RSC Advances

Toxicity of Graphene Related Materials and Transition Metal Dichalcogenides

Journal:	*RSC Advances*
Manuscript ID:	RA-REV-10-2014-012624.R1
Article Type:	Review Article
Date Submitted by the Author:	25-Nov-2014
Complete List of Authors:	Chng, Elaine; Nanyang Technological University, Chemistry and Biological Chemistry Pumera, Martin; Nanyang Technological University, Chemistry and Biological Chemistry

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Toxicity of Graphene Related Materials and Transition Metal Dichalcogenides

Elaine Lay Khim Chng, Martin Pumera*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

The dramatic rise in the development and application of graphene related materials (graphene, graphene oxide and reduced graphene oxide) as well as of nanosized layered transition metal dichalcogenides gives strong incentive to study toxicity of these nanomaterials. It was found that size, surface area, shape, number of layers and amount and type of oxygen containing groups strongly influence toxicity of the nanomaterials. Important toxicity studies are reviewed here with focus on above mentioned materials.

1. Introduction

The isolation of graphene in 2004 by Geim and Novoselov[1] has generated a tremendous amount of attention which led to a rapid development in fields ranging from engineering and materials sciences, to physics and chemistry.[2-4] More recently, the research scope on graphene has expanded to beyond electronic and chemical applications and into the frontier between biology and medicine i.e., biomedical applications where it has been postulated to be a novel nanomaterial with the potential to improve areas in therapeutic, diagnostic as well as preventive medical products. The appeal of graphene for use in bioapplications lies with their unique physiochemical properties such as enhanced thermal and electrical conductivity, high surface area and extraordinary mechanical strength.[2,5,6] These wide-ranging applications, coupled with improvements in the synthesis and versatility of graphene for surface modifications have led to the manufacture of graphene and its derivatives, collectively referred to as graphene-family nanomaterials (GFNs). Included (but not limited to) in the graphene-family nanomaterials are graphene oxide[7], reduced graphene oxide, single- or few-layer graphene[8], graphene ribbons and nanosheets.[9] Amongst these graphene-family nanomaterials are variations in properties like their purity, lateral dimensions, defect density, layer number, surface area, stiffness, shape, size, and surface chemistry; all of which can influence their interaction with biological systems considerably. In particular, surface chemistry is known to be key property in improving the biocompatibility and controlling the behaviours of nanomaterials in biological systems. It determines their hydrophobicity and hydrophilicity, which affects the stability and dispersibility of graphene-family nanomaterials under physiological conditions.[9] To illustrate, graphene oxide which is obtained via the oxidative exfoliation of graphite,[10] contain large amounts of residual carboxylic acid, epoxide and hydroxide groups on its surface resulting in its amphiphilicity and exceptional aqueous processibility. Consequently, graphene oxide demonstrates colloidal stability in biological solutions, although aggregation occurs in the presence of salts due to a charge screening effect.[5] The treatment of graphene oxide with reducing agents results in reduced graphene oxide, another graphene derivative. Reduced graphene oxide exhibits superior electrical conductivity,[11] and it can be easily enhanced with hydrophilic functional groups for use in the functionalization of given biomolecules.[12-14] Both graphene oxide and reduced graphene oxide can be produced in scaled-up quantities for suitable uses in biomedicine, and there have been many studies that detailed their significant potential in a host of applications alongside graphene.

In similar manner, but more recently, there have been dramatic development in the utilization of the layered transition metal dichalcogenides (TMDs). These materials have similar structure like graphene, they consists of the layers of the chemically bonded atoms which are stacked together and held by van der Waals interactions.[15] They have chemical composition of MX_2 where M is transition metal (i.e. Mo, W, Re) and X is chalcogen (i.e. S, Se). These materials are suggested to replace Pt in hydrogen generation schemes;[16] they are used for biosensing as well.[17] So far there is very limited toxicological testing performed on them.

The versatility and unique properties of graphene, its derivatives and TMDs have encouraged scientists to explore them as potential candidates for important biomedical applications. However, prior to their prospective use under any biological environment, it is necessary to have a detailed understanding of their possible toxicity. So even though the applications of graphene-family nanomaterials and TMDs may provide major advancements in the biomedical field, there is still a long way before such proof-of-concepts can really be applied in a real

world setting. For that reason, it is imperative to explore and examine the amount of toxicity that graphene, its derivatives and TMDs might present in a biological system as well as the degree of safety with regards to their use.[9,18] The existence of multiple graphene forms will inevitably lead to differences in their

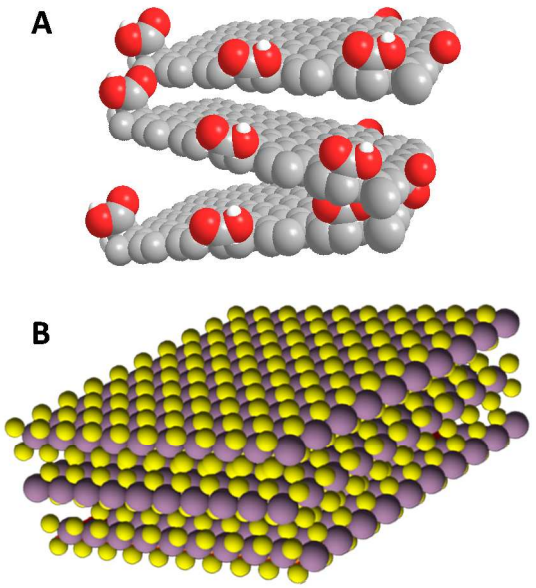


Figure 1. Example of three layers of (A) graphene oxide and (B) MoS_2.

physiochemical properties such as shapes, sizes and surface functional groups, resulting in different toxicological effects in various biological systems.[19] Therefore, systematic toxicity studies are necessary and they should be carried out on well-characterized nanomaterials in order to properly correlate the biological impact with their physiochemical variations.

