensions 114 nm) induce higher genotoxicity in the hMSCs than large rGO (3.8 ± 0.4 μm) at 0.1 and 1.0 μg/mL after 1 h exposure. The lateral size and extremely sharp edged structure of GFNs can result in higher permeability to the cell and nucleus, resulting in greater genotoxicity. Similarly, the size of GFNs is an important determinant of subcellular penetration [74]. Li et al. [75] suggested that the larger the lateral size of GO, the more severe is the pyroptosis induced by GO in Kupffer cells. Moreover, there is a strong correlation between the size of GO and the structural change in small-interfering RNAs [76]. The large GO merely reduces the A-helical pitch, while small GO inserted into the double strands can wreak havoc on the RNA conformation [77]. In addition, Kong et al. [78] proved that the DNA damage mechanism of GQDs was limited by the size of GQDs through molecular dynamics simulations. Briefly, the relatively large GQDs (61 benzene rings) tend to stick to the ends of the DNA molecule, causing the DNA to unfold, while the small GQDs (seven benzene rings) are easily embedded in DNA molecules, leading to DNA base mismatches. The planar structure of GFNs may also have an effect on DNA damage. The dsDNA bases have a stronger binding affinity with wrinkled GFNs and even cause more DNA damage than with planar GFNs [79]. Given these discordant results, it is necessary to clarify the size- and structure-related genotoxicity of GFNs.

2.3. Exposure Dose and Time

The dose–response relationship is an important principle in nanotoxicology [46]. The modified GQDs may induce DNA hypermethylation in a time and dose dependent manner [57]. The high-dose (50 mg/L) GO induces more serious DNA methylation (hypermethylation) than low-dose (10 mg/L) treatment [80]. The effective accumulation of GFNs in the nucleus is regulated by two nuclear pore complex genes (Kapβ2 and Nup98), and their cellular internalization and absorption are related to exposure time [81]. Notably, the rGO sheets with the same size or larger size, higher concentration (100 μg/mL), and longer exposure time (24 h) showed no obvious genotoxicity in the hMSCs [82]. Overall, there are few studies on the genotoxicity of GFNs doses, and especially the combination of GFNs type and dose exposure is rare.

2.4. The Resistance of Cell Structures and Biological Barriers

From an organism’s perspective, the responses of various types of cells, organs, and tissues with different structures and functions to GFNs exposure were highly diverse. Internalization and direct contact membrane stress with extremely sharp edges of GFNs are considered as important mechanisms of toxicity [83][84]. For different bacterial models to graphene toxicity, the outer membranes can better “protect” bacteria from graphene [85]. The biological barrier is crucial for mammals against the damage from GFNs [30][59]. Both GO and graphene were able to induce DNA breaks in an in vitro model simulating the human intestinal barrier [86]. Moreover, GO nanosheets could break through the first line of host defense by disrupting the ultrastructure and biophysical properties of lung surfactant membranes [87]. Combined with the routes and doses of human exposure, relevant biological barriers toxicity can be considered as an aspect of assessing GFNs genotoxicity.

3. Genotoxicity Testing of GFNs

3.1. Detection of GFNs in Cells and Organism Tissues

The detection of GFNs internalization (distribution and behavior) in model organisms and cells is a key step for a better understanding of their genotoxicity and underlying mechanisms. The most commonly used detection technique includes direct observation of localization of GFNs in organisms and cells by transmission electron microscopy (TEM) [88]. The hyperspectral imaging is also used to visualize cellular interactions with NPs [89], such as cellular uptake and binding of GFNs [90]. The label-based approaches to image GFNs exist in cells by confocal and fluorescence microscopy, reflection-based imaging, and flow cytometry. Additionally, scanning electron microscopy (SEM) can be used to detect the attachment of GFNs in the surface zone of cells [56][90]. Raman spectroscopy and atomic force microscopy (AFM) were used to evaluate nuclear area changes and the disruption of DNA chains impacted by GQDs, respectively [81]. However, these traditional techniques are limited by low observation efficiency and large errors of quantitative results, with are disadvantages in the detection of GFNs [88]. Few studies focus on GFNs nuclear detecting techniques. In the biological imaging field, most research pays attention to safe application of fluorescent GFNs nuclear images rather than assessing genotoxicity of GFNs from an environmental toxicology point of view [91][92][93]. It is necessary to further optimize and develop detection techniques of GFNs in cells and organism tissues for a better understanding of genotoxicity. For example, Chen et al. [94] used laser desorption/ionization mass spectrometry imaging to map and quantify precisely the sub-organ distribution of the carbon nanotubes, GO, and carbon nanodots in mice. The SEM–Raman spectroscopy co-located system provide both SEM and Raman data from the same area on the cell sample, which avoids sample registration issues and makes observed results more accurate [95].

3.2. Genotoxicity Assay of GFNs

There are several assays available to access the genotoxicity of GFNs, measuring various endpoints [96]. The Ames test (bacterial reverse mutation), the comet assay (single cell gel electrophoresis), the chromosomal aberration (CHA), and micronuclei (MN) are the most common tests for genotoxicity. The Ames test (bacterial reverse mutation) can provide initial testing for genotoxicity. The comet assay can detect DNA damage, while the CHA and MN can test large chromosomal abnormalities. The hypoxanthine phosphoribosyl transferase (HPRT) gene is suitable for assessing mutations induced by suspect genotoxic agents, such as NPs [96]. Oxidative DNA damage should be considered one of the causes of genotoxicity. Superoxide radicals can lead to the activation of oxidation of the guanine bases present in the DNA strands, causing rupture to these strands. The most commonly used detection techniques include 8-hydroxydeoxyguanosine and 7, 8-dihydro-oxodeoxyguanine by HPLC with electrochemical detection [97].

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