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Graphene Based Nanomaterials as Novel Nanocarriers for Nutraceuticals for the Treatment and Prevention of Chronic Diseases

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Abstract

Graphene is an extremely thin material which has received a huge interest in many areas of science and technology owing to its unique physical, chemical, mechanical and thermal properties. The more challenging aspect of synthesizing graphene in a low-cost and environmental friendly method is a big task. There are various methods to synthesis graphene but the chemical synthesis is considered as the best method because of its advantages like scalable, facile, and inexpensive method. Mostly, the chemicals used for the synthesis of graphene is toxic, corrosive and hazardous. Therefore, in the recent years researchers have been using various eco-friendly/green materials to manufacture a functionalized graphene. Moreover, the green reducers used for the synthesis of graphene is plant extracts, juices and biomolecules. In addition, graphene based nanomaterials (GBNs) have been extensively explored in the most recent years as a novel nanocarrier for the loading of variety of bioactives and these materials is used for the treatment of chronic disease. This chapter gives a brief outline of the green reduction of graphene oxide to graphene and its applications in the nutraceuticals area.

INTRODUCTION

Graphene is a two-dimensional sheet of hexagonally arranged carbon atoms which is isolated from the three-dimensional parent material graphite and it exhibits electronic, mechanical, optical and magnetic properties. These properties made graphene as an outstanding material with great potential for various applications range from energy storage to biomedical. The most fascinating material of the recent and most upcoming nanotechnology based applications is graphene based materials (GBNs) [1]. This promising material is used in almost all industries starting from electrical & electronic [2], thermal [3], aviation [4], polymer [5], pharmaceutical [6,7], biomedical [8], drug delivery [9], bioengineering [9], food and nutraceuticals [10-12]. It is more efficiently used in very recent times for the biological applications such as bactericidal [13], antiviral [14], theranostics (disease diagnosis, delivery, biosensing) [15], stem cell based tissue engineering [16], neural cell proliferation [17], differentiation [18], drug delivery [19], cancer cells imaging, targeting [20], and nutraceutical agents delivery [21], and treatment of disease [21]. Hence, the selection of appropriate material from the graphene family of materials is very important to use these materials in biological applications. Moreover, the reduced graphene oxide (RGO) is the widely used material for the bio and nutraceutical delivery because of the use of environmental friendly reducing agents (plant extract/biomolecules) [22].

The general method of preparing the eco-friendly RGO is briefed here. The dispersed GO suspension and the eco-friendly reducing agents are mixed together to produce a reaction solution. Then, the reaction solution is kept under controlled temperature for duration of a time. In the previous studies, it is mentioned that the reaction temperature and the concentration of GO is about 80-95°C and 0.5-1 mg/ml. Therefore, it is very important to maintain the concentration, temperature, time and pH of reduction of GO. The reduction is possible in both the acidic [23], and alkaline pH [24,25]. The chemicals used to regulate the pH of the solution are HCl [23,26-28], NH_4OH [29-31], and dilute NaOH [32]. In a study by Merino et al. [33], they said that the alkaline condition of the reaction is able to reduce the time of reduction, could able to deoxygenate GO sheets and stimulates strongly the colloidal durability of GO. Bosch et al.[34], also examined the effect of pH on GO sheets. They revealed that the acidic pH is responsible for the defects with sheet aggregation which leads to reduction in size of the layers [35]. Thus the acidic pH changes the sheet like structure into other nano forms of graphitic forms such as fullerene, nanoonions and multiwalled carbon nanotubes. Their study proves that for the production of defect less graphene it

is important to use alkaline pH. After reduction, the GO solution turns into brownish yellow colour to black and by filtration [27,32,36], or centrifugation [37], the graphene sheets are taken out. After centrifugation or filtration, the product should be washed and recentrifuged several times with water to remove all the unreduced graphene, excess reducers and reaction by products (Figure 1) [38,39].