In particular, "graphene" term is used in the literature for wide variety materials, not only for "semiinfinite sheet consisting of sp^2 carbon". The graphene related materials include graphene oxide (heavily oxidized graphene), reduced graphene oxide and other functionalized graphene materials (see Figure 1).[20] In addition, graphene oxides structure strongly depends on the way they were prepared[21]; the same is true about reduced graphene oxides.[22] In addition, these materials contain impurities up to several at. %.[23] TMDs do not consist of single attomic layer like graphene, but the basic unit are three layers of X-M-X which are bonded in third dimension by weak van der Waals forces. TMDs have different composition (summary formula MX_2, M=Mo, W, Re; X= S, Se, Te; see Figure 1), different number of layers and in addition, they may be partly oxidized.[24]

A thorough characterization is an important component in any nanotoxicological assessments, and it should be performed before interpreting any toxicity results induced by graphene-family and TMDs nanomaterials. Depending on the properties, numerous techniques are available for characterization. For instance, Raman spectroscopy, X-ray photoelectron spectroscopy (XPS) and Brunauer-Emmett-Teller (BET) measurements can be used to determine the surface area and chemical composition of the nanomaterials;[25,26] while shape and size can be assessed with transmission electron microscopy (TEM) and/or atomic force microscopy (AFM).[27,28]

The *in vitro* testing methods represent the ideal model systems for the analysis of graphene-family and TMDs nanomaterials toxicity because of their lower cost, lack of testing on animals, and the results can be obtained rapidly with good reproducibility.[29] In addition, these *in vitro* methods are able to provide precise and quantifiable measurements on the toxicity of the nanomaterials which can be a crucial first step towards the initial evaluation on the biocompatibility of graphene, its derivatives and TMDs. There are many widely-used *in vitro* methods for the toxicity testing of nanomaterials but they can generally be classified into two catergories: viability assays and functional assays.

Viability assays primarily provides end-point results by evaluating whether the tested nanomaterial results in cell death and depending on the cellular property being probed, different viability assays can be applied. They include metalbolic assays, haemolytic assays and apoptosis/necrosis assays which are mainly based on mitochondrial activity and membrane integrity of the cells. As for functional assays, they are used to assess and elucidate the mechanisms of cellular processes in response to nanomaterials exposure, and these cellular mechanisms can include DNA synthesis and damage, immunogenicity, oxidative stress and exocytosis.[30,31]

In the following sub-sections, we will review the available toxicology data obtained from the *in vitro* toxicity assessments on two dimensional nanomaterials, with specific focus on ***graphene***, ***graphene oxide***, ***reduced graphene oxide*** and ***transition metal dichalcogenides*** (TMDs).

2. Toxicity studies on graphene

Graphene and single-walled carbon nanotubes were compared in a cytotoxicity study where they were tested on pheochromocytoma (PC12) cells at dosages from 0.1 – 100 μg/mL. The 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactase dehydrogenase (LDH) release assays were used to evaluate the mitochondrial toxicity and cell membrane disruption respectively.[32] Both nanomaterials presented a dose-dependent toxic response after a 24 h exposure, and graphene induced greater toxicity than single-walled carbon nanotubes at lower concentrations. However, the reverse is observed at the higher concentrations. Graphene also induced a large increase in the release of LDH at 100 μg/mL, and reactive oxygen species (ROS) were generated in a time- and dosage-dependent manner which is indicative of high oxidative stress experienced by the cells. An increase in capase-3 activation also indicated the start of an apoptotic process that is time-dependent. Overall, the difference between the toxicity results suggested the shape of the nanomaterial is a key factor affecting the behaviours of graphene and single-walled carbon nanotubes.

In a similar comparative study, the proteomics of the human hepatoma (HepG2) cell was examined to understand the cellular functions in response to graphene and single-walled carbon nanotubes exposure.[33] The characterization of the

interactions between the nanomaterials and the living cells identified the differential expression of 37 proteins responsible for a range of cellular functions, including metabolic activities and cell growth. Single-walled carbon nanotubes was found to induce oxidative stress which leads to apoptosis, but only slight variations in the protein levels was observed for the cells exposed to graphene. Thus, the proteome analysis revealed graphene to be more biocompatible than the single-walled carbon nanotubes.

Macrophages are cells involved in the defence mechanisms of the immune system and a study on the biological effects of graphene on murine (RAW 264.7) macrophages was carried out by Li *et al.*[34] A dispersion of graphene in 1% pluronic F108 was incubated with the macrophages at dosages ranging from 20 – 100 μg/mL and the results showed apoptotic effect that was dose-dependent. They propose that the cytotoxicity induced by graphene was triggered by a combination of a reduction in the mitochondrial membrane potential and an increment of intracellular reactive oxygen species.

Graphene platelets have also been examined for their potential toxicity and in one work on the human glioblastoma U87 and U118 cell line; there is clearly an increase in the cell mortality and the loss of membrane integrity, alongside a decrease in cell viability which took place in a concentration-dependent manner when the cells were treated with graphene platelets with varying concentrations of 10 – 100 μg/mL.[35] Clearly, the extent of graphene platelets cytotoxicity is directly proportional to the concentrations tested. And for the U118 cells, it was interesting to note that graphene platelets were able to cause apoptosis without generating necrosis.

