International Journal of Advances in Pharmaceutics 2 (1) 2013

International Journal of Advances in Pharmaceutics

Journal home page:http://ijap.ssjournals.com

Review Article

Carbon Allotrope Graphene: Superstar In Nano- World

Shashi Kiran Misra[*1] and Himanshu Pandey[2]

[*1]*Lecturer, Uni. Inst. of Pharmacy, C.S.J.M. University, Kanpur , 228002,UP, INDIA*

[2]*Associate Professor, Department of Pharmaceutical Sciences, Faculty of Health Sciences, SHIATS, Allahabad, UP*

***Correspondence Info:**

Shashi Kiran Misra
Lecturer,
Uni. Inst. of Pharmacy,
C.S.J.M.University. Kanpur ,
228002, UP, India
Email: saushi123@rediffmail.com

Keywords:

Graphene,
Nanocarrier,
Cytotoxicity,
Nanomaterial,

Abstract

Graphene is a substance made of pure carbon, with atoms arranged in a regular hexagonal pattern similar to graphite, but in a one-atom thick sheet. It is an allotrope of carbon whose structure is a single planar sheet of sp^2-bonded carbon atoms that are densely packed in a honeycomb crystal lattice.Hence, graphene may be considered as the mother of graphite, fullerene and carbon nanotubes. Graphene can also be considered as the final member of the series of fused polycyclic aromatic hydrocarbons, such as naphthalene, anthracene and coronene It has many exceptional featureswhich make it a superstar in the world of nanotechnology as thinnest material, practically transparent (3,000,000 sheets equal to 1mm), stiffest, strongest (Young's modulus >0.5–1 TPa, tensile strength ~130 Gpa) largest surface-to-weight ratio (~2,700 m2/gram) very stretchable (stretch up to 20%), conducts heat and electricity better than any metal, impermeable to gases, large specific surface area, non-toxic, low cost and drug can attach on both sides of its sheet. Graphene can be successfully used as a non-toxic nano carrier for efficient gene transfection, a novel gene delivery,nano-vector with low cytotoxicity and high transfection efficiency, which is promising for future applications in non-viral-based gene therapy. Experimental studies have demonstrated that the shape of carbonaceous nanomaterials plays an extremely important role in how they interact with cells and potentially other biological systems, such as tissues and organisms. The cytotoxicity of graphene depends on the exposure environment and mode of interaction with cells as bacteria came directly contactwith graphene, intensive physical interactions between graphene and bacterial cells may cause physical damages on cell membranes, and result in the release of intracellular contents and cytotoxicity and genotoxicity occur.

1. Introduction

Amongnumerous commercial endeavors, nanotechnology is regarded as the key technology of the 21st century. It provides novel products and facilitates applications of innovative techniques in medicine, pharmacy, computer technology, and sensing. Therefore, it holds promise for potential global socio-economic benefits. In 2011, there were about 1100 commercial products that include nanomaterials.The interesting physical property of graphene, a novel one-atom-thick two-dimensional graphitic carbon system, has led to much excitement in recent yearsin material science and condensed-matter physics. Potential applications of graphene for nanoelectronics, sensors and nanocomposites have been actively pursued. The biological application of graphene and graphene oxideremains unexplored andwide-open[1]. There are several prerequisites for biological applications for a new material. First, rational functionalization chemistry is needed to impart graphene aqueous solubility and biocompatibility. Graphene oxide and its chemically converted derivatives form stable suspensions in pure water but aggregate in salt or other biological solutions. Second, graphene sheets with suitable sizes are desired. Size control or size separation at various length scales is necessary to suitably interface with biological systems *in vitro* or *in vivo*. Graphene and graphene oxide samples thus far aretypically microns or larger in size. Lastly, little is known experimentally about the properties of graphene with molecular dimensions, on the order of ~10nm or below. The optical properties of graphene and graphene oxide are largely unexplored, a topic offundamental interest and could facilitate biological and medical research such asimaging. It is a common belief that nanotechnology could assist in solving many

global problems that society faces. These include environmental and health concerns of the fast-growing human population, as well as access to clean waterand affordable energy[2]. The quickly growing applications of nanomaterials are due to their unique properties which offer advantages over conventional materials. Since the 2010 Nobel awards validate the importance of graphene not only in basic research but also in various commercial applications .The demand for carbon nanostructures, particularly graphene, is increasing rapidly in electrical, mechanical, and biomedical applications. This is due to their outstanding thermal, electrical, mechanical, optical and other unique properties[3].

