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Safety Considerations for Graphene: Lessons Learnt from Carbon Nanotubes

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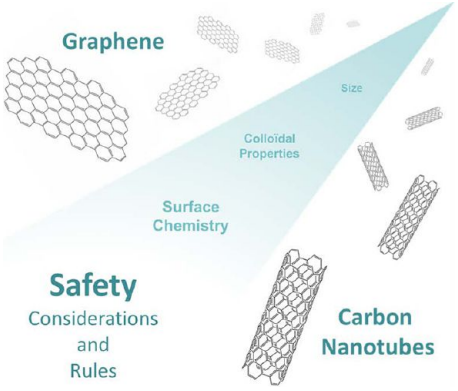
CONSPECTUS

Many consider carbon nanomaterials the poster children of nanotechnology, attracting immense scientific interest from many disciplines and offering tremendous potential in a diverse range of applications due to their extraordinary properties. Graphene is the youngest in the family of carbon nanomaterials. Its isolation, description, and mass fabrication has followed that of fullerenes and carbon nanotubes. Graphene's development and its adoption by many industries will increase unintended or intentional human exposure, creating the need to determine its safety profile. In this Account, we compare the lessons learned from the development of carbon nanotubes with what is known about graphene, based on our own investigations and those of others.



Despite both being carbon-based, nanotubes and graphene are two very distinct nanomaterials. We consider the key physicochemical characteristics (structure, surface, colloidal properties) for graphene and carbon nanotubes at three different physiological levels: cellular, tissue, and whole body. We summarize the evidence for health effects of both materials at all three levels. Overall, graphene and its derivatives are characterized by a lower aspect ratio, larger surface area, and better dispersibility in most solvents compared to carbon nanotubes. Dimensions, surface chemistry, and impurities are equally important for graphene and carbon nanotubes in determining both mechanistic (aggregation, cellular processes, biodistribution, and degradation kinetics) and toxicological outcomes. Colloidal dispersions of individual graphene sheets (or graphene oxide and other derivatives) can easily be engineered without metallic impurities, with high stability and less aggregation. Very importantly, graphene nanostructures are not fiber-shaped. These features theoretically offer significant advantages in terms of safety over inhomogeneous dispersions of fiber-shaped carbon nanotubes. However, studies that directly compare graphene with carbon nanotubes are rare, making comparative considerations of their overall safety and risk assessment challenging.

In this Account, we attempt to offer a set of rules for the development of graphene and its derivatives to enhance their overall safety and minimize the risks for adverse reactions in humans from exposure. These rules are: (1) to use small, individual graphene sheets that macrophages in the body can efficiently internalize and remove from the site of deposition; (2) to use hydrophilic, stable, colloidal dispersions of graphene sheets to minimize aggregation in vivo; and (3) to use excretable graphene material or chemically-modified graphene that can be degraded effectively. Such rules can only act as guidelines at this early stage in the development of graphene-based technologies, yet they offer a set of design principles for the fabrication and safe use of graphene material that will come in contact with the human body. In a broader context, the safety risks associated with graphene materials will be entirely dependent on the specific types of graphene materials and how they are investigated or applied. Therefore, generalizations about the toxicity of "graphene" as a whole will be inaccurate, possibly misleading, and should be avoided.

1. Introduction

The dramatic development of nanoscience and nanotechnology in recent years has offered numerous opportunities and innovative solutions in various fields and applications. Among the different types of novel materials discovered at the nanoscale, carbon-based nanomaterials are a superfamily

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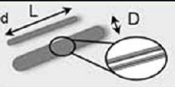
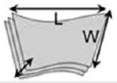
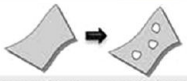
TABLE 1. Physicochemical Characteristics Relevant to Safety Considerations of Carbon Nanotubes (CNT) and Graphene

Physicochemical Characteristics	Graphene	Carbon Nanotubes	Difference
Shape	**Planar** – monolayer or multi-layer ; tunable strucuture/shape (sheet, ribbon, triangle, hexagon) 	**Cylindrical** Single-walled SWCNT; Multi-walled MWCNT 	Completely different
Dimensions (typical)	Thickness: 0.34 -100nm  Lateral size 0.3 -10µm	SWCNT: L: 10nm to 1cm D: 0.4 - 3 nm MWCNT: L: 10nm to few microns D: 2–200 nm	Similar
Surface • Area • Charge • Coating	• Up to 2675m^2/g; decrease with layer number • varies with functionalization or coating • varies with chemical nature (polymer, lipid), type, density & conformation	• SWCNT (>1000 m^2/g) ; MWCNT (100-500m^2/g) • varies with functionalization or coating • varies with chemical nature (polymer, lipid), type, density & conformation	• Quite different • Similar • Similar
Elasticity/Stiffness	Young modulus of 1100 GPa; able of bending and rippling; stiffness increases with number of layers	SWCNT from 1 to 5 TPa; able of bending MWCNT from 0.2 to 0.95 TPa	Similar
Colloidal stability • Aqueous dispersibility • Aggregation	**Dispersibility:** Graphene oxide in water  **Aggregation:** stacking 	**Dispersibility:** Oxidized CNT in water  **Aggregation:** bundling and tangling 	Very different
Durability	 Enzymatic degradation by defects in plan	 Enzymatic degradation by unzipping and decrease of L & D	Different
Impurities	Varies with manufacturing process: mainly graphite and chemical residues from processing	Varies with manufacturing process: metal catalysts (Fe, Co, Ni, Cr, Cu, Zn) ; carbon nanoparticles ; amorphous carbon	Very different