3. Toxicity studies on graphene oxide

Of all the materials included in the graphene-family nanomaterials, graphene oxide has been the most widely investigated with numerous published reports on their *in vitro* cytotoxicity. Surprisingly though, the first extensive examination on the toxicity of graphene oxide by Chang and co-workers uncovered it to be a relatively safe nanomaterial at the cellular level.[36] Examining the membrane integrity, morphology, viability and mortality at post-exposure to 10 – 200 μg/mL of graphene oxide, no obvious cytotoxic effects was observed on the adenocarcinoma human alveolar basal epithelial (A549) cells. Using TEM, no cellular uptake of the graphene oxide by the A549 cells was observed as well. They also reported that graphene oxide was able to induce oxidative stress only at high enough concentrations to result in a slight loss of cell viability. Following this, another group reported the dose-dependent cytotoxicity of graphene oxide using the same cell line, observed with 1% fetal bovine serum present in the culture medium. They discovered that the 10% fetal bovine serum usually employed for culture medium was mitigating the cytotoxic effects of the graphene oxide, and attributed it to the ability of graphene oxide to adsorb very well to proteins.[37]

The cytotoxic potential of graphene oxide has also been reported for the human hepatoma (HepG2) cell line. In this study, 1 – 16 μg/mL of graphene oxide were evaluated for their cytotoxicity with a series of assays assessing different modes of actions at the cellular level which included lysosome function, metabolic activity and plasma membrane integrity.[38] Concentration- and time-dependent cytotoxicity was observed in the HepG2 cells and at low concentrations of 4 μg/mL, and there was structural damage to the plasma membrane caused by strong physical interaction between the graphene oxide and the phospholipid bilayer. Using scanning and transmission electron microscopy, graphene oxide was shown to be internalized into the HepG2 cells where they accumulated in the cytosol, resulting in elevated levels of reactive oxygen species, altered cellular ultrastructure as well as diminished metabolic activity. Hence, the authors in this work hypothesize that oxidative stress and the impairment of the plasma membrane are two modes of actions behind the toxicity of graphene oxide.

The influence of lateral sizes on the cytotoxicity of graphene oxide was explored in HeLa cells using the MTT assay. A modified Hummer's method was used to produce the graphene oxide, which was then repeatedly oxidized to give smaller sized graphene oxides with lateral sizes averaging at 33.78 nm, 146.8 nm and 205.8 nm.[39] The uptake of graphene oxides was shown using TEM and by means of an isotope labelling and tracing procedure, a greater cellular uptake was observed for the 166.8 nm- and 33.78 nm-sized graphene oxides. Coincidentally, the increased uptake in HeLa cells corresponded with higher cell viability indicating that ultrasmall graphene oxide nanomaterials exhibited exceptional biocompatibility over their larger counterparts.

Apart from the common cytotoxicity measurements, graphene oxide-induced genotoxicity was also assessed recently by Wang *et al.*[40] Comet assay, a general method used to assess DNA damage was applied on the human lung fibroblast (HLF) cells treated with graphene oxide. Comet-like tails from the assay results represent the amount of DNA damage i.e., the longer the tail length, the greater the damage. They report an increase in the tail length as well as the tail DNA percentage along with the concentrations of the graphene oxide, and observed that genotoxicity was apparent even at a dose as low as 1 μg/mL. In contrast, the MTT revealed that the concentration-dependent cytotoxic effects of graphene oxide was only noticeable from 50 μg/mL onwards, since no reduction in the viability of the HLF cells were observed at 1 – 10 μg/mL. Likewise, evident cellular apoptosis from the flow cytometry analysis was only observed at concentrations 50 μg/mL and higher. The disparity between the results suggests that HLF cells experienced a more severe genotoxicity than cytotoxicity when exposed to graphene oxide, indicating the former may function as a more sensitive method. In addition, measurements of cellular superoxide dismutase (SOD) and reactive oxygen species (ROS) revealed a decrease in the SOD and an accumulation of ROS in a concentration-dependent manner, strongly indicative of oxidative stress. To confirm, an antioxidant (N-acetylcysteine) was added to the cells prior to graphene oxide treatment and the results showed a recovery of

10% cell viability at 50 μg/mL. Therefore, the authors report that the toxicity of graphene oxide in HLF cells is mediated an oxidative stress mechanism.

In two very similar studies describing the toxicity of graphene oxide, the human bronchial epithelial cells (BEAS-2B) and human neural stem cell lines (HBI.F3) were chosen respectively. The former was incubated with 10 – 100 μg/mL of graphene oxide and a time- and dose-dependent apoptotic effect was observed along with a corresponding decrease in the cell viability when compared with the control.[41] In an analogous manner, the MTT results showed that the decrease in the HBI.F3 cell viabilities also followed a concentration-dependent trend with concentrations of the graphene oxide nanopellet ranging from 25 – 200 μg/mL. The results were correlated by using differential pulse voltammetry which is an electrochemical technique.[42]

In this next work carried out on human retinal pigment epithelial (ARPE-19) cells, the introduction of graphene oxide seemed to cause minimal toxicity.[43] There is a decrease in the viability of the ARPE-19 cells with increasing concentrations of 5 – 100 μg/mL graphene oxide, although the percentage viability remained at a high of 60% even after being treated with 100 μg/mL graphene oxide for 72 h. No time-dependent trend is observed for the cell viability when the nanomaterials were incubated for 24, 48 and 72 h. An insignificant amount of apoptosis was also observed when the cells were incubated with 100 μg/mL of graphene oxide. Less than 8% of lactase dehydrogenase (LDH) release was measured across all the graphene oxide concentrations which suggest little structural damage to the cell membrane. This was verified when the optical micrographs showed that the ARPE-19 cells retained its cell morphology even after exposure to 100 μg/mL graphene oxide for 72 h, although it was noted that cells proximal to the nanomaterials presented signs of necrosis after 7 days. Therefore, the *in vitro* results in this work demonstrate the potential biocompatibility of graphene oxide in ARPE-19 cells.

