Review Article
ISSN:2249-1236
CODEN: IJRRBI
Eng. Bassam Al-sharif *et al., Int*. J. Res. Rev. Pharm. Appl. Sci.,*(2023) 13 (1) 012-023*
International Journal of Research and Reviews in Pharmacy and Applied sciences

Potential applications of Graphene for antibacterial treatment

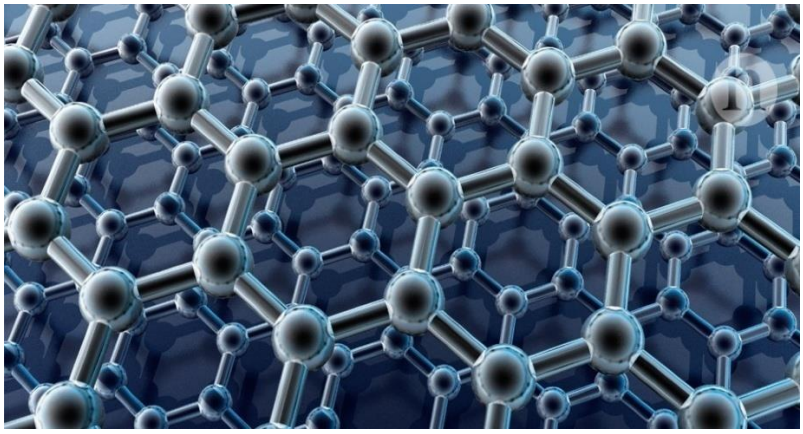
Eng. Bassam Al-sharif*
Eng. Basma Hiyari Eng.
Majid Alkhawaldeh,
Eng. Ali Alshboul
Dr. Ali Rababah

Corresponding author

Eng. Bassam Al-sharif

Article received:
26.04.2023

Article Accepted:
09.05.2023



Abstract:

Graphene, an exceptional single atom thick two dimensional nanomaterial with unique structural,electronic, and mechanical properties, has attracted a tremendous interest of researchers in many fields including biomedical applications, which concurrently raises disquieting questions, and a consequent growing concern about their potential toxicity to human and animal cells. In this article, a summary of some very recent studies is presented by describing the behavior of graphene and its derivatives both in vivo and in vitro as well as the potential mechanismsof toxicity proposed to explain this behavior. The bacterial toxicity of graphene and its derivatives is also reviewed suggesting their possible usage for antibacterial treatment. Moreover, this article reviewed the effect of functionalization of graphene on mitigating its toxicity which hold the promise of using these materials for in vivo applications. Further work aiming to examine the effects of structural and chemical variations of graphene based materials which result from processing and synthesis techniques on different living systems are needed for a better understanding of toxicity/biocompatibility of these materials.

Key words: Graphene, toxicity, bacteria, antibacterial treatment, graphene based materials, biocompatibility

Introduction:

Nanotechnology is a science that involves manipulating and control properties and structures at the nanoscale, at dimensions of roughly 1 to 100 nanometers. In the realm of medical science and technology, nanotechnology holds the promise of providing benefits for society in a wide range of medical applications including scaffolding in regenerative medicine and drug delivery systems. Graphene is a nanomaterial in the form of two dimensional atomic scale, hexagonal lattice, consisting of a single layer of sp2 network of carbon atoms as shown in figure 1b. Although scientists had theorized about graphene for decades, it was first obtained in the lab in 2003 through micromechanical exfoliation of graphite (Gt). This novel carbon based nanomaterials has attracted tremendous attention in the past few years due to its remarkable physical, chemical, and biological characteristics and hold great promise in potential applications such as lightweight, thin, flexible, yet durable display screens, field-effect transistors, solar cells and electric circuits as well as various industrial and chemical applications (1).

More recently, Ever-increasing attention has been paid to the graphene applications in biological and biomedical fields, such as biosensors, drug delivery, tumor therapy, and molecular imaging. This has been also accompanied by rapidly increasing interest in understanding the potential cytotoxicity of Graphene and its derivatives and their interaction with cell systems. The safe and efficient usage of Graphene based nanomaterials in biomedicine entails a detailed understanding, at the molecular level, of the interaction of these materials with biomolecules.There have been extensive studies recently to evaluate the in vitro and in vivo safety of Graphene and its derivatives.