Many of the scientists developed different types of reducing agents to convert GO into graphene [40-42]. Some of the chemical reducing agents such as hydrazine [43-45], sodium borohydride [46,47], hydroquinone [48], and dimethyl hydrazine [49] are found to be the toxic agents. These chemicals could adsorb on the surface of nanoparticles which leads to toxicity. Most recently, green synthesis of graphene have been extensively explored to decrease the use of toxic chemicals and to increase the biocompatibility for using these materials in biomedical applications. Recently, the researchers developed a green reducers to prepare reduced graphene sheets from GO [50]. The green reducers such as [51], amino acids [52], bacteria(*Escherichia coli*) [53], yeast [54], plant extract [51], several biological materials [55-57], wild carrot root [58], *E. fergusoni* [59], *Pseudomonas aeruginosa* [60], and the humanin peptide [57], cellulosic compounds [61], or metal powders [62] and they are free from corrosion, carcinogenicity and toxicity. It is reported that the green reducers acts as both reducing and stabilizing agents for synthesis of graphene. The organisms used in graphene synthesis includes prokaryotic systems and eukaryote (*Escherichia coli*) [63]. The synthesis of nanomaterials using plants for biological applications has received more attention as a right alternative to chemical methods [64]. Plant extracts may serve as both reducing and capping agents in synthesis of nanoparticle. Generally, plants contain many biomolecules such as polysaccharides, alkaloids, vitamins, proteins, amino acids, alcoholic compounds, polyphenols and enzymes [40].

The biomolecules reduced graphene based materials could be relevant for biological applications in particular for the delivery of nutraceuticals and the research in this area is exploring recently. There are natural antioxidants which includes amino acids and vitamins to reduce graphene oxide and to functionalize the material. These biomolecules not only acts as a reducing agents and also it produces added value to the RGO [65]. It has been shown that dextran and tea polyphenols increases the colloidal stability and biocompatibility of RGO. Furthermore, it was found that these green reducing agents have aromatic structures which could not only acts as reductants but also functionalize the graphene sheets by π-π attachment of reductant molecules onto the surface of RGO. Lately it was found that the green tea polyphenol functionalized RGO sheets decreases the reactive oxygen species (ROS) which is generated in the cell culture media whereas the chemically reduced graphene sheets (Hydrazine-RGO) induces more ROS generation [66].

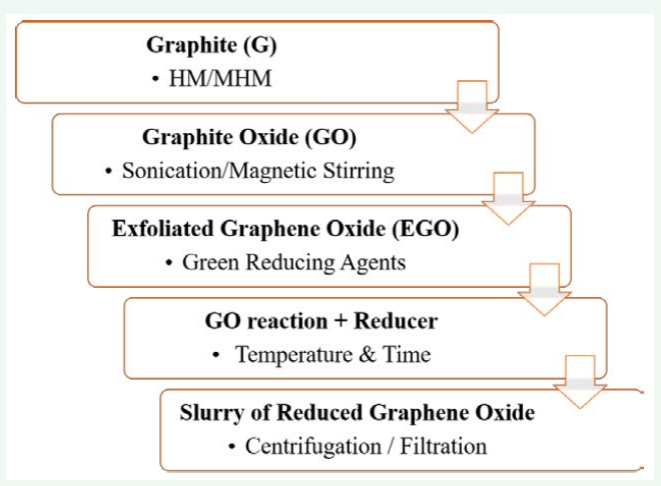

Figure 1 Schematic representation of green reduction of graphene oxide.

In the previous studies it is reported that the graphene produces cytotoxicity and geno toxicity at low concentrations of about □ 10μg/ml. Therefore, further works are necessary to incorporate these materials in biological applications especially in cancer therapy, drug and nutraceuticals delivery. It was shown recently that the functionalised graphene exhibits no or very less cytotoxic effects [67]. Regarding the biomedical applications of GBNs, it is reported that binding capability (Covalent/Noncovalent), physical and chemical properties could influence the biological responses of cells. GBNs can be either benevolent or toxic to cells and the biological responses depends on layer number, lateral size, surface functionalization, stiffness, hydrophobicity and dose [68,69]. A study by lee et al., suggested that GBNs can be used as biocompatible, transferable, and implantable platforms for stem cell culture [70]. In this chapter we explain the green synthesis method for the delivery of nutraceutical agents and its biocompatibility.

