International Journal of Biomedical and Clinical Sciences
Vol. 1, No. 1, 2016, pp. 24-29
http://www.aiscience.org/journal/ijbcs

Toxicity of Graphene and Its Nanocomposites to Human Cell Lines: The Present Scenario

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Abstract

Graphene, a two dimensional (2D) planar and hexagonal array of carbon atoms, interacts with biological systems in different ways. Pristine graphene has been found to produce different responses as compared to its nano-composites. Graphene and its derivatives have been tested for their toxic effects on human erythrocytes, skin fibroblasts and different human cell lines including HepG2, A498, Caco-2, MCF-7, MDA-MB-231, SKBR3, MGC-803 and A549. Surface charge on graphene oxide (GO) has been found to play important role in its toxicity. Higher GO concentrations were found to be associated with increase in G0/G1 phase cell proportions. Graphene and its nano-composites have also been found involved in higher reactive oxygen species production, DNA damage, cell cycle changes, interference with metabolic routes and apotosis. Though the studies on graphene toxicity on humans are rare, an attempt has been made in this paper, to compile and evaluate the studies involving graphene and its nano-composites particularly in relation to human cell lines.

Keywords

Graphene, Toxicity, Human Cell Lines, Graphene Nano-composites, Cancer, Genotoxicity

Received: August 4, 2016 / Accepted: August 11, 2016 / Published online: August 25, 2016

1. Introduction

Graphene has attracted much attention of the entire scientific community due to its enormous potential in different fields including agriculture, medical sciences, food safety and cancer research. With a range of extraordinary properties such as high surface area, high mechanical strength, and ease of functionalization, graphene is considered highly promising for applications in different fields including tissue engineering [1]. Graphene is a two dimensional (2D) planar and hexagonal array of carbon atoms. It consists of a honeycomb lattice and has a thickness of a single atom. It is a gapless material with ballistic conduction at room temperature and high carrier mobility. Related materials include few-layer-graphene (FLG), graphene oxide (GO), reduced graphene oxide (rGO), and graphene nanosheets (GNS). Though graphene and its derivatives have not been tested for their toxic effects in humans till now, but studies are available on the toxic profile of graphene and its nano-composites on human erythrocytes, skin fibroblasts and different human cell lines including HepG2, A498, Caco-2, MCF-7, MDA-MB-231, SKBR3, MGC-803 and A549. In the present paper, an attempt has been made to summarize the interactions among graphene and its nano-composites in relation to different human cell lines. It will provide an insight into the toxic associations of graphene and its derivatives and help the scientific community to predict their biocompatibilities to different human cells.

Due to their unique chemical and physical properties, graphene and its derivatives have found important places in a number of application fields as three-dimensional graphene foams have been employed as culture substrates for human mesenchymal stem cells (hMSCs) and have been found to maintain stem cell viability and promote osteogenic differentiation [2]. Graphene oxide (GO) nanoflakes incorporated gelatin-hydroxyapatite scaffolds were also

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found to enhance osteogenic differentiation of hMSCs [3]. On the same line, graphene has also been used for controlled and accelerated osteogenic differentiation of hMSCs [4]. Reduced graphene oxide-coated hydroxyapatite (Hap) composites have been found to be effectively utilized as dental and orthopedic bone fillers as these composites have potent effects on stimulating the spontaneous differentiation of mesenchymal stem cells (MSCs) [5]. Graphene-based nanomaterials are considered attractive candidates for biomedical applications such as scaffolds in tissue engineering [5]. Culture on graphene has been found to provide a new platform for the development of stem cell therapies for ischemic heart diseases by enhancing the cardiomyogenic differentiation of human embryonic stem cells (hESCs) [6]. Nanoscale topography of artificial substrates can greatly influence the fate of stem cells including adhesion, proliferation and differentiation.[7] GO film was demonstrated as an efficient substratum for the adhesion, proliferation, and differentiation of human adipose-derived stem cells (hADSCs). The enhanced differentiation of hADSCs included osteogenesis, adipogenesis, and epithelial genesis. On the other hand, chondrogenic differentiation of hADSCs was found to be decreased when compared to tissue culture polystyrene as a control substrate [7].