For example, Akhayanet al. showed that the graphene oxide was more toxic to bacteria than reduced graphene oxide, and bacteria with outer membrane exhibit more resistance to the toxicity of these materials proposing the direct contact interaction with cell membrane as a mechanism of graphene toxicity (2). Wang and coworkers showed that graphene oxide exhibits dose dependent toxicity while exploring it both in vivo and in vitro (3). The following sections in this review summarize some recent in vivo and in vitro studies that investigate the

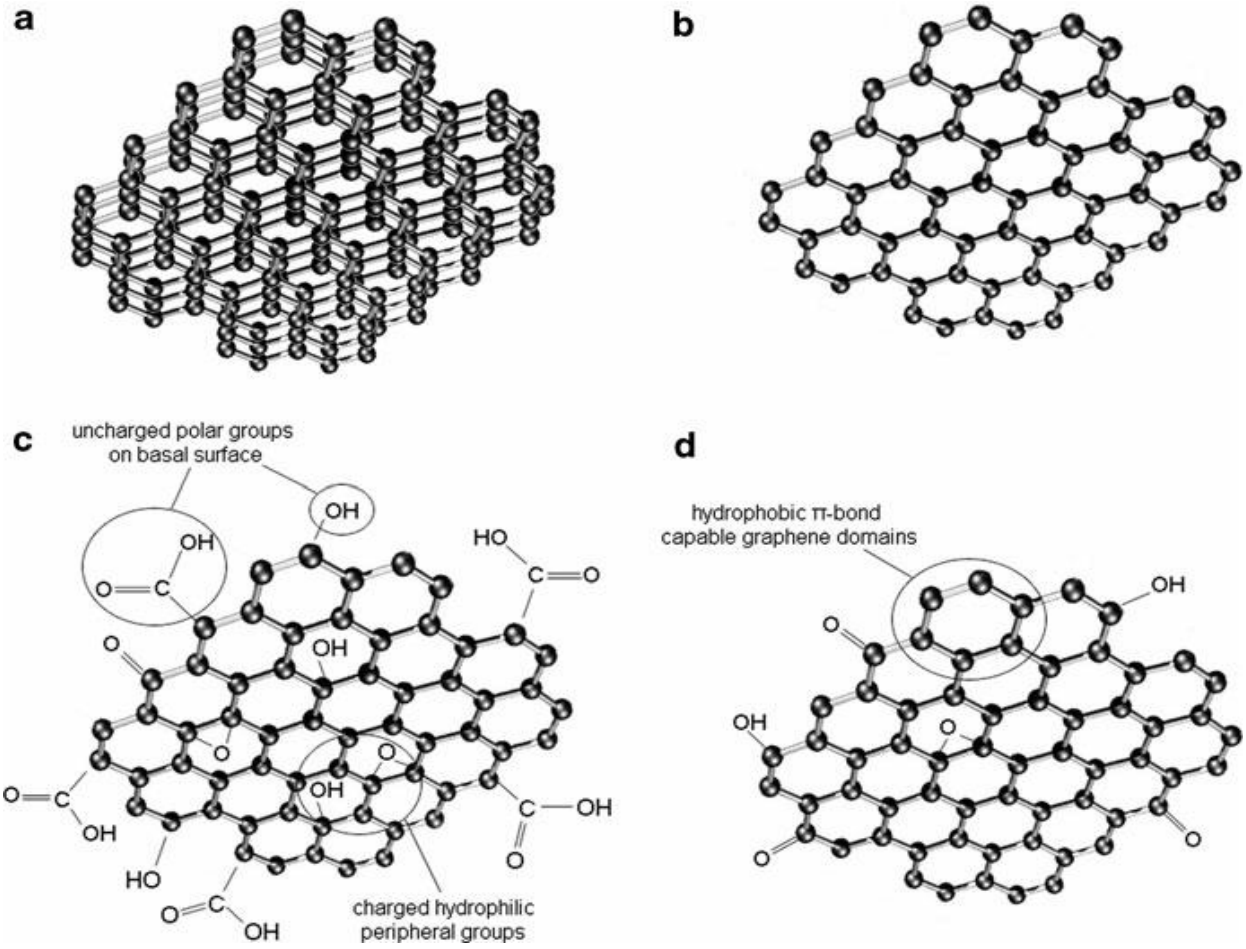


Figure 1The membrane of the graphene family materials: a) few layered graphene b)graphene nanosheet c) graphene oxide d) reduced graphene (4)

toxicity/biocompatibility of Graphene and its derivatives and the potential application for antibacterial means.