that includes fullerenes, carbon nanotubes, carbon nanohorns, diamond, and graphene. In terms of scope of applications, two of these carbon nanomaterials seem to be developed more widely and maturing faster than the rest: carbon nanotubes and graphene.

Carbon nanotubes (CNTs) are carbon-based nanostructures that were first atomically described by Iijima in 1991,[1] and can be composed of either one (single-walled; SWCNTs), two (double-walled; DWCNTs), or more (multiwalled; MWCNTs) concentric and seamless graphene sheets consisting of sp^2 bound carbon atoms rolled up in the form of thin, hollow cylinders. Those tubular structures are characterized by a high aspect ratio and a high surface area that has made them particularly attractive in various applications. Graphene is a more recent discovery, first isolated by Novoselov and Geim[2] that consists of a two-dimensional, single-atom thick sheet made of planar sp^2 bound carbons, with a high surface area that is available on both sides of the planar axis. Graphene is one of the members of a much broader graphitic family of nanomaterials that also include few-layer graphene (1−5 layers), graphene oxide (GO), reduced graphene oxide layers, and graphite. Both CNT and graphene materials have outstanding electronic, mechanical, electrical, and optical properties and a chemically tunable surface that have made them attractive candidates for a broad range of applications, spanning from composites and electronics to nanomedicine. Biosensors, tissue engineering, as well as components for the design of various types of drug delivery and release systems are among the potential applications of graphene and CNTs in biomedicine.[3]

Despite their common carbon-based elemental consistency, CNTs and graphene are two very distinct nanomaterial entities. Their shape (tubular versus planar) and dimensions (1D versus 2D) are their main structural differences, but they also differ in many other ways. The dispersibility of graphene sheets in various solvents seems to be better compared to CNTs that commonly need a surfactant to facilitate their dispersion. At the nanoscale level, while CNTs have a tendency to form bundles or entangled aggregates, graphene sheets tend to stack into few layers. Compared to CNTs, purified graphene materials usually contain less impurities, such as the metal nanoparticles originating from the metal-catalyzed fabrication of nanotubes; however, that will greatly depend on the fabrication method used. Table 1 attempts to highlight the main physicochemical characteristics for CNTs and graphene and offer a qualitative comparison of their differences.

The importance of physicochemical characteristics in relation to safety considerations of any nanomaterial type cannot be overemphasized.[4] The importance of this relationship has become even more obvious in the case of carbon nanotubes as illustrated during their development,

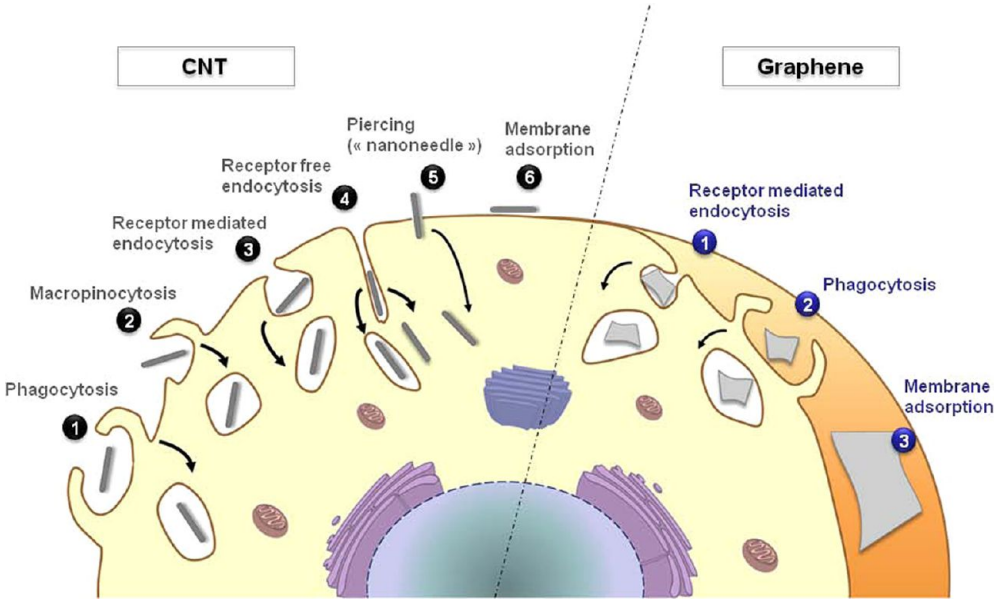


FIGURE 1. Cellular uptake mechanisms for CNTs (black 1−6) and graphene (blue 1−3).