GREEN REDUCTION OF GRAPHENE

There are many methods have been established which includes hydrothermal dehydration, solvothermal, chemical, catalytic, photo catalytic and photo reduction. By comparing all these methods, chemically reduction method is the most considered method and capable of producing graphene nanomaterials in large scale. The main disadvantages of chemical reduction method is it may lead to aggregation of graphene layers due to the Vander Walls forces between the layers [71]. In addition, other disadvantage of reduction route is highly toxic nature of reducing substances like hydrazine, hydroquinone and dimethylborohydride. These chemicals are toxic and hazardous in nature leads to detrimental effect particularly for biological applications. Therefore, the development of eco-friendly green synthetic routes for the reduction of GO is necessary to overcome the above mentioned disadvantages. In this connection, easily scalable biosynthetic and most promising methods for the synthesis of GBNs is getting higher. Recently, green and environmental friendly methods were used for the synthesis of GO and RGO by means of different biomolecules. Apart from the use of less hazardous chemicals, the salient feature of green technology is that it operates in the mild condition which makes it cost effective and affordable. Nevertheless, the important role of these biomolecules in improving the functionality of the RGO as nanocarriers is to be explored. Moreover, it is very important to understand the chemistry associated with biomolecules and graphene oxide in order to improve the reduction efficiency.

The chemical interactions will be helpful to develop the exact mechanism to destroy the bacterial and cancer cells [65].

From the literature, it was found that the seed, leaf, plant, root and juice extracts of *Gingko Biloba* [72], *Camellia Sinensis* [73], *Mesua Ferrea Linn* [51], *Colocasia Esculenta* Leaf [51], *Prunus Serrulata* [74], *Daucus Carota* [58], *Ceratophyllum Demersum* [75], *Potamogeton Pectinatus* [75], *Lemna Gibba* [75], *Cyperus Difformis* [75], *Amaranthus Dubius* [76],Coconut water [77], Rose water [78],*Citrus Sinensis* [51], Pomegranate juice [71], *Hibiscus Sabdariffa* L [79], *Spinacia Oleracea* [80], *Terminalia Chebula* [42], Grape seed extract [81], Cherry leaf extract [82], *Chlorella vulgaris* [83], *Eichhornia crassipes (Mart.)Solms* [84],*Vicia faba* L [85], Asian red ginseng [86], *gluconobacter roseus* [87], were used in the green synthesis of reduced graphene oxide. The biomolecules used in the synthesis of reduced graphene oxide are sucrose [88], glucose [89], fructose [88], starch [90], L-cysteine [91], pyridoxine [92], Riboflavin[93], citric acid [94], L-mallic acid [95], L-arginine [96], L-carnosine [97], L-glutamic acid [98], L-histidine[99], L-phenylalanine [100], L-tyrosine [101], vitamin C [102], L-glutamine [103], melatonin [104], glycoseoxidase [105], quercetin [106], and bovine serum albumin [107]. The main advantages of the green reduction by phytoextracts and biomolecules are i) efficient method for reduction ii) cost effective, iii) bulk production, iv) increased stability v) prevents agglomeration, vi) acts as nano carriers for drug delivery, delivery of bioactive components and biomedical applications [86]. Mostly, the plant extracts contains different types of polyphenols and they are converted into oxidised quinone in the presence of reactive oxygen and these have the potential to reduce GO [41,108]. In the near future, this green technology could be the most promising technology because GBNs products will be available at low-cost.

PLANT ACTIVE CONSTITUENTS DELIVERY OF GO

There are various nanomaterials such as metal, metal oxide nanoparticles, polymeric micelles, liposomes, dendrimers and carbon nanotubes have been explored as nanocarriers for the delivery of therapeutic agents. Among them, GBNs have recently emerged as a novel delivery system potentially applied for systemic, targeting and local delivery systems [109]. The GBNs properties are relevant for active components delivery and biological applications which includes surface area, layer number, lateral dimension, surface chemistry and purity. The GBNs have higher surface area (2600 $m^2 g^{-1}$) is four magnitudes higher than the surface of any other nanomaterials [110]. A monolayer of graphene allows higher loading capacity compared with other nanomaterials. If there are large number of layers it will reduce the surface area and therefore increases the rigidity of the nanocarriers required for cell penetration [111,112]. This is the important parameter in maintaining the structural integrity of carriers and if it is too rigid, they could damage the cell. Hence, it is important to reduce the rigidity of GBNs because it may be an obstacle for delivering active constituents. GBNs have size limitations related to cell uptake, renal clearance, blood brain barrier transport, biological degradation and other biological properties dependent on particle dimensions but they do not have an effect on lateral dimensions. GBNs have a unique 2-D shape with planar morphology whereas this shape is different from spherical nanoparticles and carbon nanotubes [113].