2. Graphene And Its Congeners

Graphene, a 2D carbon nanomaterial with a honeycomb-like structure, has been the subject of a considerable interest after being the subject of the 2010 Nobel Prize for Physics. Its unique properties, including ballistic electron transport at room temperature, tunable band-gap (for few-layer graphene), high chemical and mechanical stability, low electrical noise, high thermal conductivity, and bio compatibility, have led it to be used in many advanced devices ranging from ultra capacitors to spintronic devices. Although the intense interest and continuing experimental success of graphene-based devices facilitate their various applications, the reliable production of high quality samples of graphene on a large scale is very difficult. At present, great efforts have been made toward the preparation of graphenenanosheets[4]. Grapheme is a single atomic plane of graphite (Gt), which was first obtained through micro mechanical exfoliation of Gt. Graphene oxide (GO) is a graphene sheet with carboxylic groups at itsedges and phenol hydroxyl and epoxide groups on its basal plane. Graphene oxide can be chemically exfoliated from graphite oxide (GtO).

Thermal annealing or chemical treatment can eliminate functional groups on GO to produce reduced graphene oxide (rGO). Among them, the chemical reduction of exfoliated graphene oxide (EGO) is the most commonly used approach due to its low cost for large-scale production[5]. In the case of carbon nanotubes, closely related to graphene, controlling their size and diameter is still very challenging. The availability of carbon nanotubes, both in quality and quantity, has stimulated the worldwide pursuit of carbon nanotubes for technological applications. The morphology of graphene is different from that of carbon nanotube ; for example, the length of CNT influences their toxicity but graphene and graphene oxide (GO) do not have a ‘length’’ An important similarity between these carbon nano materials is that both graphene/graphene oxide and carbon nanotube structures vary according to the synthetic processes employed. Such processes can also change their physical properties, including dispersity, surface functionality, and their toxicity. In the materials science world, carbon nano structures such as fullerenes, carbon nano tubes and graphene are famous for their small dimension and unique architecture, and several possible applications in diversified areas. These graphene related materials exhibit unique electronic, thermal, and mechanical properties,and hold great promises in potential applications, such as nanoelectronics, conductive thin films, supercapacitors, nanosensors and nanomedicine. To realize their potentials, health and environmenta limpacts of graphene related materials should be thoroughly evaluated[6].

2.1 Nanotoxicity Of Carbon Allotrope Graphene

Compared to other synthetic carbon nanomaterials, such as fullerenes and carbon nanotubes, it was reported that GO and rGO exhibit strong antibacterial activity. The antibacterial activity of GO and rGO has been attributed to membrane stress induced by sharp edges of grapheme nanosheets, which may result in physical damages on cell membranes, leading to the loss of bacterial membrane integrity and the leakage of RNA. On the other hand, it was proposed that graphene may induce oxidative stress on neural phaeochromocytoma derived cell. The antimicrobial activity of graphene has been found to be the synergy of both “physical” and “chemical” effects. When bacteria directly contact with graphene, intensive physical interactions between graphene and bacterial cells may cause physical damages on cell membranes, and result in the release of intracellular contents. Grapheme may chemically increase cellular oxidative stress, which could disrupt a specific microbial process. If graphene- based materials share a similar antibacterial mechanism as that of material characteristics which influence how graphene-based materials physically interact with bacterial cells, such as solubility,dispersion, and size, should strongly influence their antibacterial activities. Moreover, material properties,which control their abilities in producing cellular oxidative stress, should also have a strong impact on their antibacterial activities[7].

The diagnostic and therapeutic applications of graphen ebased materials mentioned above will only be trialed clinically after detailed information on their environmental and health and safety effects in host biological systems. A few preliminary tests have showed that graphene are biologically benign to certain cells, tissues, and organs under limited conditions, while further studies have indicated that graphene are potential hazards that can cause both acute and chronic adverse effects to many living systems. Nevertheless, at this stage, it appears that the biological effects of graphene are sample specific and must be assessed on a case-by-case basis. The nanotoxicity of graphene, therefore, requires continuing

and extensive investigations and, indeed, this will be required by regulatory bodies before graphene can be used in clinical environments as functional biomaterials and biomedical devices[8]. Despite several years of research, definitive findings regarding the extent of toxicological risks arising from using nanotubes are far from complete. Continuing research is required to determine, for example, how graphene enter cells, where graphene are internalized, which the cytotoxic mechanisms are relevant, and how the nanotoxicity is affected by a variety of physicochemical characteristics, such as diameter, length, and presence of impurities, surface functionalization, and surface wettability[9].