Toxicity to bacteria:

Bacteria can be classified into Gram-positive and Gram negative based on the structural differences in their cell walls. Cell wall impenetrability of Gram-negative bacteria made it less susceptible to destruction by antibodies in comparison to gram-positive bacteria (5). Due to the development of microbial strains caused by drug resistance, there is an increasing interest to evaluate and develop alternative methods for antibacterial treatment. Antibacterial properties ofcarbon (i.e., nanotubes and fullerenes) and diamond nanoparticles have been examined in the

past years (6, 7).More recently, it has also been reported that a new allotrope of carbon, graphene, is more effective than some currently used therapeutic antibiotics [8].

Akhayan et al. studied the bacterial toxicity of the graphene oxide nanowalls (GONWs) and thereduced graphene nanowalls (RGNWs)(look at figure 1c &d) on two types of bacteria, Escherichia coli which is a Gram-negative type, and Staphylococcus aureus strains which is a Gram-positive type. It is found that reduced GONWs was more fatal to bacteria than unreduced GONWs. The explanation for this behavior is that thereduction of GONWsby hydrazinegrants their edges some features including sharpness, and better ability to exchange charges with bacteria. Sharpness of the edges allows them to penetrate cell membrane easily. Simultaneously, charge transfer in both direction between bacteria and reduced nanowells at their sharp edges causes increased level of reactive oxygen speciesinside the cell.This leads to cell membrane destruction and consequently more toxicity of reduced type of graphene oxide to bacteria. Moreover,closelyinvestigating the effect of nanowells on Escherichia coli and Staphylococcus aureus by performing biological assays to measure the efflux of RNArevealed the effectiveness of these materials in bacterial inactivation. However, it is noticed that the destruction of cell membrane of Escherichia coli bacteria was less than that of Staphylococcus aureus. This can be attributed to the structure of Staphylococcus aureus lacking the outer membrane in comparison to Escherichia coli owning this membrane (3).

Liu et al.studied and compared the bacterial toxicity of four derivatives of graphite; Graphite (Gt), and its oxide form (GtO), as well as graphene oxide (GO), and its reduced form (rGO)on Gram-negative Escherichia coli. Colony counting method based on estimating the number of viable cells that is able to form colonies in samples containing one of these materials and prepared at the same condition, showed the highest efficacy of GO in inactivating Gram-negative Escherichia coli. Reduced graphene oxide was in the second rank, followed by graphite and finally graphite oxide which exhibited the least effect on bacteria among all other materials. Scanning electron microscope (SEM) images highlight the destruction of bacterial cells following their interaction with graphene-based materials (9).

The XTT tests show that none of graphene based materials exhibits toxicity as a result of producing hyperoxides. This is because none of these materials produced this toxic anionduring interaction with bacteria. On the other hand, adding glutathione, which is an antioxidant, to the samples showed the ability of each material to oxidize glutathione. It can be concluded that the interaction between bacteria and graphene based materials which leads to membrane destruction can be explained as a result of both direct contact and oxidative stress mechanisms of toxicity. The authors proposed that the toxicity of these materials takes place in several stages, starting by deposition of cell on the material surface, and followed by applying stress on the membrane as a result of nanosheets penetration and culminating with oxidative stress caused by charge transfer between sharp nanosheets and membrane.Authors also proposed that changing physiochemical features of graphene based materials using different processing techniques that allow adding functional groups or changing the conductivity can be of clinical value and can allow introducing a graphene materials as a strong candidate for biomedical application(9).

Although the above mentioned studies suggest the applicability of graphene in the field of bacterial treatment in the near future, but recent studies have indicated thatgraphene oxide is not toxic andcause a growth of bacteria in comparison to samples with control substances (10, 11).For these debatableresults, it may be the differences in production methods, processing of the surface and other features of GO that cause this controversial biological behavior. It is clear that further studies should be conducted more carefully for a better understanding of the detailed mechanisms and regulating factors influencing the interactions between graphene materials andbacteria.

In vitro studies:

Given that graphene and its derivatives have caughtsignificant interest for their potential biomedical applications such as drug delivery, tissue engineered scaffold, bio sensing and so on,scientific analysis of their biosafety in cultured mammalian cells becomes critically important. Wang et al. studied the impact of graphene oxides on human fibroblast cellsaiming to investigatethe biocompatibility of graphene oxides. The human fibroblast cells were cultured with different doses of graphene oxides prepared by the modified Hummers method for day 1 to day 5. The results indicated that graphene oxides exhibit dose-dependent toxicity to cells.It is noted that the exposure of human fibroblast cells to graphene oxide with dose less than 20µg/mL revealed no adverse effects on them. However, the concentration of graphene oxide that exceeds 50 µg/mL showsan obvious influence on cultured cells and apparent cytotoxicity reflected in inducing cell apoptosis, decreasing cell adhesion, penetrating the internal cell organelles such as mitochondrion,lysosomes, endoplasm, and cell nucleus (4).