application, and mass production in the past few years. Graphene materials are younger in development, surrounded by great enthusiasm and expectations, as well as a variety of unanswered questions regarding their safety considerations. Below, we attempt to distill the safety considerations important for these two types of carbon nanomaterials at three different levels (cell, tissue, whole body), highlight the lessons learnt, even if yet inconclusive, from the carbon nanotube experience, and try to correlate with that of graphene. The role of the physicochemical characteristics from Table 1 will be addressed throughout the subsequent discussion on the ensuing safety considerations for both types of materials.

2. Safety Considerations at the Cellular level

Interaction with Membranes. The interaction of carbon nanomaterials with membranes is the first point of contact during their contact with cells. Experimental evidence[5] and molecular dynamics simulations[6] have supported the proposition that spontaneous insertion of nanotubes can occur through the lipid bilayer of the plasma membrane in a piercing, needle-like fashion. Different mechanisms have been evoked to explain this phenomenon, with the amphiphilic nature of chemically functionalized CNT surfaces thought crucial in facilitating such molecular interactions with the lipid bilayer. Surface charge is also a critical parameter in such interactions, with positively charged material being more favorable (due to electrostatic attraction forces) for membrane insertion. Contrary to such processes, if the nanotube surface is modified by coating with a macromolecule (polyethylene glycol, large protein, block copolymer), the capacity for spontaneous insertion is lost as has repeatedly been shown,[7] and interaction will largely depend on the cell type studied and their natural machinery to internalize material (e.g. by phagocytosis or not). Moreover, colloidal (dispersion) and structural (size, diameter, or graphitic defects) properties are thought also critical in these interactions, even though poorly investigated.

Yue et al. studied the interaction of graphene oxide (GO) of different lateral dimensions (350 nm and 2 μm) with peritoneal macrophages.[8] Based on transmission electron microcsopy (TEM) of cell sections, the initial interaction was seen different between the two types of GO, with the 350 nm GO wrapped by the active filopodia of the macrophages, while the 2 μm GO seen perpendicular to the plasma membrane. Mu et al. looked into the interaction between protein-coated GO and mouse mesenchymal progenitor cells, and found that interaction with cell membranes occurred for all GO materials.[9]

A comparison of the mechanisms and processes is reported (Figure 1), and the lack of consideration for the different physicochemical material characteristics among these investigations (that can lead to seemingly contradicting reports) is revealed. Biological investigations using graphene can avoid this recurrent problem if well-characterized graphene material is used to draw structure−function relationships. For example, much more systematic work is currently needed in order to elucidate the effect of graphene

sheet dimensions and surface properties on interaction with cellular and bacterial membranes.

Cellular Uptake. Following their interaction with membranes, cell internalization may occur by various processes. In Figure 1, the most commonly described mechanisms of internalization of CNTs and graphene are described. Previous studies from our laboratory and others reported that ammonium functionalized CNTs can directly pierce the cell membrane and translocate freely (outside any vesicular compartment) into the cytoplasm.[5,10] We also showed that various chemically functionalized CNTs were able to enter a wide variety of cell types (mammalian and prokaryotic), independently of the (small molecular weight) functional groups at the surface of the CNTs.[11] The presence of CNTs in the perinuclear region was observed just few hours after the initial exposure and even under endocytosis-inhibiting conditions.

A few research groups have attempted to study the mechanism of cellular uptake of GO in yet inconclusive observations. Mu et al. studied the cellular internalization of BSA-coated GO and suggested size-dependent uptake in C2C12 cells.[9] Smaller BSA-coated GO in the range of 0.5 μm diameter were thought to be uptaken via clathrin-mediated endocytosis, while their larger counterparts of around 1 μm were internalized via both clathrin-mediated endocytosis and phagocytosis. In contrast, using TEM, Chang et al. did not observe any uptake of GO in A549 cells.[12] In one of the most systematic studies today, Yue et al. studied the effect of lateral dimension on the internalization of GO in phagocytic cells and nonphagocytic cells.[8] While no uptake was reported in nonphagocytic cells, high internalization was observed in phagocytic cells. Interestingly, no size-dependent phagocytosis was reported as both the 350 nm and 2 μm GO sheets were uptaken to the same extent, and showed identical intracellular accumulation over 24 h.

