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The current graphene safety landscape – a literature mining exercise†

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As for any novel nanomaterial, the development of applications and industrial adoption of graphene-based materials will be subject to the confirmation of their safety profile and risk assessment. The analysis performed here maps the current knowledge of the safety of graphene-based materials as extracted by a literature mapping exercise of studies investigating these materials in preclinical animal models. We attempt to identify gaps for future studies and elucidate the critically important structure–function correlations between reported biological effects and graphene-based material physicochemical characteristics.

Background

The successful adoption of graphene in a range of industrial applications (electronics, optics, energy storage, alloys, concrete, filtration) will be dependent on the determination of its safety from exposure, as well as its environmental sustainability.[1] There is an ongoing broader discussion whether nanomaterials, including graphene, can give rise to previously unknown health risks due to their dimensions and their interaction with biological matter.[2,3]

The currently available knowledge about health risks associated with graphene-based materials (GBM) is limited and inconclusive. Information on human and environmental exposure is also almost non-existent because no industrial-scale adoption of graphene has taken place yet. To compound the complexity of the '*graphene safety landscape*', inconsistency in the conclusions of the reported studies is attributed to the large varieties of GBMs used, all incorrectly or misleadingly capped under the generic term 'graphene'. Recently, there have been propositions for the adoption of a more precise nomenclature to distinguish GBMs,[4] and a classification framework to correlate their safety profile with key physicochemical characteristics.[5]

With this drawback, the overall objective of this work was to offer an illustration of the emerging landscape about graphene safety by mining the published studies that generated primary experimental data using different types of GBM and preclinical *in vivo* models.

Literature mining exercise

The starting point of this analysis was the selection of all the published reports that studied the interaction of GBM using *in vivo* models. Then, the physicochemical characteristics of GBM in each study were carefully determined based on the data and information provided in the published reports (see Table S1† and references therein). The design parameters in each study, such as the maximum administered dose of GBM, or the maximum exposure were then mapped against the material characteristics. This data mining approach was applied to 34 original research articles representing 45 materials, all different types of GBM. Studies containing pristine graphene (G), reduced graphene oxide (rGO), graphene oxide (GO) or functionalised graphene (fG) were selected. The fG encompassed materials from one of the first three categories bearing one further type of surface functionalisation (for example PEGylated graphene oxide materials) but with no distinction between covalent or non-covalent functionalisation.

Results

Each GBM was plotted as an individual cube according to the average thickness and lateral dimension reported (Fig. 1). Most of the studies used fG (47% of all GBM studied) or GO (38%), while the minority used G (13%) or rGO (2%). GO materials varied in lateral dimension (between few nm to few μm; the majority were below 100 nm) and most GBM were 1 nm thick, whereas fG varied in thickness. It should be emphasised that no material studied today *in vivo* fulfilled the archetypal definition of graphene (*i.e.* a one-atom-thick hexagonal arrangement of carbon atoms). All of the materials that could be categorised as *'pristine graphene'* composed of at least 2, and up to 15, layers. Our analysis revealed a lack of safety