There are three important parameters to successfully design GBNs in applications of bioactive components delivery. Firstly, surface modification is required to build an efficient nanocarrier with optimised active moiety loading capacity. Secondly, to improve or to confirm the biocompatibility and toxicity, preclinical and clinical studies should be undertaken. Thirdly, we should design a system which could able to release the actives in a controlled manner at a targeted site with an optimum dosage form. To address these issues there are limited number of *in vitro* and *in vivo* studies has been reported with promising results [112,114].

GBNs have been extensively explored in the recent years as a novel nanocarrier for the loading of variety of bioactives such as anticancer agents, poorly soluble compounds and the molecules which has low bioavailability [115,116]. Multi drug delivery is also applicable for GBNs because of the large specific surface area. Surface modification and conjugation strategies improves *in vivo* biocompatibility and circulation. Some of the examples from this research will be presented to show the application of GBNs for targeted, controlled and simulated delivery systems. By physisorption method (π-stacking), hydrophobic molecules are loaded in GBNs with selective killing of cancer cells. The π-π stacking and electrostatic interactions of GBNs can assist in high loading of poorly soluble components without compromising its efficiency [117,118].

GBNS FOR BETTER NUTRIENTS AND THERAPEUTICS DELIVERY (FOOD)

The effectiveness of nutraceutical products in preventing diseases depends on the protection and effective delivery of the active ingredients that should be bioavailable. There are innovative technologies which focus in the formulation methods to increase the bioavailability of active molecules. However, there are number of challenges associated with the nutraceutical functional food formulation in disease prevention and supplementing a food. Before formulating the food nutrients into the nanocarrier, it is important to measure the physiochemical and physiological characteristics (solubility, bioavailability, chemical stability, melting point) of the bioactives. For instance, the interdisciplinary research is needed in the area of drug delivery scientists and food engineers for the development of new nutrient delivery systems [119]. The development of food grade delivery system offers the possibility to manufacture products to protect, control and target the release of the bioactive ingredient in its desired location. Mostly, the key methodologies such as entrapment, encapsulation, coating etc. which protects the nutraceuticals from the environment and release the active components in a controlled manner. Moreover, they can also be delivered by wide range of formulations such as tablets, granules, micro/nanoparticles, emulsion, suspensions as per the recent developments in the area of drug delivery research [120,121]. The European commission (EC) recommends that a nanomaterial can be defined as the fullerenes, graphene and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials. By using GBNs as a delivery vehicle, it is possible to provide or enhance the optimal characteristics of a nutraceutical component which includes stability, incorporation of hydrophobic and hydrophilic

substances, high loading capacity and suitable for various routes of administration such as oral, pulmonary and topical application. GBNs gained a considerable interest in the scientific community due to their superior physico-chemical properties, large surface area which is available for interaction with biologically active agents, low cost and ability in crossing biological barriers [10].

Like drugs, the efficacy of food and food components is the primary function of the bioavailabity. By changing the size of materials into nanoscale, solubility and stability of active molecules of food is enormously improved. The research in the field of developing food based delivery system is an emerging stage [12,122]. Recently, the nutrients are encapsulated and entrapped in the GBNS for the effective delivery and increased bioavailability. The GBNs are generally mentioned as "rolled up structures" with one or more layers of graphene sheets which increase their chemical stability, thermal stability and mechanical strength. The bioactive compounds involve the association of non bioactive moieties GBNs and polymers which can be dissociated within the body. Thus, they have been shown to enhance the potency and dose efficiency of both hydrophobic and hydrophilic molecules [123]. The bioactive compounds are grafted by means of functionalization reactions or by adsorbing onto the surface for the delivery of food components, proteins, vitamins and minerals. In a recent study they have shown that GBNs exhibits strong antimicrobial activity by destructing the cell membrane in direct contact. Application of using GBNs as building blocks for antimicrobial materials opens a new way to be utilised in food safety area [64]. The GBNs have been tailored for the efficient and fully controllable release of flavours, nutrients and other food bioactives. The functionalised GBNs could find acceptance in food market because of the new developments of more available production. The GBNs have shown many advantages allowing to carry off water or fat insoluble nutrients to the blood stream and make them available to cells [124].