Size and individualization of the nanotubes have also been revealed to play an important role in their cellular uptake. According to our data, short, chemically functionalized CNTs (about 200 nm) may favorably enter via clathrin or caveolae dependent pathways, while long tubes (>500 nm) and bundles or aggregates of CNTs are probably internalized via macropinocytosis in nonphagocytic cells. In addition, well-individualized CNTs with lengths around 300−400 nm can enter the cell via direct cytoplasmic translocation. Size and individualization are two characteristics that can be chemically modified, the former by carboxylation reactions and the latter by different chemical functionalization strategies that enhance dispersibility. While the size of graphene would probably prove an equally important factor in the ensuing cellular uptake processes, graphene dispersibility is generally considered better than that of CNT. Moreover, close attention should be placed on the degree of GO sheet stacking and their conformation (open sheets or collapsed bundles) in the biological environment as major parameters that may affect their cellular uptake.

Intracellular Trafficking. Fate after cellular internalization is also of great importance in safety considerations, since it will determine the intracytoplasmic compartments (e.g., endoplasmic reticulum, Golgi, lysosome, mitochondria, and nucleus) within which interactions or accumulation of the nanomaterial will occur.

In the case of CNTs, we have been previously able to show the intracellular trafficking of chemically functionalized SWCNT and MWCNT and their accumulation in vesicular compartments of the perinuclear region.[13] Throughout our studies CNTs have not been observed inside the nucleus or in mitochondria, ruling out potential risk from interference with the genomic or respiratory blueprint of the cell. Using 3D electron tomography imaging, we have also reported that, at early time points, shortened ammonium functionalized MWCNTs were mainly entrapped into intracellular compartments,[10b] while after 14 days in phagocytic cells these nanotubes were able to escape from phagosomes, indicating that their capacity to pierce through plasma membranes could also persist intracellularly. Overall, intracellular trafficking will greatly depend on the ability of the material to interact with membranes. Beside their shape and aspect ratio, surface properties of CNTs are believed to be critical parameters. In addition, size and the individualization of CNTs are also key features for their intracellular trafficking. Shortened well-individualized, ammonium functionalized MWCNTs were shown to cross cell membranes and therefore escape endosomes much more readily. At the same time, we have also observed CNTs that remain entrapped in endosomal vesicles and might further be exposed to the lysosomal enzymatic digestion process.

Various cellular responses leading eventually to cytotoxicity can result from the membrane interaction, cellular uptake, and intracellular fate of carbon nanomaterial.[14] Induction of oxidative stress via generation of reactive oxygen species appears as the main toxicity mechanism that can trigger inflammatory, genotoxic, and cytotoxic damages. Dimensions, metal impurities, colloidal properties, and surface chemistry (including oxidative defects)[15] have been identified as key physicochemical features that can drive biological responses.[14a] Published reports on the

intracellular trafficking of GO are almost absent from the literature currently, and this may be because of the challenging task to image and visualize graphene sheets intracellularly. Even though a comparison between CNTs and graphene at this stage may be premature, the chemical functionalities at the GO surface are predominantly negatively charged (hydroxyl and carboxyl groups) and their interaction with the cellular membranes should be expected to be different than that of CNTs. Moreover, the question of whether the shape and structure of graphene sheets remains intracellularly will need to be addressed.

3. Safety Considerations at the Tissue Level

Unintended exposure or intended administration of carbon nanomaterials may result in either elimination from the body through physiological processes (glomerular, GI tract) or accumulation within tissues. In the first case of elimination, acute barrier and tissue damage should be considered. In the second case, identification of tissues where material deposition mainly occurs, residence time of accumulation, and any unwanted effects due to contact with host cells that may potentially induce local histopathological responses have to be investigated.

Hemocompatibility and Hematotoxicity. Once in the bloodstream, one important issue to be considered is the pro-thrombotic activity of the nanomaterials. In a well-designed study, the pro-coagulation activity of MWCNT has been reported in vitro, concluding that it may be enhanced by amidation or carboxylation.[16] In the same study, MWCNTs activated platelets in vitro, with amidated MWCNTs exhibiting greater platelet activation than carboxylated or pristine MWCNTs. However, opposite results were reported in vivo with functionalization diminishing pro-coagulant activity. In another study, the coating of SWCNTs with human serum proteins was shown to improve their blood compatibility.[17]

The interaction of graphene oxide with human red blood cells (RBCs) has also been investigated.[18] Hemolytic activity was reported with small graphene oxide sheets, however, improving their dispersion using chitosan almost eliminated this. A different study also described the hemolytic activity of GO at doses as low as 2−10 μg/mL,[19] in contrast to amidated GO shown to be hemocompatible even at doses up to 50 μg/mL. No pulmonary thrombosis was observed when the amidated GO were injected into mice compared to native GO. Zhang et al. observed no hemolysis or change in the shape of erythrocytes after 4 h of incubation with 10 μg/mL GO,[20] but at higher concentrations (80 μg/mL) RBCs were ruptured. Sasidharan et al. also studied the hemocompatibility of graphene and acid-treated graphene,[21] reporting compatibility with RBCs.[21] Singh et al. studied the effect of GO on platelets in vitro,[22] with no LDH release observed with large (0.2−5 μm) 2−3 layered GO sheets, but reported the production of reactive oxygen species was increased in a concentration-dependent manner after 15 min of incubation. In addition, the same authors in a different study showed that native GO but not amidated GO caused platelet aggregation.[19] Overall, it appears that amidation of CNTs and graphene leads to a significant improvement in the hemocompatibility and hemotoxicity profile of both types of material, but more systematic in vivo studies are required.