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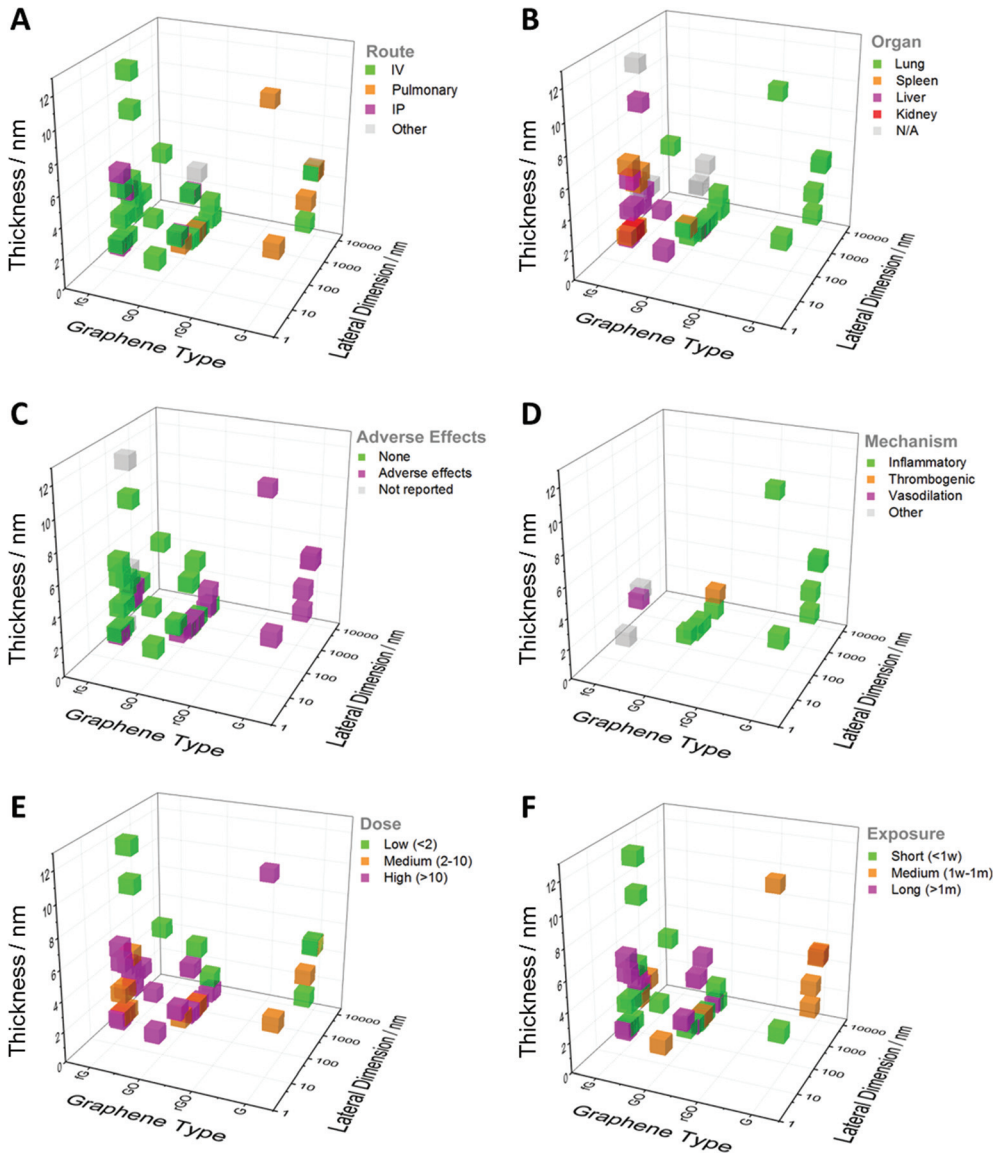

Fig. 1 Current landscape of *in vivo* safety for graphene-based materials. Graphs have been plotted along the same axes: graphene type, lateral dimension and thickness. Each reported GBM is represented by a cube and positioned in the landscape according to the type of functionalisation qualitatively described and its average thickness and lateral dimension (as reported in the original work). Different graphs represent: (A) route of administration; (B) organ of highest accumulation; (C) reported adverse effects; (D) biological mechanism responsible for adverse effects; (E) maximum administered dose; and (F) duration of exposure. In (A) 'IV' is intravenous, 'Pulmonary' includes intra-tracheal instillation, pharyngeal aspiration and inhalation and 'IP' is intraperitoneal. In (B), 'N/A' refers to studies that do not report or do not specify organ of highest accumulation. In (E) 'Low' dose is less than 2 mg kg^{-1}; 'Medium' is between 2 and 10 mg kg^{-1} and 'High' is above 10 mg kg^{-1}. In (F) 'Short' is exposure for less than 1 week; 'Medium' is between 1 week and 1 month; and 'Long' is exposure to GBM for longer than 1 month. (see ESI† for an animated version of this graph).

studies using preclinical *in vivo* models for pristine graphene (only 13%), even though some of these GBM may be closer to industrial adoption as components of various types of composites (metallic alloys, concrete). Future research would need to focus on the hazard assessment of such GBM.

The landscape for GBM safety was first drawn in correlation to the routes of administration, commonly considered as potential exposure routes (Fig. 1A). The intravenous route of administration has been predominant (64% of all materials), followed by the intraperitoneal (17%) and pulmonary routes (including instillation, aspiration and inhalation; 15%). This reflects the fact that the safety studies of GBMs are primarily performed by researchers that aim to develop a specific biomedical application (*e.g.* a blood-circulating drug delivery platform). Inhalation, ingestion and skin deposition are the main routes most relevant to hazard assessment in the context of occupational health or environmental protection. In this context, systemic blood circulation can only be considered relevant as a secondary route after translocation from a primary entry (*e.g.* lung, skin) to the vascular compartment.