In another study, Duch et al. studied the impact of GO on mouse alveolar macrophages and epithelial cells. The results revealed that GO increased the rate of aerobic respiration that takes place inside mitochondria. This consequently led to generation of chemically reactive free radical molecules, reactive oxygen species (ROS), activating a cascade of molecular signals that lead to apoptosis and inflammatory response. Authors demonstrated that thepulmonary toxicity of GO is attributed to covalent bonding of oxygen atoms or hydroxyl groups with graphene (12).

A systematic study was conducted by Chang et al. to evaluate the toxicity/biocompatibility of graphene oxide by investigatingits impact onA549 cells, which is extensively used for in vitro toxicity studies, taking into account any change in several important aspects such as viability,morphology, mortality, and membrane integrity of these cells. The results showed that GO did not enter the cells, neither did it express any cytotoxicity. However, GO affect cells byinducing an imbalance between generation and elimination of reactive oxygen species. This imbalance is referred to it as oxidative stress.In addition, high concentration of GO causes inconsiderabledecrease in cell viability (13).

Hu et al. have also performed a systematic study to investigate the influence of graphene oxide nanosheets on human and animal at cellular level. They investigated the impact of changing the concentration of fetal bovine serum (FBS) in the culture medium on the toxicity of graphene oxide nanosheets. Two levels of concentration were used, a usually used 10% concentration of

FBS in comparison to 10 fold lower concentration (1%). It is noticed that the toxicity of graphene oxide nanosheets reflected in cell mortality was greatly reduced at higher concentration (10%) as opposed to lower concentration (1%) of FBS.This is because of the high ability of GO surface to adsorb proteins existed in FBS. The authors also carried out the study at two levels of temperature, 37°C as opposed to 4°C. The results presented no differences in cell viability at either levels of temperature when an FBS coated GO was employed. However, when the GO was used with FBS free medium, the cell viability was 30% lower at 37°C in comparison to 4°C. This can be explained by the lower permeability of cell membrane at lower temperature, supporting the proposed direct interaction mechanism of toxicity (14).

Another in vitro study employed human blood platelets, which is an important type of human cells that should be considered before incorporating of graphene and its derivatives into biomedical devices especially those coming in contact with blood. Singh et al. demonstrated that GO was nearly as thrombin in inducing platelet aggregatory response. Whereas, reduced graphene oxide was much less effective in activating platelets, suggesting the dependence of toxicity on the density of charges which is much higher at the surface of GO in comparison to that at the surface of reduced GO. As a result,GOshould be carefully investigated against its serious thrombogenic threat before using it in biomedical applications, whether diagnostic or therapeutic (15).

Liao et al. explored the relationship between the cytotoxicity of graphene and its derivatives and size of graphene oxide sheets. Authors found out that toxicity of graphene reflected bythe outflow of hemoglobin from red blood cells was greatest at the smallest size of graphene oxide and lowest with aggregated graphene sheets. In the same study, it is revealed that graphene oxide coated with chitosan, a type of polysaccharide,has great effect in reducing the breakdown of red blood cells. Authors also employed human skin fibroblasts in this study to investigate graphene toxicity, but measuring mitochondrial activity was the adopted parameter to reflect the degree of toxicity. It is revealed that the less densely packed graphene oxide are lessdamaging to mammalian fibroblasts thancompacted graphene sheets (16).

In attempting toexamine the interactions of graphene or its derivatives with neural cells,Li et al. used a mouse model which is suffering from hippocampal sclerosis to explore the impact of graphene on neurite during the development until maturation. The results revealed that Grapheneisnon-toxic, ascell viability and morphologywere within normal levels. As well as that, graphene efficiently increased the growth of neurite, which in turn suggests the potential application of graphene as a promoter for neural interfacing (17).