2. Graphene Based Detection Platforms for Human Derived Samples

A rapid, sensitive, selective and highly stable electrochemical detection platform for beta-nicotinamide adenine dinucleotide (NADH) based on uncapped Au nanoparticle/reduced graphene oxide (rGO) nanocomposites in human urine was reported [8]. The sensor displayed a high sensitivity of 0.916 μA/μMcm^2 and a wide linear range of from 50nM to 500μM with a limit of detection of 1.13nM (S/N=3). Interferences from glutathione, glucose, ascorbic acid and quanine were found to be negligible revealing good biocompatibility of the platform. On the same line, an ultrasensitive DNA biosensor based on graphene/Au nanorod/polythionine was developed for human papillomavirus (HPV) detection in human serum samples [9]. The DNA biosensor showed excellent results for HPV DNA detection over the range from 1.0x10^{-13} to 1.0x10^{-10} mol/L with a detection limit of 4.03x10^{-14} mol/L. Similarly, He et al. [10] employed rGO electrodes modified by folic-acid for detection of folic acid protein in human serum. The platform was fabricated by electrophoretic deposition of rGO onto a gold electrode and post-functionalization of rGO with folic acid, and a detection limit of 1pM was achieved. A facile and flexible thin-film sensor was demonstrated based on thermoelectric effects of graphene. It was shown to be highly conductive, flexible and compressible all-graphene passive electronic skin which can be used for sensing human touch [11]. Graphene has also been used as adsorbent for spectrofluorimetric determination of glutathione in human plasma [12]. A simple and sensitive immunoassay has been reported for the determination of human chorionic gonadotropin (hCG) by graphene-based chemiluminescence resonance energy transfer (CRET) method [13]. Similarly, label-free amperometric immunosensor based on nanoporous gold and graphene for the detection of hCG has also been reported. The immunosensor exhibited a specific response to hCG in the range of 0.5-40.00 ng ml^{-1} with a detection limit of 0.034 ng ml^{-1} under optimal conditions [14]. Recently, an electrochemical biosensor based on rGO-tetraethylene pentamine-BMIMPF6 hybrids has been reported for the detection of alpha 2,6-sialylated glycans in human serum [15].

3. Toxicity of Graphene and Its Nano-composites

The potential for widespread human exposure raises safety concerns about graphene and its derivatives. Graphene interacts with biological systems in different ways. Pristine graphene produces different responses as compared to its nano-composites. Even pristine GO (p-GO) was not found to enter into the cell or damage the membrane but its presence was found to increase reactive oxygen species (ROS), DNA damage and T lymphocyte apoptosis. Our previous studies also reported higher ROS levels [16-18] and genotoxicity [16, 19, 20] among humans due to different environmental aspects. The toxic mechanism is that p-GO interacts directly with the protein receptors to inhibit their ligand-binding ability, leading to ROS-dependent passive apoptosis through the B-cell lymphoma-2 (Bcl-2) pathway [21]. The toxicity of p-GO and functionalized GO (GO-COOH and GO-PEI) to primary human peripheral blood T lymphocytes and human serum albumin (HSA) was assessed. The results indicated that p-GO and GO-COOH have good biocompatibility to T lymphocytes at the concentration below 25 μg mL^{-1}, but result in notable cytotoxicity above 50 μg mL^{-1}. GO-PEI was found to exhibit significant toxicity even at 1.6 μg mL^{-1}. GO-PEI showed severe hematotoxicity to T lymphocytes by inducing membrane damage. The binding of GO-COOH resulted in minimal conformational change in HSA and HSA's binding capacity to bilirubin remained unaffected. On the other hand, binding of p-GO and GO-PEI exhibited strong toxicity on HAS [21]. The effect of using a self-supporting graphene hydrogel (SGH) film to induce the osteogenic differentiation of human adipose-derived stem cells (hADSCs) was examined and it was found that film

alone could stimulate the osteogenic differentiation of hADSCs, independent of additional chemical inducers. Moreover, SGH film was found to be non-toxic and biocompatible in the experiment [1]. GO, thermally reduced graphene oxide (TRGO) and nitrogen-doped graphene (N-Gr) were investigated for several biological toxic effects including cytotoxicity, oxidative stress, cellular and mitocondrial membrane alterations on human dental follicle stem cells. Cytotoxicity was found to be in the order TRGO > N-Gr > GO [22].