Tissue Distribution (including Excretion and Organ Retention). In the past few years, we have attempted to identify the design parameters and factors that determine tissue distribution of carbon nanotubes. The intravenous injection of different CNT types has indicated that purified, pristine MWCNTs coated with serum proteins mainly accumulate in the lungs, liver, and spleen.[23] On the other hand, covalently functionalized MWCNTs with increased degree of surface functionalization can maintain individualization of the nanotubes in vivo. The higher the degree of amino-functionalization, the lower the tissue accumulation and the higher their urinary excretion obtained.[24] Throughout these investigations, no histological or physiological damage of major tissues (kidneys, liver, spleen, and lungs) in which CNTs transited through or accumulated have been observed.

The in vivo tissue distribution and excretion studies using graphene are very limited and at the proof-of-concept stage. Zhang et al. intravenously administered single-layered GO sheets of 10−800 nm in lateral size using mice.[20] No pathological alterations were observed with the low dosing (1 mg/kg) after 14 days postinjection. However, at high dose (10 mg/kg), lung accumulation and its slow clearance caused granulomatous lesions, pulmonary edema, inflammation, as well as fibrosis. Yang et al. looked into the pharmacokinetics of PEGylated graphene oxide sheets in tumor-bearing mice, reporting high tumor accumulation.[25] The same group subsequently studied the long-term biodistribution of ^{125}I-labeled PEGylated GO sheets of 10−30 nm reporting accumulation in the liver and spleen after intravenous administration that was gradually cleared by both renal and fecal excretion.[26] No changes in blood biochemistry, hematological analysis, and histology of organs were observed, even after 90 days postinjection. A different study looking into the biocompatibility of GO after intravenous administration in mice injected various doses of GO (0.1, 0.25, and 0.4 mg).[27]

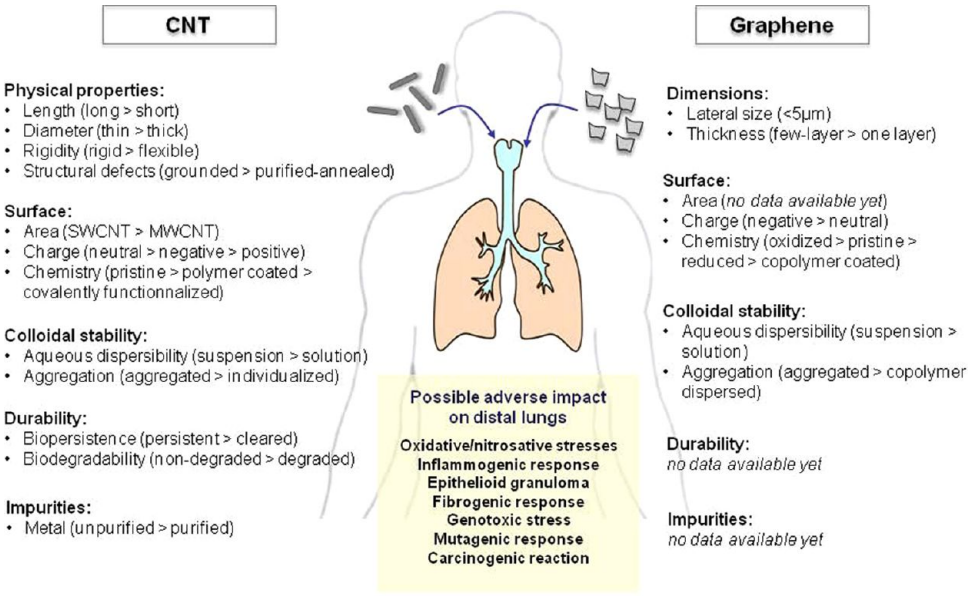


FIGURE 2. Physicochemical characteristics of CNTs and graphene relevant to pulmonary exposure and adverse effects. The first characteristic in brackets induces the most severe biological response.

In the low and medium dose regime, the animals did not show any signs of toxicity. However, at the highest dose, chronic and severe toxicity in four out of nine mice was observed, manifested by a dose-dependent inflammatory response in the lung and the formation of granulomas and lesions.