2.2 Mechanism Of Cellular Uptake Of Graphene

The uptake of graphene into cells plays a critical role in determining their cytotoxicity and genotoxicity. The outermost layer of the cell, the cellular membrane, consists of a phospholipid bilayer, which serves to segregate the subcellular compartments from the external medium, and to regular the transport of foreign materials, including graphene into cells. Experimental results indicate that graphene can be internalized by a variety of cells. Although systematic knowledge is still lacking, it is in general considered that there are two possible pathways for graphene to cross the cellular membrane and enter cells. One pathway is passive transport,which includes diffusion, membrane fusion, and direct pore transport. Individually dispersed graphene in aqueous solutions have been experimentally demonstrated to be able to enter the cytoplasm of cells by directly crossing the membrane, despite recent modeling showing that the energy cost of entering the cellular membrane via rupture and diffusion was high compared to that of the energy of thermal motion of graphene.A more common pathway for the cellular uptake of graphene is active transport via *endocytosis*, which includes phagocytosis and pinocytosis. Endocytosis involves the enclosing of foreign objects in vesicles orvacuoles pinched off from the cellular membrane.

In general, long graphene (>1 μm in length) were taken up by phagocytosis,which was mainly conducted by macrophages, monocytes, and neutrophils. Shorter graphene of length from a few to several hundred nanometers, on the other hand, were mainly internalized by pinocytosis, such as macropinocytosis, clathrin-mediated endocytosis, and caveolin-driven endocytosis. Endocytosis is an energy-dependent process,and the orientation of graphene entry can be controlled bythe interplay between the tip recognition through receptorbinding and the rotation driven by asymmetric elastic strain at the nanotube-phospholipid bilayer interface, as demonstrated recently by numerical modeling. Inthe most common case, a near-perpendicular orientation resulted in a minimum energy barrier. The exact cellular uptake pathway of graphene is complex and depends on many experimental parameters, such as the size, length, hydrophobicity, surface chemistry, and the cell culture medium. Additionally, the hydrophobic surface of graphene can interact with components in cell growth medium and affect the cellular uptake. Serum proteins in the cell growth medium can bind to graphene walls through π-π interactions or electrostatic attractions, forming a protein coating. The "screening effect" of such protein coatings, known as the "protein corona," allows functionalized graphene to experience a similar cellular uptake pathway. The cytotoxic effect of size and shape of graphene is best represented by their high aspect ratio, which results in incomplete phagocytosis by the mononuclear cells because the graphene are too large. This induced incomplete or frustrated phagocytosis can result in macrophage activation and granulomatous inflammation[10]. In fact, it has been hypothesized that the failure of resident macrophages to clear graphene is the main reason for the activation of pro inflammatory pathways that induce lung fibrosis, lung cancer, and malignant mesothelioma. In addition, the aggregation of graphene by van der Waals' interactions could also affect profibrogenic cellular responses and contribute to the pulmonary toxicity of graphene *in vivo*[11].

The cytotoxicity level of graphene to that of carbon nanotubes founded that toxicity was shape and composition dependent, with graphene overall having a lower toxicity than CNTs; however the toxicity of graphene was curiously found to be inverse to concentration, with graphene exhibiting a higher toxicity than CNTs at low concentrations[12]. However, sharp graphene nanosheet edges have been shown to cause considerable damage to the cell membrane of bacteria,although this antibacterial property has the potential tobe useful. Moreover, hydrophilic carboxyl-functionalized graphenes have been shown to be able to be internalized incells without any toxic effects, in contrast to hydrophobic pristine graphene.

2.3 Biodistribution And Nanotoxicity Of Graphene In Other Organs

Many *in vivo* studies have shown that graphene delivered to a specific area in the body are not confined to that area. For example, intravenously injected graphene were shown to be taken up both by the liver and the spleen and then excreted rapidly through the kidney. Because of the migration of graphene in biological systems,their toxicity to a variety of other organs should also be tested. In many cases, macrophages, which form the first line of defense against foreign materials, will interact with the administrated grapheme [13]. This is why macrophages are one of the mostly studied cells in *in vitro* investigations of graphene toxicity. After graphene have been ingested by macrophages,they can enter into the blood and lymph circulation at alater stage. Carbon nanotubes can also be dispersed by mucins (glycosylated proteins produced by

epithelial tissues)in certain cases and cleaned away in a physiological solution,before they can interact with cells[14].