In vivo studies:

Graphene and its derivatives are emerging materials that could be promising candidates for important biomedical applications. In vitro studies is a fairly simple and cost-effective process to initially screen the toxicity of these materials,but it is nearly impossible to imitate a complimentaryin vivo system. Consequently, it is essential to study the overall effects of these materials in vivo on animal models to investigate their toxicity. Wang et al. injected three test

groups of 9 mice each, with 0.1, 0.25, and 0.4 mg graphene oxides. When compared with the low (0.1 mg) and middle (0.25 mg) dose group which did not exhibit obvious toxicity, high dose group (0.4 mg) exhibited chronic toxicity reflected in 4/9 mice death and lung granuloma formation, mainly found in liver, lung, spleen and kidney, almost could not be cleaned by kidney (4). Mouse model was also used in a study to assess the hazard of graphene nanoplatelets (GP) to the lungs. At 24 h post-exposure to GP by pharyngeal aspiration at a dose of 50 µg per mouse, an Acute Pulmonary Inflammatory Response characterized by granulomatous lesions in the bronchiole lumen and near the alveolar region was observed (18).

Zebrafish is another important preclinical model for in vivo toxicity studies owing to its high transparency during embryonic stage and genetic similarity with mammals such as rat, mouse, and humans as well as its faster embryonic development within 120 h.Gollavelli and Ling investigated the biocompatibility/toxicity of multi-functional graphene (MFG) on zebrafish embryos during different stages of embryogenesis. Zebrafish embryos at 2-cell stage were injected with different concentrations of MFG and maintained until complete development (72 hours-post fertilization). The results revealed that MGF was biocompatible to zebrafishand did not affects the survival rate nor induce any significant malformations in zebrafish (19).In a similar study, zebrafishwas also used as a vertebrate models from the embryo through the hatchling stage to assess the cytotoxicity of graphene based nanomaterials. Toxicity assessment was performed by considering several aspects including survival of the embryo and the severity of phenotypic and gross morphological differences. Authors observed that graphene based nanomaterialsexerted negligible influence on the development of zebrafish from embryos to larvae. In other words, graphene complexes were not toxic to Zebrafish model during different stages of development (20).

A study based on examining the effect of coating nanographene sheets with one of the most familiar type of biocompatible polymers, which is polyethylene glycol (PEG), was conducted in vivo on mouse models of cancer. The mice survived without significant loss in body weight, which mean that they did not experience any adverse toxic effects. Analyzing different mice tissues histologically showed that their organs were free of damage. Healthy mice that survived for 3 months after injection of PEG coated NGS were tested and blood samples were taken to perform blood chemistry and complete blood panel tests. No significant abnormalitieswere observed suggesting the potential usage of NGS coated with PEG for in vivo applications (21).

Singh et al. administered intravenously Graphene oxide (250 µg/kg body weight) or amine-modified graphene G-NH2 (250 µg/kg body weight) into different groups of mice to study the effect of them on thrombus formation in vivo. The results showed that Graphene oxide (GO) induced platelet aggregatory response when it come in contact with mouse blood. This means that GO is toxic in mouse as it promotes thrombus formation. These results do not contradict with previously discussed in vitro results that show the thrombogenic effect of GO. On the other hand, amine-modified graphene (G-NH2) was found to be non-toxic, non-thrombogenic and non-hemolysis, which may be attributable to decreased interaction of G-NH2 with the cells. Comparatively, Authors observed that G-NH2 is the safest graphene derivative with potential for biomedical applications owing to its hemocompatibility unlike other graphene derivatives (22).

In an intraocular biocompatibility study of Graphene oxide (GO), different concentrations of GO were injected into one eye of eachJapanese white rabbit enrolled in the study. The results showed that the toxicity of GO on eyes is not sever and has good intraocular biocompatibility. Together, the in vivo and in vitro results suggest the promise of potential ophthalmological application of GO (23).

Combined analysis of data from multiple studies provide important information regarding the biocompatibility of graphene related materials. Avoiding generalization about data is important due to Inconsistency of studies' results and the significant variability of the material under study. Different types of graphene resulting from physiochemical or structural modifications must be studied thoroughly to compare between them and find out their biological impact.

Potential mechanisms of toxicity

Recent findings on the toxicological effects of graphene-family nanomaterials in bacteria and mammalian cells, both in vivo and in vitro have been discussed previously. However, there are too much uncertainty about the effects and mechanisms of toxicity of Graphene based materials.This toxicity is relevant to several parameters including size, agglomeration state, surface area, surface charge and purity of the samples. In this review, two potential mechanisms that have been proposed in the literature will be summarized.

One of the common mechanisms is the provoking of oxidative stress insidehuman or animal cells caused by direct or indirect production of free radicals and reactive oxygen species (ROS) that are ready to intervene in cellular internal reactions. These ROS are reactive free radical molecules which contain oxygen including oxygen ions and peroxides that play significant roles in communication, signaling and maintaining balance inside cell. Cells maintain hemostasis through production and elimination of ROS by using many complex mechanisms such as enzymatic and nonenzymatic antioxidant systems. Any abnormality or impairment in the function of these complex mechanisms can greatly raise the level of ROS inside cell and consequently oxidative stress. Oxidative stress in turn plays a crucial role in alteration of macromolecules such as membrane lipids, protein denaturation, and ultimately DNA destruction leading to sever and irreversible oxidative damage and consequently cell death (24).