The haemocompatability of any graphene-family nanomaterials with human blood components is a crucial toxicological consideration, given their potential in drug delivery applications which allow the entry of these nanomaterials into the blood system. To address the concern of blood compatibility, Liao and co-workers performed a haemolysis assay using graphene and graphene oxide with varying sizes and discovered that all of them resulted in a concentration-dependent haemolytic activity, in which the loss of red blood cells membrane integrity led to the efflux of haemoglobin (Figure 2).[44] Among the graphene oxide samples, the smallest sized graphene oxide triggered the greatest extent of haemolysis; whereas graphene exhibited the lowest haemolytic activity. The authors reasoned that the lack of oxygen-containing groups on the surface of graphene and its tendency to form aqueous aggregates limits their interaction with the red blood cells membrane. Confirming this was optical micrographs showing that red blood cells treated with graphene did not demonstrate significant lysis, but graphene oxide treatment resulted in a lower cell count with evidence of cell lysis and morphological change, as opposed to the normal biconcave shape for normal red blood cells.

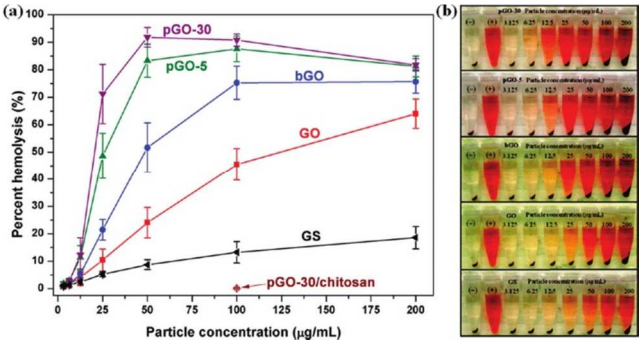


Figure 2. Toxicity of graphene and graphene oxide (a) Percent hemolysis of RBCs incubated with different concentrations (3.125 to 200 μg mL_1) of GO (red), bGO (blue), pGO-5 (green), pGO-30 (purple), and GS (black) for 3 h at 37 °C with agitation. Data represent mean from at least five independent experiments. Also included is the percent hemolysis of RBCs incubated with pGO-30/chitosan at 100 μg mL^{-1} for 3 h at 37 °C with agitation. (b) Photographs of RBCs after 3-h exposure to GO, bGO, pGO-5, pGO-30, and GS at different concentrations (3.125 to 200 μgmL^{-1}). The presence of red hemoglobin in the supernatant indicates RBCs with membrane damage. (+) and (-) symbols represent positive control and negative control, respectively. Reprinted with permission from Ref.[44]

They also explored the cytotoxicity effects of graphene and graphene oxide on human skin fibroblast (CRL-2522) cells but encountered vast discrepancies in the viability data which was later found to be false-positive results due to the reaction between the nanomaterials and the MTT assay reagent. This has important implications for future works since the high surface area and surface reactivity of these graphene-family nanomaterials may result in their interference with viability assays, producing unreliable toxicity data.[45] By using alternate assessments, the authors were able to show that both graphene and graphene oxide had a dose-dependent cytotoxic effect on the CRL-2522 cells, although the former was found to be more toxic because of their ability to form compact graphene aggregates which reduced the nutrients available to the adherent cells. Additionally, oxidative stress was suggested to be the mechanism behind the greater cytotoxicity of graphene as they were able to induce a higher level of reactive oxygen species than graphene oxide.[44] As a result, it is clear that whilst the cytotoxicity of graphene and graphene oxide towards human skin fibroblast cells are governed by their particulate behaviour (i.e., formation of aggregation) and the way they interact with the type of cells (i.e., adherent or suspended cells); the toxicological behaviour towards the red blood cells are influenced by the size and surface oxygen content of the graphene-family nanomaterials. The method of preparation of graphene oxide (permanganate or chlorate route) has strong influence on density of defects and amount of oxygen containing groups of graphene oxide and thus on toxicity of graphene oxide sheets.[46]

4. Toxicity studies on reduced graphene oxide

A comparative study on the biocompatibility of reduced graphene oxide and single-walled carbon nanotubes were carried out by culturing pheochromocytoma (PC12) cells on films that were coated with the respective nanomaterials.[47] A confluent growth and proliferation of the PC12 cells were observed on the reduced graphene oxide film, as opposed to sparse clusters with little cell growth seen on the single-walled carbon nanotube film.