RECENT STUDIES OF GBNS IN NUTRACEUTICALS DELIVERY

In the functional food category, plant derived polyphenols are entrapped in GBNs and it shows high antiproliferative activity against colon and ovarian cancer cells (Abdolahad et al., 2013). The author unveiled the effect of reduced grapheme oxide in HT29 and SW48 cells (82% destruction) however, they used 3mg/l RGO nanosheet solution and the different methods were adopted [125]. In another report, GO loaded SN 38 with a concentration of 1mg/ml was used against HCT 116 cell lines [126]. Akhavan et al., disclosed that RGO-ginseng properties improved ascribed to its higher compatibility, hydrophilicity and the presence of ginsenoside molecules acts as a powerful antioxidants on the surface of the reduced sheets [86].

In this regard, an interesting method involves the reduction of GO by polyphenols or plant extracts and concurrent establishment of π-interaction between the two species. In our recent work we have reported that GBNs with grape seed extract (GSE) shows high anti-inflammatory and anti-cancer activity against colon cancer cells. GSE contains oligomeric proanthocyanidins (PCs) and these PCs are called as polymers of catechin. The PCs contain free -OH groups and these groups could act as reactive sites for functionalizing and reducing GBNs. The antimicrobial activity of RGO with a concentration of about 4-5 µg/ml which proves the complete death of *E. coli* and *S.aureus*. By AFM analysis, it is further substantiated well regarding the cell wall damage (Figure 2). In our experiments, we have used 100 to 500 µg of RGO-GSE and found that they are very effective in killing the cells around 88% within the span of 24 h and also to prevent the other cells from proliferating. *In vitro* antiproliferative results exhibits that the RGO-GSE has shown efficient activity with 500 µg against the colon cancer cells and also they act against inflammation produced by the cancer cells (Figure 3) [81].

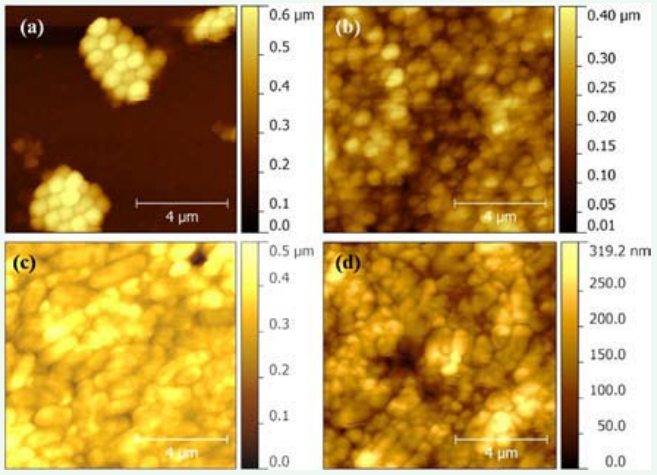

Figure 2 Atomic force microscopy images (a*) S. aureus* control (b) RGO treated *S. aureus*(c) *E. coli* control and (d) RGO treated *E. Coli* (Figure reprinted from Materials Science and Engineering - Copyright permission).

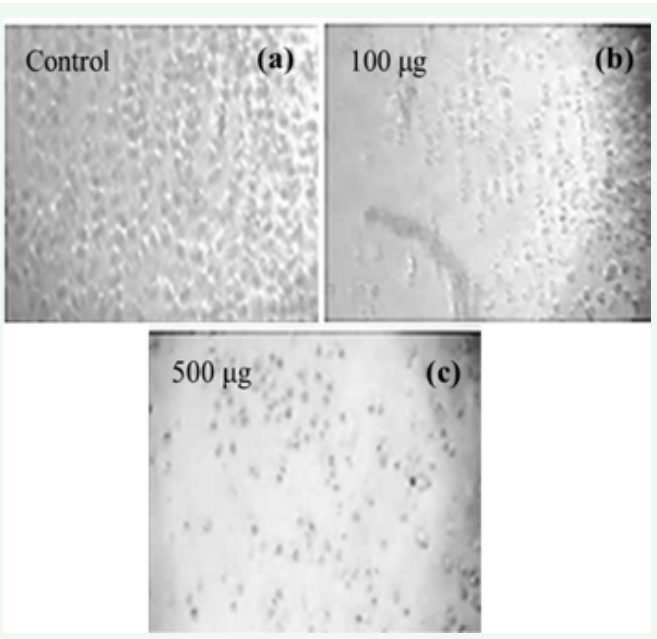

Figure 3 Microscopy images of HCT-116 cells with RGO (a) Control (b) 100 µg and (c) 500 µg (Figure reprinted from Materials Science and Engineering C - Copyright permission).