Even with the very limited number of in vivo studies available, it has become apparent that graphene materials do not exhibit a common distribution profile. Some of the critical factors to consider here will be the lateral size and surface modifications of the graphene sheets.[28] Individualization of the material is also a critical parameter that influences pharmacokinetics. Inadequate individualization of materials leading to poor dispersibility will tend to form aggregates in vivo, and compromise their blood circulation and their ability to interact with biological barriers (such as the glomerular filter). Structural features (size, shape) and surface properties (surface charge, functionalization, lattice defects) of graphene will influence their in vivo degree of individualization from intact, separate entities to aggregates or stacks of graphene sheets.

4. Safety Considerations at Whole Body Level

Exposure to graphene will involve two major aspects that will determine their overall safety at the systemic, whole body level: immunoreactivity and inflammogenicity. Even though scarce data exists for graphene on the safety considerations at this level, we will attempt to offer speculative comparisons based on the knowledge accumulated for carbon nanotubes.

Immunoreactivity. In one of the first studies to be performed on the immunogenicity of chemically functionalized CNTs, ammonium-functionalized CNTs were exposed to B and T lymphocytes and also macrophages.[29] No cytotoxicity and no effect on lymphocyte activation or macrophage secretion were detected for the cells exposed to ammonium-functionalized CNTs. MWCNTs functionalized by either oxidation or oxidation followed by the 1,3-dipolar cycloaddition reaction (ammonium functionalization) were all found to be nontoxic following interaction ex vivo with different human immune cells (i.e., B and T lymphocytes, natural killer cells, or monocytes).[30] On the other hand, two groups have reported that the presence of CNTs (either pristine or acid-purified) in the lung lumen (introduced via instillation or inhalation to mimic occupational exposure) could induce a decrease in splenic T cell proliferation, due to activation of cyclooxygenase enzymes in the spleen, in response to signaling from the lungs.[31] Taken together, these findings demonstrate the advantage of chemical functionalization over pristine or purified materials. Figure 2 schematically depicts a correlation of the possible physicochemical characteristics of CNT and graphene in the context of lung exposure.

Inflammation and Carcinogenicity. Most of the studies performed to determine the inflammatory profile of CNTs are based on the pathogenic fiber paradigm.[32] According to this, fibers which are long, thin, and biopersistent are likely to induce inflammation if they remain in the lungs and pleural cavity. Considering the well-known pathogenic

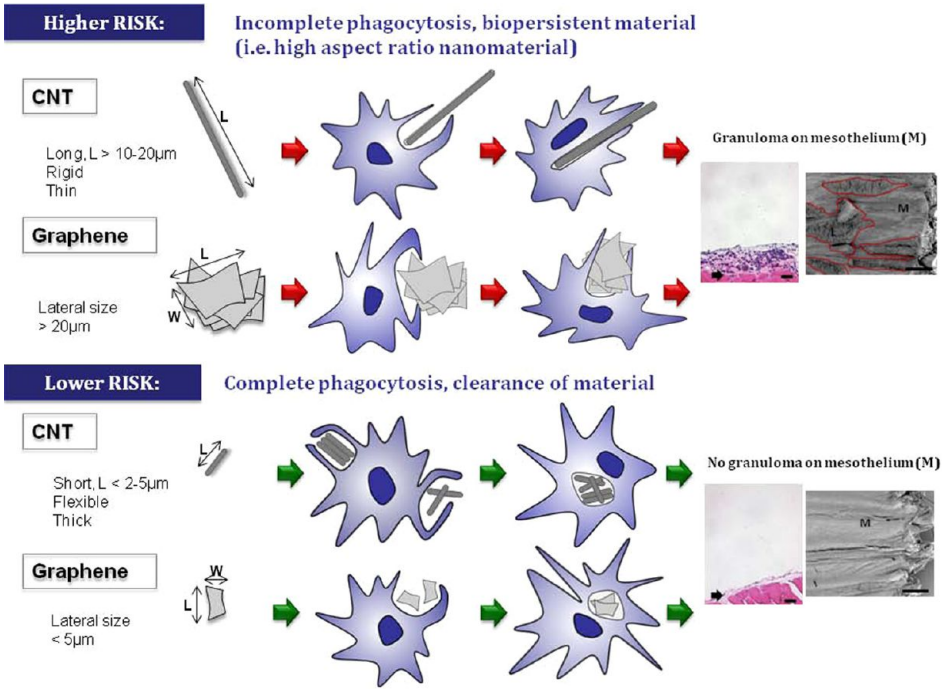


FIGURE 3. Inflammation and carcinogenicity (higher and lower risk) based on macrophage responses. The key physicochemical characteristics to determine the potential risk of CNTs (length, diameter, and stiffness) and graphene (lateral dimension) are highlighted.

effect of long asbestos fibers on lungs and the mesothelium, the comparison has raised concerns about a similar carcinogenic risk upon unwanted inhalation of CNTs.

