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Short Communication

Do Graphene Worship Cerebellar Granular Neuron Cells?

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DESCRIPTION

Graphene has actually fostered material science and is one of the most probed Two-Dimensional (2D) materials [1]. The intriguing properties of graphene have surely caused research on to other 2D materials and notably to modified forms of graphene. Reduced graphene oxide, is one such chemically modified form of graphene which is widely explored on various grounds. Reduced graphene oxide is obtained by chemically reducing the oxygen moieties of graphene oxide using various reducing agents like sodium borohydride [2], hydrazine [3], L-ascorbic acid [4] etc. Complete reduction of Graphene Oxide (GO) yields 2D graphene sheets but practically complete reduction is not usually achieved since some sp3 bonds remain resilient to change to sp2 hybridization [5]. The choice of reducing agent determines the composition and amount of carbon to oxygen ratios in finally obtained rGO [6]. Reduced Graphene Oxide (rGO) finds suitable applications in electronic, opto-electronic, sensor [7], electrochemical [8] and biomedical applications. The available literature and research updates file the feasibility of rGO for various applications such as sensor, as delivery agent of drug molecules, Photothermal Therapy (PTT), tissue engineering, antibacterial coating etc., rGO has been interestingly explored for stem cell differentiation, 3D printing

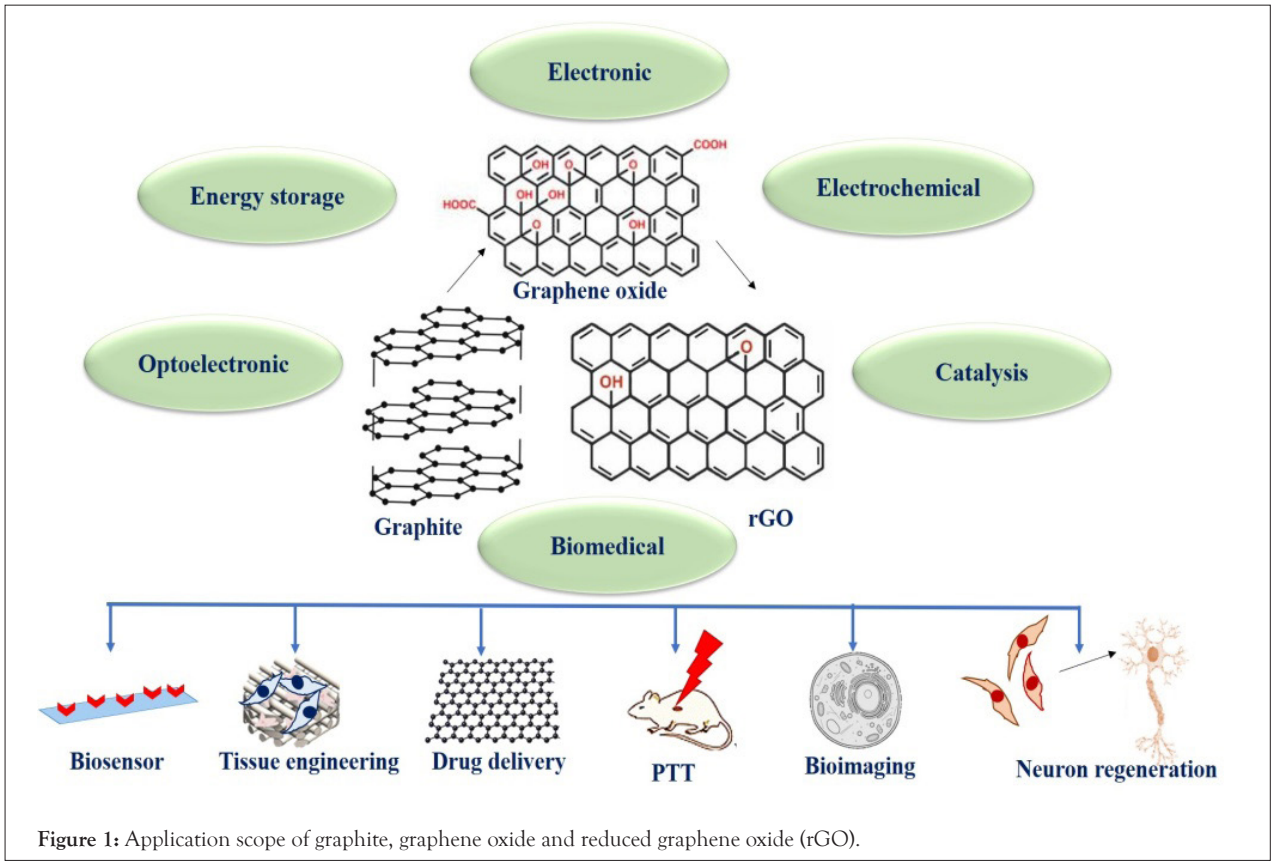

Figure 1: Application scope of graphite, graphene oxide and reduced graphene oxide (rGO).

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and neuron regeneration (Figure 1).

Due to its high hydrophobicity, rGO has a propensity to irreversibly agglomerate or even restack into graphite via van der Waals interactions between individual layers in the absence of a stabilizer. These make further fabrication/ processing difficult for various biomedical applications. Chemical or surface modification is a resolution to overcome the above-discussed limitation and also improve the biocompatibility as well functionality of rGO. Wei et al., reported the functionalization of rGO using p-aminobenzoic acid followed by modification with the aid of Polyethyleneimine (PEI) and folic acid. The functionalized rGO was found to enrich rGO with amazing water dissolvability and targeting capability. The modified rGO as drug delivery framework showed higher loading capacity for drug molecules. The loaded medication was discharged at the target malignant cell site in a pH and salt dependent manner. The results endow functionalized rGO as excellent drug delivery agents capable of targeted and controlled release [9].