Abdullah et al., used an eco-friendly and safer reducing agent green tea extract (GTE) for the reduction of GO. Green tea have been used as a common drink which offers many health benefits such as cancer prevention, heart disease, lowering of high blood pressure and lowering of high blood cholesterol levels. Recently, green tea attracted a considerable interest for this applications in manufacture of nanomaterials. It contains high composition of polyphenolic compound which is used as a reducing agent because of its ability to donate electron or hydrogen atom. They have evaluated the biocompatibility and cytotoxicity of GO and RGO-GT against human fibroblast cells (CCD-18Co). The authors revealed that RGO-GTE at low concentration, the cell proliferation was inhibited (45-60%). The cellular morphology of the cells treated with RGO-GTE and GTE were found to be almost similar to that of control [66].

A bioactive flavonoid, quercetin is one of the most important and well known dietary antioxidants used as health supplement. Its chemopreventive action is confirmed by strongly inhibiting breast, lung, colon and ovarian cancer cell growth. Quercetin has low aqueous solubility, instability in intestinal fluids and exhibits first pass metabolism before reaching systemic circulation which leads to poor oral bioavailability. To avoid these complications, it is entrapped/adsorbed into GO nanoparticles and these are efficient in crossing permeability barriers. The loading efficiency of quercetin over GO sheet was around 44%, it means that 1 g of GO is able to release 0.44 g of quercetin. They reported high loading capacity compared to the other nanocarriers like liposomes, solid lipid nanoparticles, nanostructured lipid carriers, polymeric micelles. Their results indicates that no toxicity was observed up to the concentration of 120 µg/ml of GO (Figure 4). Therefore, it is necessary to study the toxicology in more detail at higher doses using different animal models before the clinical applications of GO [11,106].

Kakran M et al., developed a GO-ellagic acid (EA) by functionalising with hydrophilic and biocompatible pluronic F38, Tween 80 and maltodextrin for loading of poorly water soluble anti-cancer compound. EA is a polyphenolic compound found in many fruits and vegetables, which has antioxidant, anticarcinogenic, antiproliferative and chemopreventive activities. EA shows low bioavailability because it is less soluble in water. Thus, the functionalised GO not only acts as nanocarrier but also it helps to increase the solubility and cellular uptake by enhancing its ability to target cell membrane. The release of EA from GO-EA was found to be pH dependent release by increasing order neutral pH < pH 4 < pH 10. The high concentration of GO-F38-EA, GO-T80-EA and GO-MD-EA were incubated with the MCF7, HT29 cells and observed no significant toxicity. The maximum concentration of EA loaded onto the functionalised GO was about 500 mg/L which corresponds to the concentration of about 200 mg/L. Therefore, GO-F38, GO-T80, GO-MD were found to be the ideal nanocarriers and they did not show cytotoxicity. The authors found that the antioxidant activities of EA in all the three carriers were similar to that of free EA, which indicates that nanocarrier GO doesn't hinder antioxidant activity [127].

Suresh et al., disclosed the reduction of GO by spinach leaves (*Spinacia oleracea*) and it consists of rich source of essential nutrients such as vitamin A, vitamin C, vitamin K, folate, iron, manganese, flavonoids, coumarin, protein and they are found to be antioxidant. These significant amounts of antioxidant phyto-constituents in spinach acts as reducing agent for the reduction of GO. They proved that RGO is strong in inhibiting the DPPH free radical scavenging activity with IC50 (Inhibitory Concentration) of 1590 mg/ml [80]. Geummi L et al., studied seven plant extracts such as cherry, magnolia, platanus, persimmon, pine, maple and ginkgo and compared for their abilities to reduce GO. They disclosed that this environmental friendly reduced GO could be used in various areas such as food and biomedical applications [82].