The next parameter to be mapped was major accumulation of tissue (Fig. 1B), with the highest accumulation reported for lungs (38%), liver (28%) and spleen (13%). This accumulation pattern suggested that the quality of GBM dispersions used for intravenous administration was poor, resulting in the aggregation of the material and subsequent entrapment within the pulmonary vascular bed and its capillaries. Several studies on carbon nanotube safety have highlighted that the degree of functionalisation and quality of the dispersion will greatly influence potential risks.[6]

The reported adverse effects from GBM administration were then mapped (Fig. 1C). The majority of GBM were reported to cause no deleterious effects (55%; Fig. 1C; green cubes), while there was a significant minority of studies that offered no data on adverse effects (11%; Fig. 1C; grey cubes). Some correlations became apparent when comparing the adverse effects with the route of administration and the main organ of accumulation. First, the GBM that were administered directly into the pulmonary cavity (7 materials out of 45; Fig. 1A; orange cubes) led to lung accumulation (Fig. 1B; green cubes), and most interestingly induced adverse effects (Fig. 1C; magenta cubes). All other pulmonary adverse effects corresponded to the GBM that were administered intravenously, but were still principally found to accumulate in the lungs. The mechanisms behind most of the reported adverse effects were mainly associated with inflammatory responses of the pulmonary system (Fig. 1D). In addition, there was no direct correlation between the occurrence of adverse effects (Fig. 1C) and the highest administered doses (Fig. 1E) or longevity of exposure (Fig. 1F).

An interesting fact revealed by this mapping exercise is that most of the GBM that have been reported to induce adverse effects in the lungs were materials with a low degree of functionalisation. For two of the fG materials (both at high doses and long exposure times) for which significantly adverse reactions were reported, no possible mechanism was mentioned, while another fG material was reported to induce vasodilatation. These analyses further suggest that chemical functionalisation can be a strategy to improve the safety profile of GBMs, as previously shown for carbon nanotubes.[6,7]

Discussion

One of the shortcomings of this analysis stems from the inherent challenges in the accurate measurement of the critical GBM properties, such as the mean lateral dimension and degree of surface functionalisation. Throughout this analysis we used size data for GBM as reported in the published reports, including errors, to reveal the apparent uncertainty (Fig. S1†) that prevails. Such analysis highlighted the urgent need for the development of methodologies and techniques that can reliably and precisely characterise the populations of 2D materials in bulk.

Despite these caveats, the analysis undertaken indicated that inadequately dispersed GBM in physiological environments can result in aggregate formation, increase the risk of entrapment in the pulmonary capillaries upon entry into the systemic blood circulation and result in eventual adverse effects. Throughout the current literature, the quality of GBM dispersions has been scarcely considered. Another indication from the present landscaping exercise is the improvements of the overall safety profile that surface-modified GBMs exhibit. However, the most appropriate strategies and types of surface GBM functionalisation will need to be revealed because some surface modification strategies and functional groups may prove to be more biologically reactive than others.

Attempting to draw the *in vivo* safety landscape for GBMs based on mining the current literature is considered an initial effort that follows earlier recommendations regarding the importance of revealing material structure–biological function relationships.[5] More sophisticated methodologies based on computational and systems biology models will certainly offer further contributions towards such efforts. However, great attention should be placed on the quality of the data used to feed-in such models as they have been previously found to be of insufficient quality for inclusion in sophisticated nanomaterial hazard assessment exercises.[8,9]

Conclusion

This analysis aimed to offer a snapshot of the current landscape around the *in vivo* safety profiling of GBMs, based on (a non-computational) data mining approach of the existing literature. The main outcome of this exercise revealed pulmonary as the tissue of highest risk. Lungs were the organ in which there was the highest accumulation of GBMs larger than 100 nm in (reported) lateral dimension, and they were the site of reported adverse effects, regardless of administration route. The quality of dispersion and the surface functionalisation (no distinction between covalent or non-covalent was considered in our analysis) were also identified as key factors. The limited amount of reported *in vivo* studies demonstrated the urgent need for more research in this area, combined with improvements in the methodologies for the characterisation of bulk GBMs and their administered dispersions.

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