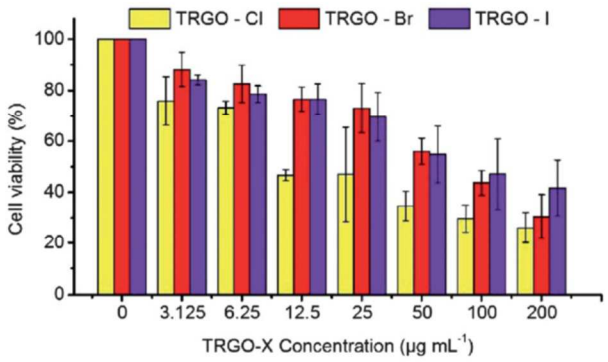


Figure 3. Percentage cell viability of the human lung carcinoma epithelial cells derived from the MTT assay measurements after 24 h exposure of the cells to halogenated graphenes (TRGO-X; X = Cl, Br or I) of varying concentrations. The results shown are relative to the response of the control cells and they represent mean (±standard deviation) of at least three repeat experiments, with four wells per treatment per experiment. Reprinted with permission from Ref.[50]

Neuritogenesis of the PC12 cells was also investigated on the treated films and results showed that the percentage of neuronal differentiated PC12 cells and the average length of extended neurites are both substantially higher on the reduced graphene oxide than on the single-walled carbon nanotube film. The MTT assay results also revealed reduced graphene oxide to be significantly less cytotoxic than the single-walled carbon nanotubes. In the same work, human fetal osteoblasts (hFOB) and oligodendroglia (HOG) cells were also cultured on the coated films, and almost 100% confluence was observed for the HOG cells on both nanomaterials on day 4. But for the hFOB cells, negligible growth was observed on the single-walled carbon nanotube film as in the case of PC12 cells, but proliferation was observed with 60% confluence on day 4 for the reduced graphene oxide film. Overall, the biocompatibility of reduced graphene oxide and its ability to support cell growth has been demonstrated in this study.

The toxicity profile of reduced graphene oxide was evaluated in a comprehensive investigation that involved the cytotoxicity and genotoxicity effects of reduced graphene oxide sheets and nanoplatelets on the human mesenchymal stem cells (hMSC), which is a multi-potent cell that is essential in tissue engineering. The sizes of the reduced graphene oxides were characterized using atomic force spectroscopy and the average lateral dimensions were 3.8 μm, 418 nm, 91 nm, 11 nm.[48] The reduced graphene oxides with the larger dimensions (3.8 μm and 418 nm) were termed as nanosheets, and the smaller ones (91 nm and 11 nm) were termed as nanoplatelets. The viability assay showed a dose- and size-dependent cytotoxicity effect for all the reduced graphene oxide samples. The factor of size had an obvious impact on the cytotoxicity as 1.0 μg/mL of the 11 nm reduced graphene oxide nanoplatelets was able to induce cytotoxicity within a short exposure period of 1 h. Conversely, the largest sized reduced graphene oxide sheets at 3.8 μm exhibited the lowest cytotoxicity among all the samples, even with 24 h exposure at the highest concentration of 100 μg/mL. The extent of oxidative stress was also examined and the 11 nm nanoplatelets were found to generate 26 times more reactive oxygen species than the control, as compared to an increment of 13 times observed for the reduced graphene oxide sheets. While the results of the oxidative stress seemed to correlate with the viability data for the sheets, the same cannot be said for reduced graphene oxide nanoplatelets which suggest to the authors that additional mechanisms other than oxidative stress must be involved in the cytotoxicity of the nanoplatelets. Following this, the RNA efflux was monitored to determine if there is any loss of hMSC cell membrane integrity and the measurements indicated that cells exposed to the reduced graphene oxide nanoplatelets showed significantly higher RNA effluxes, whereas a negligible amount of RNA efflux was detected from cells treated with the reduced graphene oxide sheets. In the genotoxicity assessment, it was revealed that even after 1 h of exposure to 0.1 μg/mL and 1.0 μg/mL of nanoplatelets, the cells showed signs of DNA damage and chromosomal aberration; whilst for the reduced graphene oxide sheets, only slight DNA fragmentation was observed at 100 μg/mL and there were no signs of chromosomal aberration at all the tested concentrations. Therefore, the lateral size is a major factor governing the toxic effects of reduced graphene oxide, and in addition of oxidative stress and cell membrane damage, smaller sized nanoplatelets were able to induce genotoxic effects as well.[48]

Hu *et al.* also looked into the cytotoxicity of reduced graphene oxide found that they were more cytotoxic than their graphene oxide counterparts. The cell viability of the adenocarcinoma human alveolar basal epithelial (A549) cells was reduced to 47% and 15% when they were incubated with 20 μg/mL and 85 μg/mL of reduced graphene oxide for 24 h, respectively. The main focus of this paper was to investigate the anti-bacterial activity of graphene-family nanomaterials; hence the cytotoxicity aspect formed a brief component in their study.[49] Teo et al studied toxicity of chloro, bromo and iodo graphenes and found out that chlorographene is the most toxic variety from all halogengraphenes (Figure 3).[50] Study of highly hydrogenated graphene (graphane[51]) was also performed. Hydrogenated graphene proved to be more cytotoxic than graphene oxide.[52]

As important as the blood compatibility is to the growing bioapplications of graphene-related nanomaterials, the thrombus inducing property is also a crucial consideration which is why graphene-related nanomaterials was also examined for their impact on human blood platelets. In the study, reduced graphene oxide was discovered to be considerably less effective than graphene oxide at eliciting an aggregatory response of the platelets as the results showed the response observed for 2 μg/mL of reduced graphene oxide is merely 10% of the aggregation

induced by graphene oxide at the same dose.[53] Further evidence was provided using scanning electron microscopy, which showed that the appearances of the reduced graphene oxide-treated platelets to be largely similar to the normal resting state of the platelets. In contrast, the platelets exposed to graphene oxide showed signs of pseudopods which resulted in the formation of aggregates. Because reduced graphene oxide not as capable at activating the platelets, it lowers the risk of thromboembolism which could potentially lead to medical issues which include stroke and heart diseases.