Gurunathan et al. proposed oxidative stress as one of the mechanisms that answers the questions raised about how GO and rGOcan cause toxicity to cells and biological systems. Authors used NBT assays to measure the levels of ROS produced inside cultured cells. The results indicated that the levels of ROS inside cells treated with two types of graphene derivatives, GO and rGO, were much higher than that level in control cells, being3.8-fold and 2.7-fold higher, respectively. In addition, ROS generation by GO and rGO could be prevented and consequently, cells could be protected from toxic compounds if these cells were pre-incubated with antioxidant compounds such as glutathione (GSH) or N-acetylcysteine (NAC) (25).The findings of several

studies suggest that ROS are produced inside cells depending on two parameters, time of exposure and concentration of GFM, pointing to the oxidative stress mechanism of toxicity which depends also on these parameters (26).

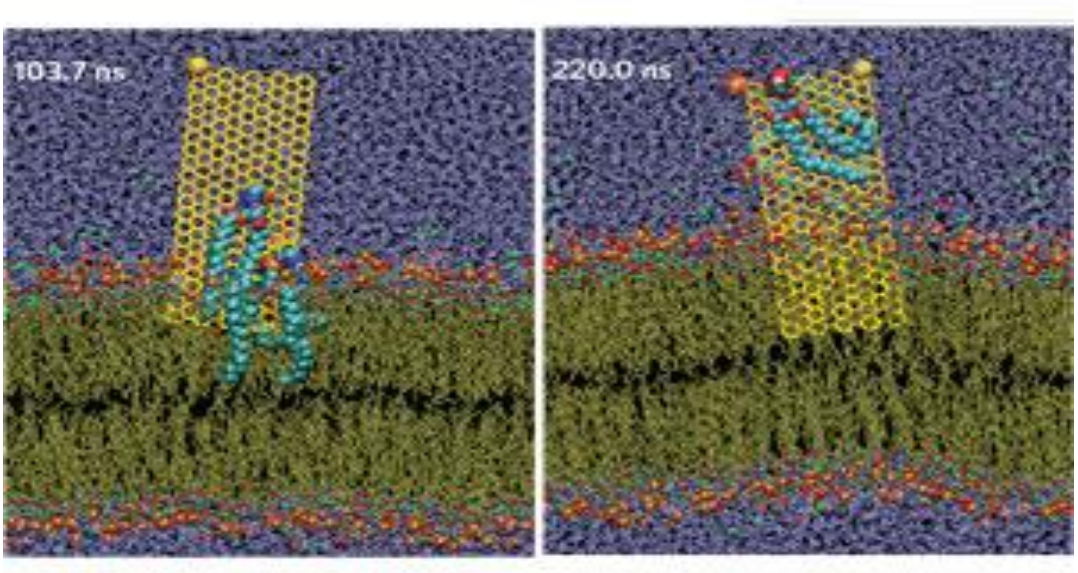


Figure 2: Graphene kills bacteria by slicing through their membranes and yanking out their phospholipids (27).

In comparison to the studies mentioned above, some studies proposed another mechanism of toxicity represented by cell membrane damage through physical contact interaction with the extremely sharp edges of graphene. Akhavan et al. compare graphene oxide nanowells' toxicity in gram-positive and Gram-negative models of bacteria. It is found that the cell membrane of Gram-negative bacteria owing the outer membrane experienced less damage than that of Gram-positive bacteria lacking the outer membrane (3). This is caused by the direct contact interaction of the bacteria with the very sharp edges of the nanowells leading to slicing through their membranes and yanking out their phospholipids as shown in figure 2. Liu et al. also showed that thecell membrane destruction was caused by direct contact of cell membrane with sharp nanosheets (9).Hu et al. demonstrated how the cytotoxicity of graphene nanosheets is reduced significantly when they are surrounded by proteins such as serum proteins. Therefore, although they are lethal to bacteria, they are less toxic to human or other mammalians (14). This confirms the mechanism of toxicity discussed previously because serum proteins reduce the physical interaction between graphene and cell membrane.