3. Future Prospects

As mentioned above for any biological-related application, particularly *in vivo* applications, great care must be taken to ensure that the toxicity of the nanomaterial is well characterized and understood. There are many challenges ahead that must be addressed before graphene and its derivatives can be successfully integrated into biomedical devices and technology. The main advances required advanced techniques and facile methods increase the sensitivity of grapheme biosensors towards single-molecule detection followed by more efficient loading and unloading methods for drug delivery would refine overall performance of carbon allotropeas carriers. Further research is required into various carbon allotropes to promote cell adhesion, growth, differentiation,and proliferation. The novel graphitic nanostructures, combined with multi-functionalities including biocompatibility, photoluminescence and drug loading and delivery, suggest promising applications of graphene materials in biological and medical areas.

4. Conclusion

Having unique mechanical, electrical, optical, and thermal properties, graphene and its congeners show great promise for advancing the fields of biology and medicine. Many reports have demonstrated that of these carbon nanostructures and their hybrid structures (composites with polymers, ceramics, and metal nanoparticles) for a variety of biomedical areas ranging from biosensing, drug delivery, and diagnostics, to cancer treatment, tissue engineering, and bioterrorism prevention. However, the issue of the safety and toxicity of these carbon nanostructures, which is vital to their use as diagnostic and therapeutic tools in biomedical fields, has not been completely resolved. Nanoscalegraphene and graphene oxide have immense potential in nanomedicine as biocompatible and supportive substrates, and as a novel tool for the delivery of therapeutic molecules. Graphene can be successfully used as a non-toxic nano-vehicle for efficient gene transfection, a novel gene delivery nano- vector with low cytotoxicity and high transfection efficiency, which is promising for future applications in non-viral-based gene therapy.

Acknowledgement

I would like to acknowledge Dr. HimanshuPandey(Department of Pharmaceutical Sciences, Faculty of Health Sciences, SHIATS, Allahabad, UP for his valuable information and technical assistance.

References

1. Jain S, Singh SR, Pillai S Toxicity Issues Related to Biomedical Applications of Carbon Nanotubes, *J Nanomed Nanotechol* 2012, 3(5): 140-155.
2. Yi Wang *et al*., Controlled drug release characteristics and enhanced antibacterial effect of graphene oxide/drug intercalated layered double hydroxides hybrid films , *J. Mater. Chem*., 2012. (online)
3. Tapan Kumar Das, *et al*., Graphene: A Revolution in Nanobiotechnology, *Journal of Research in Nanobiotechnology* (2012) 1(1): 019-030.
4. Zhuang Liu, *et al*., PEGylated Nanographene Oxide for Delivery of Water-Insoluble Cancer Drugs, *J. Am. Chem. Soc*. 2008, *130*: 10876–10877.
5. Shaobin Liu, *et al*., Lateral Dimension-Dependent Antibacterial Activity of Graphene Oxide Sheets, *Langmuir* 2012, 28: 12364−12372.
6. Santos Catherine M , Mangadlao Joey, *et al*., Graphenenanocomposite for biomedical applications: fabrication, antimicrobial and cytotoxic investigations, *Nanotechnology*, 2012, (online)
7. Caitlin Fisher, *et al*., Applications and Nanotoxicity of Carbon Nanotubes and Graphene in Biomedicine, *J. Nanomaterials,* 2012, (online)
8. Mei Yang , Jun Yao *et al*., Graphene and its derivatives for cell biotechnology, *Analyst* , 2012, (online)
9. Sivasankar, M.,BogaPramod Kumar, Role of Nanoparticles in Drug Delivery System, *Int. J. of Res. Phar. and Bio. Sci.* 2010, 1 (2): 41-67.
10. Lovelyn, C., Attama, A., Current State of Nanoemulsions in Drug Delivery, *J. of Bio. and Nanobiotech*. 2011, 2: 626-639.
11. Jiraporn C.,Nanosuspension Technology for Drug Delivery, *Walailak J Sci & Tech* 2007; 4(2): 139-153.
12. Sailaja, A.K, Amareshwar, P, Chakravarty, P,Chitosan nanoparticles as a drug delivery system, *Res. J. of Phar., Bio.and Chem. Sci.* 2010, 1(3): 474-485.
13. Ochekpe, N.A, Patrick O,Ngwuluka, C., Nanotechnology and Drug Delivery Part 2: Nanostructures for Drug Delivery, *Tro. J. of Phar. Res.* 2009; 8 (3): 275-287.
14. Mohanraj V.J., Chen. Y., Nanoparticles – A Review. *Tro. J. of Phar. Res.* June 2006; 5 (1): 561-573.