5. Toxicity of Transition Metal Dichalcogenides

Layered transition metal dichalcogenides are large group of inorganic materials; the most studied is MoS_2 but its analogues from VIB group, such as $MoSe_2$, WS_2 and WSe_2 have been widely studied for various applications. There are very limited amount of studies on toxicity of these materials. Study by Teo et al. compares toxicity among exfoliated (few layered) MoS_2, WS_2 and WSe_2.[54] MoS_2 and WS_2 induced very low toxicity to the lung cancer cells (A549) while WSe_2 exhibited larger toxicity (Figure 4). It was found that studied TMDs exhibited significantly lower toxicity than graphene oxide tested on the same cells in the same conditions (see Table 1 for a comparison). Chng et al. demonstrated on the case of MoS_2 that exfoliation method and number of layers of final exfoliated product does matter. The three different exfoliation methods were compared and it was concluded that with more exfoliated MoS_2 the toxicity increases.[55]

Table 1. Comparison of toxicity of graphene oxides, halogen graphenes and transition metal dichalcogenides. Normalized percentage of viable cells measured using MTT / WST-8 assays, after 24 h exposure with either 125 μg/mL of GO synthesized using the Hoffmann (GO-HO) and Hummers method (GO-HU), or 200 μg/mL of chlorine-doped graphene (Cl-TRGO), iodine-doped graphene (I-TRGO), and exTMDs. Reprinted with permission from Ref.[54]

Materials	Cell Viability / %						
	GO-HO	GO-HU	Cl-TRGO	I-TRGO	MoS_2	WS_2	WSe_2
MTT Assay	40.0	60.0	26.1	41.7	66.5	90.6	52.0
WST-8 Assay	35.0	5.0	25.8	54.4	80.7	83.6	45.0

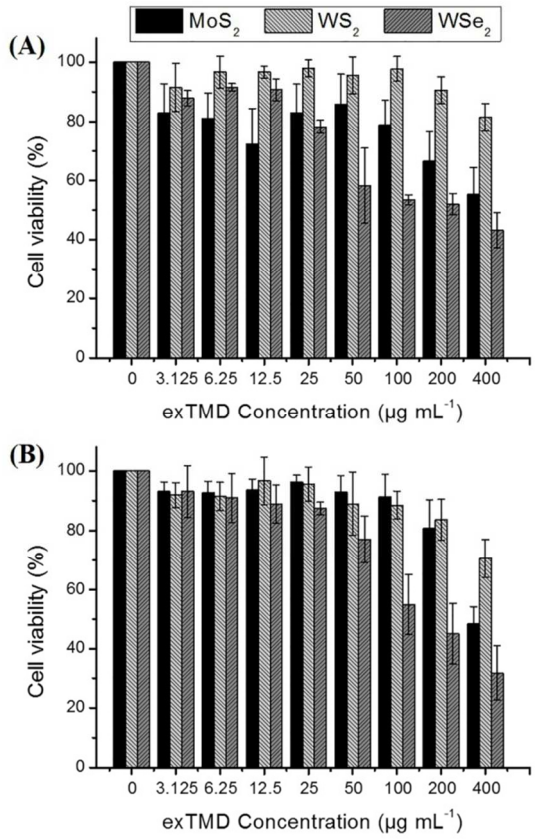


Figure 4. Toxicity of transition metal dichalcogenides. Percentage cell viability of human lung epithelial cells (A549), as measured with (A) MTT assay and (B) WST-8 assay, following 24 h exposure to varying amounts of exTMDs (MoS2, WS2 and WSe2). The percentages shown are normalized to data obtained from the control cells that are not exposed to any exTMDs and they are mean values ± standard deviations of a minimum of three repeat experiments, each consisting of four wells per treatment per experiment. Reprinted with permission from Ref.[54]

6. Conclusions

This reviews showed that the toxicity of the graphene related material strongly depends on the fabrication method, amount of oxygen containing groups and the size of the sheets. We also showed that this is similar in case of transition metal dichalcogenides where toxicity depends on the preparation method. We also discussed that toxicity of TMDs is much lower than of graphene oxide materials. We shall hope that more toxicity studies, especially in under-researched are of TMDs and other 2D inorganic materials, will be performed to assess potential dangers originating in these materials before they are deployed in commercial products.