Conclusions:

Physical and chemical properties uniqueness of graphene and its derivatives made them promising candidates for biomedical applications including drug delivery, biosensing, tissue

engineering, anddiagnosis. However, it is essential to evaluate these materials extensively both in vivo and in vitroto ensure their safety and efficacy to human health. Little is known about the adverse effects of graphene based materials and further studies are needed. This review reveals that the toxicity of GO on human fibroblast cells is dose dependent, and can be mitigated by increasing the concentration of fetal bovine serum. It is also clear that the toxicity vary with the type of material,Gt, GtO, GO, orrGO.In addition, adding functional groups to graphene would change its characteristics. For example, amide modified graphene is non-toxic, non-thrombogenic and non-hemolysis material. This variability of graphene based materials entails avoiding generalization and further detailed studies for better understanding of their safety. Finally, direct contact interaction and oxidative stress are the most important mechanisms proposed to explain the toxicity/biocompatibility of these materials. Direct contact interaction mechanism suggests that the interaction between graphene sharp edges and cell membrane causes the toxicity. However, Oxidative stress mechanisms attribute the toxicity to the increased level of ROS inside the cell induced by graphene based materials.

References:

1) Chodos A.Discovery of Graphene. APS physics. [Online]. 2009, **18** (9), pp. 2. Available from: http://www.aps.org.
2) Akhavan O, Choobtashani M, and Ghaderi E. Toxicity of graphene and graphene oxide nanowalls against bacteria. ACS Nano, [Online]. 2010, **4** (10), pp 5731–5736. Available from: http://pubs.acs.org.
3) Wang K, Ruan J, Song H, Zhang J, Wo Y, Guo S, and Cui D. Biocompatibility of Graphene Oxide. Nanoscale research letters. [Online]. 2011, **6** (8), pp. 9751-9756. Available from: http://www.nanoscalereslett.com.
4) Jastrzębska AM, Kurtycz P, and Olszyna AR. Recent advances in graphene family materials toxicity investigations. Journal of nanoparticle research: an interdisciplinary forum for nanoscale science and technology. [Online]. 2012, **14** (12), pp. 1320. Available from: http://www.ncbi.nlm.nih.gov/.
5) Defen. Gram-negative bacteria vs. Gram-positive bacteria. [Online]. 2015. Available from: http://www.diffen.com.
6) Brunet L, Lyon DY, Hotze EM, Alvarez PJ, and Wiesner MR. Comparative Photoactivity and Antibacterial Properties of C60 Fullerenes and Titanium Dioxide Nanoparticles. Environmental science & technology. [Online]. 2009, **43** (12), pp. 4355–4360. Available from: http://alvarez.blogs.rice.edu/.
7) Sawosz E, Chwalibog A, Mitura K, Mitura S, Szeliga J, Niemiec T, Rupiewicz M, Grodzik M, and Sokołowska A. Visualisation of morphological interaction of diamond and silver nanoparticles with Salmonella Enteritidis and Listeria monocytogenes. Journal of nanoscience and nanotechnology. [Online]. 2011, **11** (9), pp. 7635-7641. Available from: http://www.ncbi.nlm.nih.gov/.
8) Krishnamoorthy K, Veerapandian M, Zhang L, Yun K, and Kim SJ. Antibacterial efficiency of graphene nanosheets against pathogenic bacteria via lipid peroxidation. Journal of physical chemistry C. [Online]. 2012, **116** (32), pp. 17280–17287. Available from: http://pubs.acs.org.