Barua S et al., synthesized RGO –Ag nanohybrid using *Colocasia esculenta* leaf extract and the material possess good cytocompatibility profile in mammalian PBMC and RBC, excellent antimicrobial activity and studied the acute dermal toxicity study on rats. The histopathological result shows that RGO –Ag nanohybrid did not cause any abnormality to the vital organs of the host, such as liver, kidney, skin, brain and heart (Figure 5). The fine skin sections exhibited well defined cellular structure with different epithelial layers. They are biocompatible and it can be used as topical ointments and bandages [64]. Agarkhar et al., reviewed and summarised the reduction of GO by green methods by using biomolecules, plant extracts and microorganisms. They have also explained the issues and challenges particularly in the area of food, drug delivery, catalysis and biomedical applications. Hence, it is important to measure the clinical status of these materials and this method is said to be a highly promising for the effective use in humans [65].

Hatamie S et al., used curcumin as a natural reducing agents and it is one of the very effective natural antioxidants. It has successfully been used in therapeutic applications like antioxidants, anti-inflammatory and anticancer agent. They have utilised curcumin for the synthesis and functionalization of RGO sheets (exfoliation). The π-π attachment of curcumin molecules onto the RGO sheets was studied by spectroscopical methods such as Raman and Fourier transform infrared spectroscopies (FTIR). They have also found that with concentrations up to 100 µg/ml exhibits dose dependent action of curcumin functionalised graphene sheets against human breast cancer cell lines and a normal mouse cell line [128].

Muthoosamy et al., prepared the mushroom-extract-reduced RGO and studied its biocompatibility. They found that RGO did not shows antiproliferative effects towards colon and brain cancer cell lines because they doesn't possess selectivity against cancer cells. They mention that it is added value because it can be used as a tool for gene transfection and as a drug delivery vehicle by conjugating anticancer molecule. However, based on their findings that the mushroom reduced RGO exhibits good aqueous stability and biocompatibility compared to the functionalised ones [129]. Gurunathan S et al., utilised Ginkgo Biloba as bioreductant for the preparation of graphene. The reduction method used by them is very simple, cost-effective, avoidance of toxic chemicals and they used a different route for the preparation of reduced graphene. Moreover, they have assessed the biocompatibility of synthesised graphene in cancer cell lines. And also they have determined the cell viability, TUNEL and ALP activity in human breast cancer cells. They indicated that the GO shows dose dependent toxicity

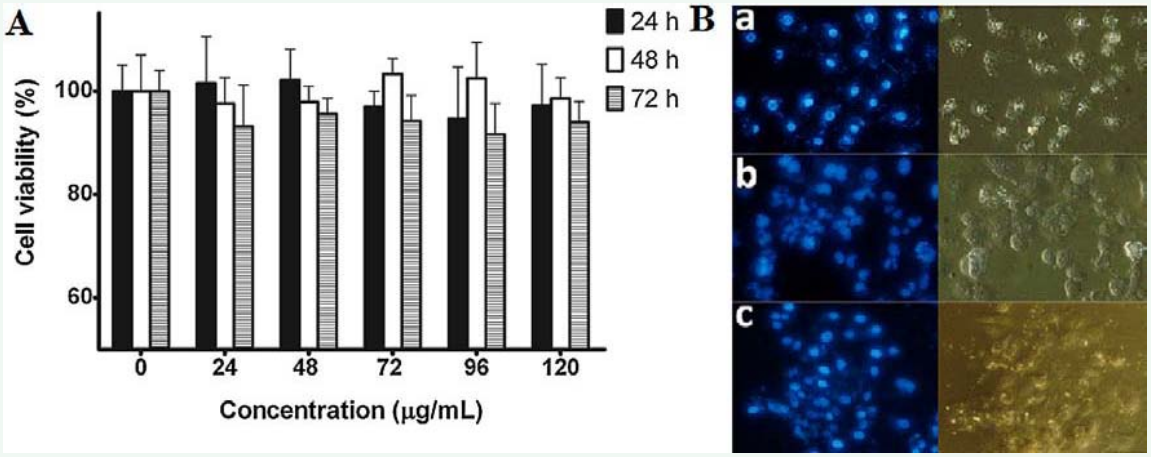

Figure 4 (A) Relative cellular viability of A549 cells after 24 h, 48 h, and 72 h treatment with nano GO and (B) light and fluorescent microscopy images of A549 cells stained with DAPI; Untreated cells (a), DMSO-treated cells as positive control (b), and nano GO-treated cells (120 µg/mL) (c) (data are shown as mean ± standard deviation, n = 6) (Figure reprinted from Colloids and Surfaces B: Biointerfaces- Copyright permission).