Compared to short entangled CNTs, nonfunctionalized CNTs that were long and rigid injected intraperitoneally were found to induce frustrated macrophage phagocytosis, leading to local inflammation followed by formation of mesothelial granuloma (Figure 3).[32b] We previously demonstrated using whole body imaging that short, chemically functionalized CNTs can rapidly migrate from the pleural space.[33] We have also confirmed that the pathogenicity of the long fibers is a result of length-dependent retention on the parietal pleura that can lead to localized inflammation, granuloma, and fibrosis. From such studies, chemically functionalized MWCNTs exhibit a safer profile compared to their long pristine counterparts (>15−20 μm). In a separate study using nonfunctionalized CNTs of different diameters and rigidities,[34] the deleterious effect of CNTs on mesothelial cells was also associated with the injury of the cell membrane. Thick and entangled MWCNTs were found to be safer compared to thin and rigid nanotubes that were more inflammogenic and of higher carcinogenic risk.

Regarding whole-body in vivo toxicity studies using graphene, Duch et al. showed that a better dispersion of graphene with Pluronic decreased peribronchiolar fibrosis,[35] using aggregated and oxidized graphene. Schinwald et al. studied the respiratory risks associated with large, unmodified graphene nanoplatelets (multiple stacks of graphene) of 25 μm in diameter reporting that these nonfunctionalized,[36] respirable graphene particles were inflammogenic to the lungs and the pleural space. Very recently we reported a methodology for the production of well-dispersed purified graphene oxide sheets in biological media with no evidence of in vitro cytotoxicity and in vivo pathogenicity.[37]

5. Degradation and Biopersistence

Tissue accumulation and clearance of graphene are two processes that, from the safety consideration point of view, are closely related to biodegradation. If carbon nanomaterials are non-biodegradable, their safety profile will be risk-free only if body clearance and excretion of the vast majority of the administered dose are achievable. In contrast, if carbon nanomaterials are readily biodegradable, clearance may not be needed, provided that any degradation by-products will not be toxic. As mentioned in Section 3 of this Account, elimination from the body after systemic administration has been observed for specific types of CNTs[23,24] and graphene.[20,26] In both cases, surface chemistry and size of the materials determined their degree of excretion. In general, eventual biodegradation or elimination of those