Acknowledgements

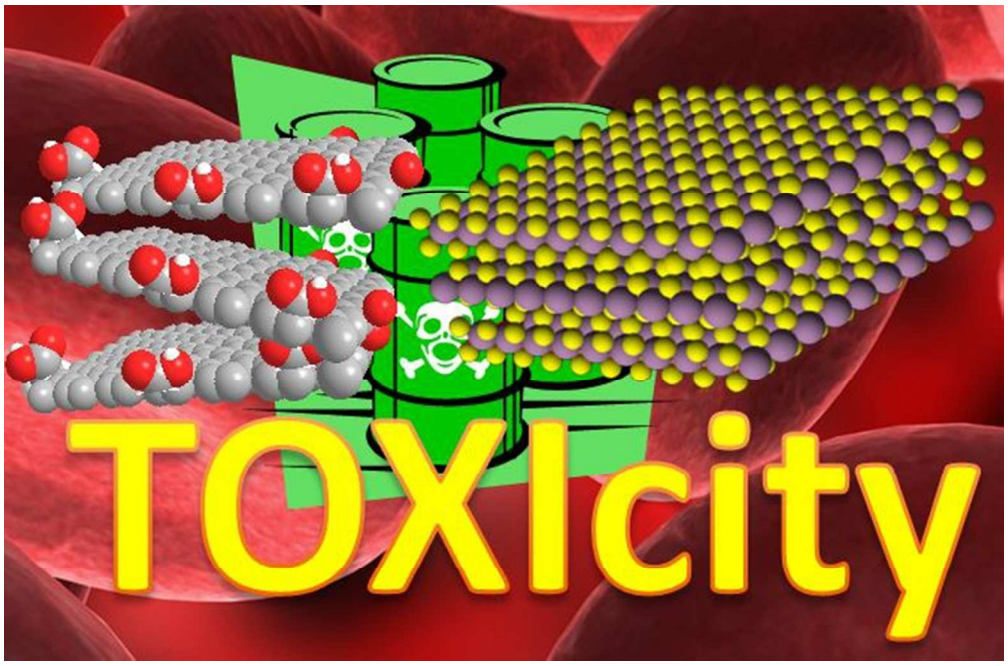
M.P. acknowledges Tier 2 grant (MOE2013-T2-1-056; ARC 35/13) from Ministry of Education, Singapore.

Notes and references

[a] *School of Physical and Mathematical Science, Division of Chemistry and Biological Chemistry, Nanyang Technological University, 21 Nanyang Link, Singapore E-mail: pumera.research@gmail.com*