9) Liu S, Zeng TH, Hofmann M, Burcombe E, Wei J, Jiang R, Kong J, andChen Y. Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress. ACS Nano,[Online]. 2011, **5** (9), pp 6971–6980. Available from: http://pubs.acs.org/.
10) Ruiz ON, Fernando KA, Wang B, Brown NA, Luo PG, McNamara ND, Vangsness M, Sun Y,andBunker CE. Graphene oxide: a nonspecific enhancer of cellular growth. ACS Nano, [Online]. 2011, **5** (10), pp 8100–8107. Available from: http://pubs.acs.org.
11) Zhanga Y, Moa G, Lia X, Zhanga W, Zhangb j, YeaJ,Huangc X, andYu C. A graphene modified anode to improve the performance of microbial fuel cells. Journal of power sources.[Online]. 2011, **196** (13), pp. 5402–5407. Available from: http://www.sciencedirect.com.
12) Duch MC, Budinger GR, Liang YL, Soberanes S, Urich D, Chiarella SE, Campochiaro LA, Gonzalez A, Chandel NS, Hersam MC,andMutlu GM. Minimizing Oxidation and Stable Nanoscale Dispersion Improves the Biocompatibility of Graphene in the Lung. Nono letters. [Online]. 2011, **11** (12), pp. 5201-5207. Available from: http://pubs.acs.org.
13) Chang Y, Yang S, Liu J, Dong E, Wang Y, Cao A, Liu Y, andWang H. In vitro toxicity evaluation of graphene oxide on A549 cells. Toxicology letters.[Online]. 2011, **200** (3), pp. 201-210. Available from: http://www.sciencedirect.com.
14) Hu W, Peng C, Lv M, Li X, Zhang Y, Chen N, Fan C, andHuang Q. Protein Corona-Mediated Mitigation of Cytotoxicity of Graphene Oxide. ACS nano. [Online]. 2011, **5** (5), pp. 3693-3700. Available from: http://pubs.acs.org.
15) Singh SK, Singh MK, Nayak MK, Kumari S, Shrivastava S, Gracio J, andDash D. Thrombus inducing property of atomically thin graphene oxide sheets. ACS nano. [Online]. 2011, **5** (6), pp. 4987-4996. Available from: http://pubs.acs.org.
16) Liao KH, Lin YS, Mascosko CW, andHaynes CL. Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts, ACS applied materials & interfaces. [Online]. 2011, **3** (7), pp. 2607-2615. Available from: http://www.ncbi.nlm.nih.gov.
17) Li N, Zahng X, Song Q, Zahng Q, Kong T, Liu L, Jin G, Tang M, andCheng G. The promotion of neurite sprouting and outgrowth of mouse hippocampal cells in culture by graphene substrates. Biomaterials. [Online]. 2011, **32** (35), pp. 9374-9382. Available from: http://www.ncbi.nlm.nih.gov.
18) Schinwald A, Murphy FA, Jones A, Macnee W, andDonaldson K. Graphene-based nanoplatelets: a new risk to the respiratory system as a consequence of their unusual aerodynamic properties. ACS nano. [Online]. 2012, **6** (1), pp. 736-746. Available from: http://pubs.acs.org.
19) Gollavelli G, andLing YC. Multi-functional graphene as an in vitro and in vivo imaging probe. Biomaterials.[Online]. 2012, **33** (8), 2532-2545. Available from: http://www.ncbi.nlm.nih.gov.
20) Liu CW, Xiong F, Jia HZ, Wang XL, Cheng H, Sun YH, Zhang XZ, Zhuo RX, andFeng J. Graphene-based anticancer nanosystem and its biosafety evaluation using a zebrafish model. Biomacromolecules. [Online]. 2013, **14** (2), pp. 358-366. Available from: http://www.ncbi.nlm.nih.gov.

21) Yang K, Zhang S, Zhang G, Sun X, Lee S, andLiu Z. Graphene in mice: ultrahigh in vivo tumor uptake and efficient photothermal therapy. Nano letters.[Online]. 2010, **10** (9), pp. 3318-3323. Available from: http://pubs.acs.org.
22) Singh SK, Singh MK, Kulkarni PP, Sonkar VK, Gracio JJ, andDash D. Amine-modified graphene: thromboProtective safer alternative to graphene oxide for biomedical applications. ACS nano. [Online]. 2012, **6** (3) pp. 2731-2740. Available from: http://pubs.acs.org.
23) Yan L, Wang Y, Xu X, Zeng C, Hou J, Lin M, Xu J, Sun F, Huang X, Dai L, Lu F, andLiu Y. Can graphene oxide cause damage to eyesight? Chemical research in toxicology. [Online]. 2012, **25** (6), pp. 1265-1270. Available from: http://www.ncbi.nlm.nih.gov.
24) Trachootham D, Lu W, Ogasawara MA, Valle N, andHuang P. Redox regulation of cell survival. Antioxidant & redox signaling.[Online]. 2008, **10** (8), pp. 1343-1374. Available from: http://www.ncbi.nlm.nih.gov.
25) Gurunathan S, Han JW, Dayem AA, Eppakayala V, andKim JH. Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in Pseudomonas aeruginosa. International journal of nanomedicine.[Online]. 2012, **7**, pp. 5901-5914. Available from: http://www.ncbi.nlm.nih.gov.
26) Zhang L, Xia J, Zhao Q, Liu L, andZhang Z. Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. Small.[Online]. 2010, 6 (4), pp. 537-544. Available from: http://www.ncbi.nlm.nih.gov.
27) Chemistry world. Green graphene band-aid. [Online]. 2013. Available from: http://www.rsc.org.