Genetic toxicity in animal models represents a potential health hazard that may lead to different health related ailments including cancer. Authors have reported genotoxicity in human stem cells [23] and human lung fibroblast cells [24]. Genotoxicity of GO to human lung fibroblast (HLF) cells was also reported to be concentration dependent [25]. The cytotoxicity effects of GO and bacterially reduced graphene oxide (B-rGO) on the inhibition of cell viability, ROS generation and membrane integrity in human breast cancer cells were investigated and the results suggested that both GO and B-rGO exhibit toxicity to MCF-7 cells in a dose-dependent manner. A dose >60 µg/mL exhibited obvious cytotoxicity effects including decreased cell viability, increased ROS generation and release of lactate dehydrogenase [26]. The cytotoxic effects of biologically reduced GO using *Ganoderma spp.* mushroom extracts in MDA-MB-231 human breast cancer cells were determined. Biologically derived *Ganoderma* extract (GE)-reduced GO (GE-rGO) was found to be more toxic to cancer cells than GO [27]. Curcumin-reduced GO sheets were evaluated for their effects on two human breast cancer cell lines (MDA-MB-231 and SKBR3). At 70 µg/mL, initiation of some cell morphological changes was observed but at higher concentrations of near to 100 µg/mL, slight cytotoxic effects (resulting in ~15-25% cell destruction) were detected by MTT assay. The interaction of the Curcumin-rGO sheets and cells also resulted in apoptosis and morphological transformations of the cells [28]. Nguyen et al. [29] investigated the antibacterial properties of GO against human intestinal bacteria and in-vitro cytotoxicity using the Caco-2 cell line derived from a colon carcinoma but found no toxicity of GO at different concentrations (10 to 500 µg/ml) against the selected bacteria and only a mild cytotoxic action on Caco-2 cells after 24 h of exposure was observed suggesting its biocompatibility and scope to be used in food science. Wu et al. [30] studied the potential cytotoxicity of GO nanosheets on human breast cancer MDA-MB-231 cell line and suggested that higher concentrations of GO (>/=100 µg/mL) exhibited time- and dose-dependent cytotoxicity against MDA-MB-231 cells. In vitro cytotoxicity analysis performed on A498 human kidney epithelial cells showed CD_{50} values of graphene nanoplatelets (GNPs) followed by reduction with hydroiodic acid (rGNP-HI) to lie between 179-301 µg/ml [31]. Wu et al. [32] evaluated the cytotoxicity in human multiple myeloma cells (RPMI-8226) treated with GO, doxorubicin (DOX), and GO loaded with DOX (GO/DOX) and revealed that cells treated with GO, DOX and GO/DOX for 24 hours showed a decrease in proliferation. GO/DOX was found to significantly inhibit cell proliferation as compared to pure DOX ($P<0.01$).

Cytotoxicity of GO and carboxyl graphene nanoplatelets in the human hepatocellular carcinoma cell line (HepG2) were investigated and a dose- and time-dependent cytotoxicity in HepG2 cells was found. Plasma membrane damage and induction of oxidative stress were also observed. No toxicity was found at low concentrations (< 4 µg/ml) [33]. Similarly, Liao et al. [34] investigated cytotoxicity of graphene and GO in human erythrocytes and skin fibroblasts. Graphene sheets were found to be more damaging to mammalian fibroblasts than GO. On the other hand, GO showed higher hemolytic activity than graphene sheets and coating of GO with chitosan was found to eliminate the hemolytic activity. Wang et al. [25] examined the role of surface charge and oxidative stress in cytotoxicity and genotoxicity of GO towards HLF cells and surface charge on GO was found to play an important role in its toxicity to HLF cells. Higher GO concentrations were also found to increase G0/G1 phase cell proportion, intracellular ROS production and induce LDH release [30]. Comparative protein profile of human hepatoma HepG2 cells treated with graphene and single-walled carbon nanotubes (SWCNTs) was investigated and was found that oxidized SWCNTs induced oxidative stress; interfered with intracellular metabolic routes, protein synthesis and cytoskeletal systems and perturbed the cell cycle. A significant increase in the proportion of apoptotic cells was also observed [35].