Infact, apart from the biosensor, PTT and anti-bacterial applications, one of the most encouraging and looked up application of rGO is neuronal regeneration. rGO act as a mechanically solid and cytocompatible platform for neuronal cell regeneration in vitro. With its phenomenal cytocompatibility and protein adsorption capacity, the nanostructured rGO microfiber can uphold neuronal attachment and differentiation. Guo et al., reported rGO fibers of thickness 100 μm as capable of acting as an excellent scaffold that can promote neural cell adhesion, proliferation and differentiation into neurons. The results demonstrate that rGO fibers offer a more impressive substrate for forming thick neural organization encompassing the microfiber. The outcomes validate its applicability as a framework for neuron regeneration [10].

The research conclusions highlight the multifunctionality of rGO for various healthcare applications notably for neuron regeneration applications. The prime focus on exploiting rGO for neuronal application demands an equal focus on its toxicity and potential side effects. Clear cut idea about the actual impact of rGO and its modified forms on neurons is still lacking and needs more validation before making solid inferences. One way to get more reliable analysis is by proper selection of test systems for in vitro neurotoxicity analysis. Primary neurons notably Cerebellar Granule Neurons (CGNs) are an ideal test system for neurotoxicity studies. CGNs constitute 50% of the central nervous system [11].

In this background, Cherian et al., synthesized pluronics coated reduced graphene oxide (P-rGO) and evaluated its biocompatibility with neuroregeneration point of view. For obtaining pluronics functionalized rGO, graphite was initially oxidized to GO using Hummer's process followed by reduction using ascorbic acid and surface functionalization using pluronics. The pluronics coated rGO was well characterized using various instrumentations like Transmission Electron Microscope (TEM) and X-Ray Diffraction (XRD). The effect of P-rGO exposure on CGNs isolated from Wistar rat pups was assessed to check its feasibility and compatibility for neuron regeneration applications. P-rGO exhibited a dose-dependent toxicity on isolated CGNs cells according to MTT and neutral red uptake assays. Live dead assay also confirms an increase in cell death at the highest concentration of 80 μg/ml. Increased cellular uptake of rGO-P with increased exposure also compromised the cellular morphology and initiated cytoskeletal disturbance along with a decrease in mitochondrial membrane potential (MMP). From the DCFH-DA and Griess reagent assay, it was concluded that the oxidative stress developed as a result of reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation

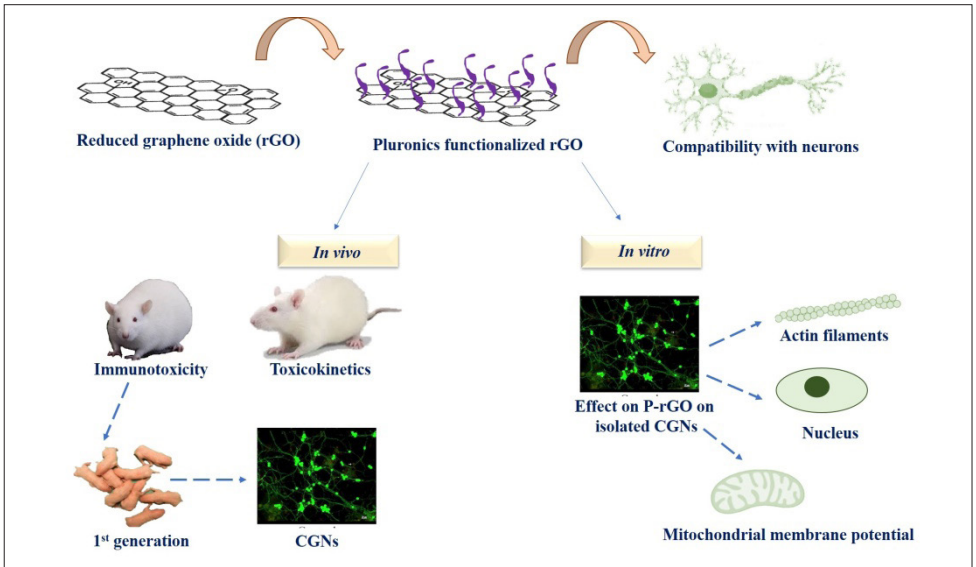

Figure 1: Application scope of graphite, graphene oxide and reduced graphene oxide (rGO).

post particle exposure was responsible for the dose-dependent decrease in cell viability (Figure 2) [12].

To get a wider perspective on the upshot of P-rGO on neurons, Cherian et al., also evaluated the effect of P-rGO on *in vivo* systems [12]. P-rGO exposure on male Wistar rats did not instigate any toxic activity. Additionally, confocal Raman spectra analysis showed that the P-rGO was eliminated from the blood within a time period of 21 days post exposure.

CONCLUSION

The findings also include the immunotoxicity analysis data that shows no remarkable splenocyte proliferation. The group also evaluated the chance of developmental toxicity in neuron cells of 1st generation following the exposed parent mother. For this evaluation, female rats were exposed to P-rGO on their 7th day of gestation and the CGNs were isolated from the 7-day old newborns. No evident morphological changes or toxic reactions were observed in the isolated CGNs and the yield was comparable to normal unexposed rat offspring. As far as the results indicate, P-rGO does not persuade any harm or disintegration in neurons at *in vivo* level, which is hopefully a good assurance for its potential neuroregeneration applications.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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