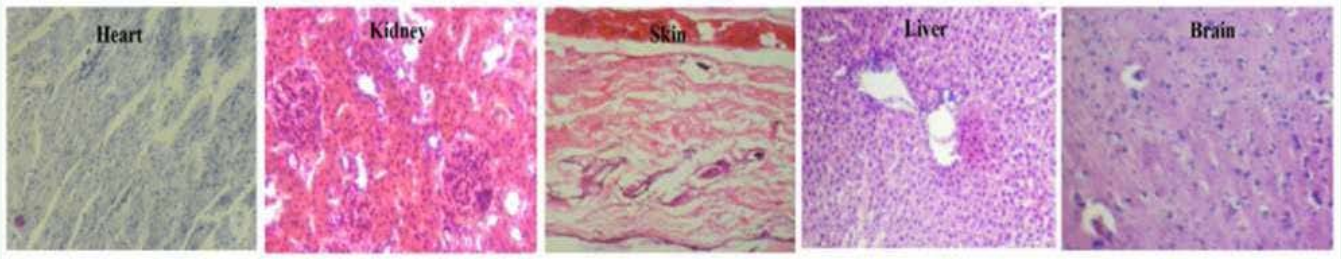

Figure 5 Histopathological sections of the Ag-RGO treated Wistar rats (Figure reprinted from RSC Advances- Copyright permission).

whereas Ginkgo Biloba-RGO shows significant biocompatibility even at higher concentration (100 µg/ml) [40].

Liao R et al., developed tea polyphenols reduced graphene oxide (TP-RGO) and they found that TPs were employed as an environmentally friendly and highly efficient reducer and stabilizer for graphene oxide. The molecular backbone of TPs is mainly contains rigid aromatic rings which could lock up particle and aggregate the layers through their steric hindrance [41].

Akhavan et al., studied the efficacy of the glucose-reduced GO sheets in photothermal therapy of cancer cells. For that, they have developed a green method for the reduction and functionalization of graphene oxide using glucose. The process of functionalization of reduced sheets by gluconate ions produced during the reduction by glucose in the presence of Fe catalyst, without any PEGylation. Moreover, the GRGO-Fe was used as a biocompatible graphene-based nanomaterial suspension for a highly efficient NIR photothermal therapy of LNCaP prostate cancer cells *in vitro*. The authors found that the GRGO-Fe with a high concentration of 1 mg mL^{-1} requires only 12 min for complete destruction of the cancer cells under irradiation of an 808 nm laser source with power density of 7.5Wcm^{-2}. It is an effective NIR photothermal nanotherapy of cancer cells [89].

CONCLUSIONS

The reduction of GO by chemical method is found to be a very promising technique to produce large scale graphene. The use of environmental friendly reducing agents to synthesis graphene attains an excessive interest in the scientific community. There is continuous increment of research publications in the area of green reduction of GO. The green reduction is considered as one of the most versatile method and the reagents used here is an alternate of hydrazine or other poisonous reducing agents. They are also safe to handle and the reaction coproduces are biocompatible. Most of the researchers uses the reduced graphene oxide for the biological applications and in particular for the functional foods and nutraceuticals, they are using this green technology. Like drugs, the efficacy of food and food components is the primary function of the bioavailabity. The research in the field of developing food based delivery system is an emerging stage. GBNs as a delivery vehicle, it is possible to provide or enhance the optimal characteristics of a nutraceutical component which includes stability, incorporation of hydrophobic and hydrophilic substances, high loading capacity and suitable for various routes of administration such as oral, pulmonary and topical application. The replacement of the toxic chemicals will be a big achievement and more studies can help to understand the exact reduction mechanism of GO using most of the reported green reducing agents in the nutraceuticals area.

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