4. Toxic Investigations Using Graphene Quantum Dots

Graphene quantum dots (GQDs) have attracted much attention in recent biological and biomedical applications due to their super properties. But their potential toxicity investigations have rarely been done. Cytotoxicity of GQDs has been assessed by few researchers on human gastric cancer MGC-803; breast cancer MCF-7 cells [36] and human A549 lung carcinoma cells; human neural glioma C6 cells [37]. The molecular interaction between GQDs and human serum albumin was characterized by the combination of multispectroscopic and electrochemical approaches. Thermodynamic parameters suggested that GQDs interacted with HSA through van der Waals interactions and hydrogen

bonding. GQDs were found to cause local conformational change of HSA indicating the potential toxicity risk of GQDs to humans [38]. A neural-stem-cell-based chip was fabricated to electrochemically detect the toxic effects of GO nanopellets on HB1.F3 cells. A negative correlation was observed between the GO nanopellet concentration and the cell viability, verified via MTT assay and microscopic imaging tool [39]. Author's previous paper also describes the toxicity related to GQDs [40]. Cytotoxicity of three kinds of GQDs with different modified groups (NH_2, COOH, and CO-N $(CH_3)_2$, respectively) in human A549 lung carcinoma cells and human neural glioma C6 cells was investigated using thiazoyl blue colorimetric (MTT) assay and trypan blue assay. GQDs were found to randomly disperse in the cytoplasm but not get diffused into nucleus. The study suggested the three modified GQDs to have good biocompatibility even at the concentration of 200 µg/mL [37].

5. Conclusion

Graphene is a novel 2D material which has enormous potential in different application fields of biomedical sciences, agriculture, food safety, cancer research and tissue engineering. Though graphene is being employed in varied biomedical fields, its widespread human exposure raises safety concerns over health impacts of graphene and its derivatives. Different testing assays including microscopic imaging tools, MTT assay and trypan blue test are being employed for detecting the toxicity of graphene based materials. As these materials are involved in genotoxicity, higher ROS production, cell cycle alterations and apotosis, to complete their toxic profile in humans, should be the priority of the future studies by the scientific community.

Abbreviations

Bcl-2: B-cell lymphoma-2

B-rGO: Bacterially reduced graphene oxide

CRET: Chemiluminescence resonance energy transfer

DOX: Doxorubicin

FLG: Few layer graphene

GE-rGO: *Ganoderma* extract -reduced GO

GNPs: Graphene nanoplatelets

GNS: Graphene nanosheets

GO: Graphene oxide

GQDs: Graphene quantum dots

hADSCs: Human adipose-derived stem cells

Hap: Hydroxyapatite

hCG: Human chorionic gonadotropin

hESCs: Human embryonic stem cells

HLF: Human lung fibroblast

hMSCs: Human mesenchymal stem cells

HPV: Human papillomavirus

HSA: Human serum albumin

MSCs: Mesenchymal stem cells

NADH: Nicotinamide adenine dinucleotide

N-Gr: Nitrogen-doped graphene

p-GO: Pristine GO

rGNP-HI: Graphene nanoplatelets reduced with hydroiodic acid

rGO: Reduced graphene oxide

ROS: Reactive oxygen species

SGH: Self-supporting graphene hydrogel

SWCNTs: Single-walled carbon nanotubes

TRGO: Thermally reduced graphene oxide

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