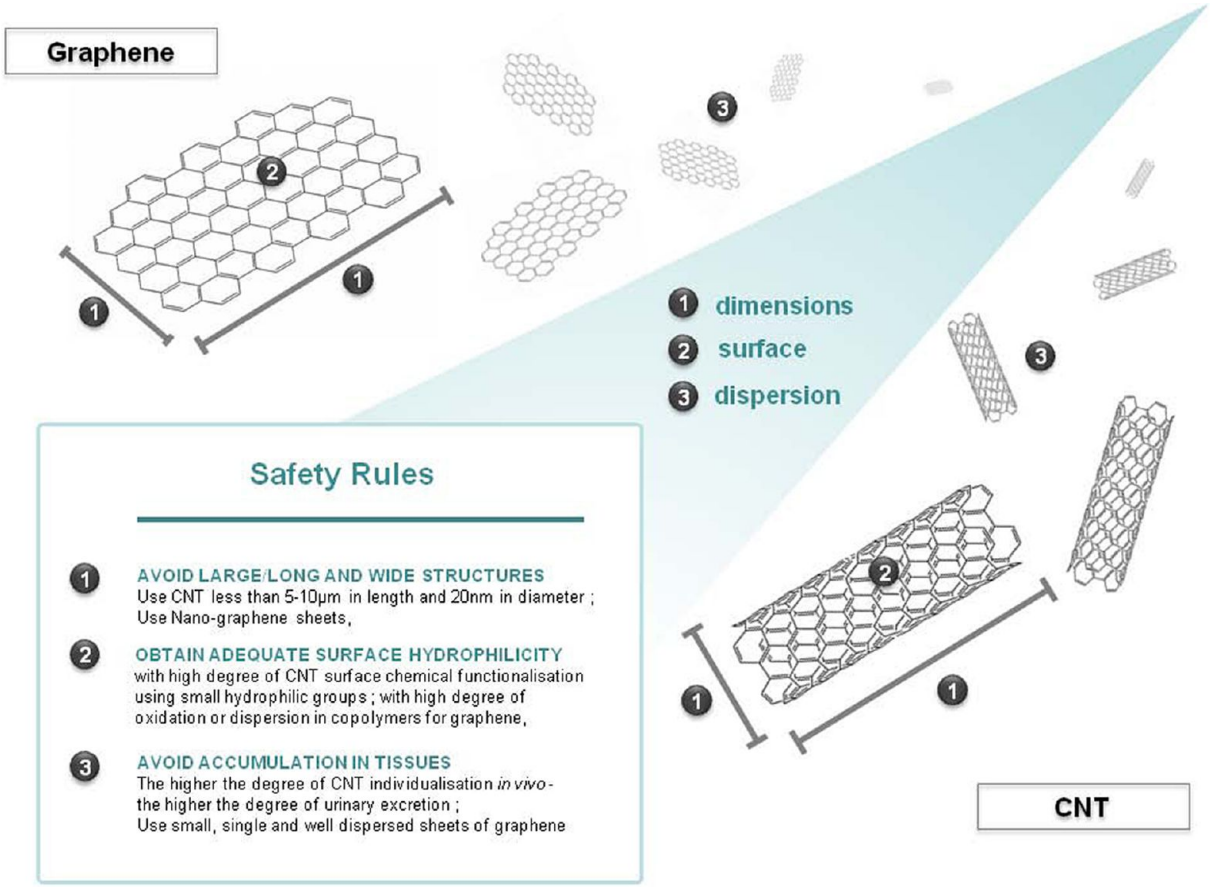


FIGURE 4. Primary safety rules for the development of CNT and graphene.

materials is seen as the ideal scenario for human exposure, especially toward their biomedical use.[38]

A few studies have investigated the critical physicochemical parameters that would allow degradation of CNTs. Using in-test-tube (acellular) methods,[39] it has been demonstrated that CNTs (SWCNT and MWCNT) could be degraded by horseradish peroxidase (HRP) enzyme in the presence of low concentration hydrogen peroxide. More relevant to human exposure, CNT biodegradation also occurred inside isolated human phagocytes (in neutrophils and to a lesser extend in monocyte derived macrophages) for engulfed CNTs, probably inside phagolysosome.[39] Very recent data have also reported the occurrence of in vivo CNT degradation in lung[40] and brain[41] taking place inside resident or recruited macrophages that engulf the material after local administration.

In-test-tube studies have also described the enzymatic oxidation of single layer of GO by HRP which resulted in the formation of holes on its basal plane.[42] Interestingly, reduced graphene oxide (RGO) was not oxidized by HRP which was thought to be due to orientation of the HRP around the basal plane instead of the edges of the GO and RGO. This is the only study that investigated the degradation ability of GO, and more systematic work is warranted to assess the degradation kinetics of the material in vitro and in vivo.

6. Conclusion

Safety Considerations for Graphene. Consideration of the physicochemical features of graphene and its derivatives is characterized by a lower aspect ratio, larger surface area, and overall better dispersibility in most solvents compared to CNTs. However, the studies that compare graphene with CNT are very rare, making comparative considerations of their overall safety and risk assessment challenging. Dimensions, surface chemistry, and impurities are equally important for graphene and CNTs to determine both mechanistic (aggregation, cellular processes, biodistribution, and degradation kinetics) and toxicological outcomes. The fact that colloidal dispersions of individual graphene (or graphene oxide and its derivatives) sheets can easily be engineered devoid of metallic impurities, with high stability and less aggregation, and the fact that graphene

nanostructures are not fiber-shaped can theoretically offer significant advantages in terms of safety over inhomogeneous dispersions of fiber-shaped CNTs. Nevertheless, a lot of work is needed to: (a) elucidate that desired function using graphene is preserved or improved compared to CNT, and (b) determine any safety risks associated with these planar sheet nanoscale structures.

Despite the structural differences between graphene and carbon nanotubes, we believe that invaluable lessons have been learnt during the development of carbon nanotubes in the past decade that can guide the safer development of graphene. In Figure 4, we attempt to offer a set of three primary rules that can be applied in the development of graphene-based biomedical applications to enhance the overall safety from exposure of the material and minimize the risks for adverse responses: (1) use of small, individual graphene sheets that macrophages can efficiently internalize and remove from the site of deposition; (2) use of hydrophilic, stable colloidal dispersions of individual graphene sheets to minimize aggregation in vivo; and (3) use graphene material that can be excreted or chemically modified graphene that can be degraded effectively. Obviously, such "rules" can only act as guidelines at this infant stage in the development of graphene-based technologies.

Considering that safety risks will be entirely dependent on the specific properties, characteristics, and use of the material in each study, generalization about the toxicity profile of genres of materials needs to be avoided. Systematic studies designed to reveal both the biological responses to graphene and their correlation with key physicochemical material properties related to toxicity (structure, surface, and colloidal properties) are very much needed. These future findings will be essential for the design and manufacturing of safe, or more biocompatible, graphene-based materials used in applications that consider unintended or intentional human exposure. Research efforts should also be devoted to reveal the long-term adverse health impact and long-term fate of those materials in the environment. These long-term aims are crucial to accurately determine risks to public health and safety in all possible exposure scenarios (i.e., environmental, occupational, or user exposures).

BIOGRAPHICAL INFORMATION

Dr. Cyrill Bussy obtained a Ph.D. in Toxicology from the Université Paris-Diderot. After a postdoctoral research at the UTC (UMR7338) and the INSERM (U955), he joined the Nanomedicine Laboratory at UCL as a Marie Curie Fellow.

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FOOTNOTES

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The authors declare no competing financial interest.

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