1) K. S. Novoselov, A. K. Geim, S. Morozov, D. Jiang, Y. Zhang, S. Dubonos, I. Grigorieva, A. Firsov, Science 2004, 306, 666.
2) A. K. Geim, K. S. Novoselov, Nat. Mater. 2007, 6, 183.
3) A. K. Geim, Science 2009, 324, 1530.
4) M. J. Allen, V. C. Tung, R. B. Kaner, Chem. Rev. 2009, 110, 132.
5) D. Li, M. B. Müller, S. Gilje, R. B. Kaner, G. G. Wallace, Nat. Nanotechnol. 2008, 3, 101.
6) C. N. R. Rao, A. K. Sood, K. S. Subrahmanyam, A. Govindaraj, Angew. Chem., Int. Ed. 2009, 48, 7752.
7) C. K. Chua, M. Pumera, Chem. Soc. Rev., 2014, 43, 291.
8) M. S. Goh, M. Pumera, Chem Asian J. 2010, 5, 2355.
9) V. C. Sanchez, A. Jackhak, R. H. Hurt, A. B. Kane, Chem. Res. Toxicol. 2012, 25, 15.
10) W. S. Hummers, R. E. Offeman, J. Am. Chem. Soc. 1958, 80, 1339.
11) K. P. Loh, Q. Bao, P. K. Ang, J. Yang, J. Mater. Chem. 2010, 20, 2277.
12) J. T. Robinson, F. K. Perkins, E. S. Snow, Z. Wei, P. E. Sheehan, Nano Lett. 2008, 8, 3137.
13) S. Stankovich, D. A. Dikin, G. H. B. Dommett, K. M. Kohlhaas, E. J. Zimney, E. A. Stach, R. D. Piner, S. T. Nguyen, R. S. Ruoff, Nature 2006, 442, 282.
14) Y. Shao, J. Wang, H. Wu, J. Liu, I. A. Aksay, Y. Lin, Electroanalysis 2010, 22, 1027.
15 M. Chhowalla, H. S. Shin, G. Eda, L.-J. Li, K. P. Loh, H. Zhang, Nat. Chem. 2013, 5, 263
16) M. Pumera, Z. Sofer, A. Ambrosi, J. Mater. Chem. A 2014, 2, 8981
17) M. Pumera, A. H. Loo, Trends in Analytical Chemistry 2014, 61, 49.
18) S. Liu, T. H. Zeng, M. Hofmann, E. Burcombe, J. Wei, R. Jiang, J. Kong, Y. Chen, ACS Nano 2011, 5, 6971.
19) A. M. Jastrzebska, P. Kurtycz, A. R. Olszyna, J. Nanopart. Res. 2012, 14, 1320.
20) M. Pumera, Electrochem. Commun. 2013, 36, 14.
21) C. K. Chua, Z. Sofer, M. Pumera, Chem. Eur. J. 2012, 18, 13453.
22) a) C. K. Chua, M. Pumera, J. Mater. Chem. A 2013, 1, 1892. b) C. K. Chua, A. Ambrosi, M. Pumera, J. Mater. Chem. 2012, 22, 11054. c) A. Ambrosi, C. K. Chua, A. Bonanni, M. Pumera, Chem. Mater. 2012, 24, 2292.
23 a) C. H. A. Wong, Z. Sofer, M. Kubešová, J. Kucera, S. Matejková, M. Pumera, Proc. Natl. Acad. Sci. 2014, 111, 13774. b) C. K. Chua, A. Ambrosi, Z. Sofer, A. Mackova, V. Havranek, I. Tomandl, M. Pumera, Chem. Eur. J. 2014, 20, 15760.
24) a) A. Ambrosi, Z. Sofer, M. Pumera, Small 2005, DOI: 10.1002/smll.201400401; b) A. Y. S. Eng, A. Ambrosi, Z. Sofer, P. Simek, M. Pumera, ACS Nano 2015, DOI: 10.1021/nn503832j
25) Z. Ji, X. Jin, S. George, T. Xia, H. Meng, X. Wang, E. Suarez, X. Zhang, E. M. V.Hoek, H. Godwin, A. E. Nel, J. I. Zink, Environ. Sci. Technol. 2010, 44, 7309.
26) K.E. Sapsford, K. M. Tyner, B. J. Dair, J.R. Deschamps, I. L. Medintz, Anal. Chem. 2011, 83, 4453.
27) S. A. Love, C. L. Haynes, Anal. Bioanal. Chem. 2010, 398, 677.
28) M. A. Maurer-Jones, Y.-S. Lin, C. L. Haynes, ACS Nano 2010, 4, 3363.
29) S. Sharifi, S. Behzadi, S. Laurent, M. L. Forrest, P. Stroeve, M. Mahmoudi, Chem. Soc. Rev. 2012, 41, 2323.
30) S. A. Love, M. A. Maurer-Jones, J. W. Thompson, Y.-S. Lin, C. L. Haynes, Annu. Rev. Anal. Chem. 2012, 5, 181.
31) M. Pumera, Chem. Asian J. 2011, *6*, 340.
32) Y. Zhang, S. F. Ali, E. Dervishi, Y. Xu, Z. Li, D. Casciano, A. S. Biris, ACS Nano 2010, 4, 3181.
33) J. Yuan, H. Gao, C. B. Ching, Toxicol. Lett. 2011, 207, 213.
34) Y. Li, Y. Liu, Y. Fu, T. Wei, L. Le Guyader, G. Gao, R.-S. Liu, Y.-Z. Chang, C. Chen, Biomaterials 2012, 33, 402.
35) S. Jaworski, E. Sawosz, M. Grodzik, A. Winnicka, M. Prasek, M. Wierzbicki, A. Chwalibog, Int. J. Nanomedicine 2013, 8, 413.
36) Y. Chang, S. T. Yang, J. H. Liu, E. Dong, Y. Wang, A. Cao, Y. Liu, H. Wang, Toxicol. Lett. 2011, 200, 201.
37) W. Hu, C. Peng, M. Lv, X. Li, Y. Zhang, N. Chen, C. Fan, Q. Huang, ACS Nano 2011, 5, 3693.
38) T. Lammel, P. Boisseaux, M. L. Fernández-Cruz, J. M. Navas, Part. Fibre Toxicol. 2013, 10, 27.
39) H. Zhang, C. Peng, J. Yang, M. Lv, R. Liu, D. He, C. Fan, Q. Huang, *ACS Appl. Mater. Interfaces*, 2013, 5 , 1761
40) A. Wang, K. Pu, B. Dong, Y. Liu, L. Zhang, Z. Zhang, W. Duan, Y. Zhu, J. Appl. Toxicol. 2013, 33, 1156.
41) N. V. Vallabani, S. Mittal, R. K. Shukla, A. K. Pandey, S. R. Dhakate, R. Pasricha, A. Dhawan, J. Biomed. Nanotechnol. 2011, 7, 106.
42) S. M. Kang, T. H. Kim, J. W. Choi, J. Nanosci. Nanotechnol. 2012, 7, 5185.
43) L. Yan, Y. Wang, X. Xu, C. Zeng, J. Hou, M. Lin, J. Xu, F. Sun, X. Huang, L. Dai, F. Lu, Y. Liu, Chem. Res. Toxicol. 2012, 25, 1265.
44) K. H. Liao, Y. S. Lin, C. W. Macosko, C. L. Haynes, ACS Appl. Mater. Interfaces 2011, 3, 2607.
45) A. L. Holder, R. Goth-Goldstein, D. Lucas, C. P. Koshland, *Chem. Res. Toxicol.*, 2012, 9, 1885.
46) E.L.K. Chng, M. Pumera, Chem Eur J 2013, 19, 8227.
47) S. Agarwal, X. Zhou, F. Ye, Q. He, G. C. K. Chen, J. Soo, F. Boey, H. Zhang, P. Chen, Langmuir 2010, 26, 2244.
48) O. Akhavan, E. Ghaderi, A. Akhavan, Biomaterials 2012, 33, 8017.
49) W. Hu, C. Peng, W. Luo, M. Lv, X. Li, D. Li, Q. Huang, C. Fan, ACS Nano 2010, 4, 4317.
50) W. Z. Teo, E. L. K. Chng, Z. Sofer, M. Pumera, Nanoscale, 2014, 6, 1173.
51) M. Pumera, C. H. A. Wong, Chem. Soc. Rev., 2013, 42, 5987.
52) E. L. K. Chng, Z. Sofer, M. Pumera, Chem. Eur. J. 2014, 20, 6366.
53) S. K. Singh, M. K. Singh, M. K. Nayak, S. Kumari, S. Shrivastava, J. J. A. Grácio, D. Dash, ACS Nano 2011, 5, 4987.
54) W. Z. Teo, E. L. K. Chng, Z. Sofer, M. Pumera, Chem. Eur. J. 2014, 20, 9627.
55) E.L.K. Chng, Z. Sofer, M. Pumera, Nanoscale 2014, 6, 14412.

Review: the size, surface area, shape, number of layers and amount and type of functionalities strongly influence toxicity of nanomaterials.



118x77mm (144 x 144 DPI)