

# Functionalized graphene process in biotechnology: a brief landscape

## Abstract

Carbon-based nanomaterials, as graphene have caused increased interests of the scientific community due to their unique physical and chemical properties with potential applications in biotechnology and other fields. Graphene has awakened in medicine a new perspective in prophylactic, diagnostic and therapeutic areas. The chemical modification of graphene and its derivatives, plays an important role in their solubility, biocompatibility that allows better interaction with several biological systems, this subject is the main contribution in this brief review.

**Keywords:** Graphene, Chemical modification, Biocompatibility, Nanomedicine applications, Medicinal chemistry

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**Abbreviations:** CVD, chemical vapor deposition; r-GO, reduced graphene oxide; GNS, graphene nanosheets; ODA, octadecylamine; GNS-CS, chitosan modified graphene nanosheets; PEI, polyethyleneimine; PEG, polyethylene glycol

## Introduction

Recent studies have shown that the r-GO, a nanomaterial that exhibits high biological compatibility and low toxicity,<sup>1</sup> is able to create a temporarily opening in the hematoencephalic barrier. This barrier is responsible for protecting the central nervous system, strictly selective in the transition of substances that cross and can be deposited in the brain region. Thus, r-GO became an efficient carrier of drugs where other substances it was not previously possible. In this way, the study of diseases such as Parkinson's and Alzheimer's suffered great impact with the advance of this new area.<sup>2</sup>

Graphene can be prepared by different techniques,<sup>3</sup> as mechanical exfoliation, epitaxial growth on SiC, chemical vapor deposition (CVD) and chemical oxidation from graphite.<sup>4</sup> However, the application of graphene nanosheets in high performance nanomaterials in biodevices depends on their interaction and compatibility in different biochemical environments, which frequently demands functionalization steps previously to their use.<sup>5</sup> The chemical modification of graphene nanosheets (GNS) has been studied for several researches using different types of functional groups as carboxylic, alcohol, sulfonates, amines, amides, carbamates.<sup>4</sup> For instance, the functional groups containing nitrogen have attracted attention due to the broad range of reactions using nitrogen atoms that can be performed with different oxygen groups from GO structure.<sup>6</sup>

Duo to its high variability functionality, graphene has been shown to be ideal for delivery of nucleic acid modified (for RNA recognition),<sup>7</sup> biosensors,<sup>8</sup> bio-nanocomposites based electrochemical immunosensing,<sup>9-10</sup> template in cholesterol sensors,<sup>11</sup> among others. However, due to some inherent disadvantages, such as hydrophobicity and its easy aggregation in aqueous solution, the applications of graphene have been seriously limited.<sup>8</sup> One strategy used to improve the dispersion of GNS is the oxidation and functionalization of the carbonaceous surface, which increases their wettability. The oxidation

process has frequently been carried out using Hummers methods.<sup>12</sup> The surface functionalized of GNS not only plays an important role in the process of GO exfoliation as well as, allows its potential application in different types of biomaterials. The covalent functionalization with oxygen functional groups on GNS surfaces, including carboxylic acid groups at the edge and epoxy/hydroxyl groups on the basal plane can be utilized to change the surface functionality of GO. The presence of oxygen-containing groups in GNS renders it strongly hydrophilicity in water.<sup>13</sup>

## Materials and methods

ZrO<sub>2</sub> thin films were deposited onto n-type silicon (100) and quartz substrates maintained at room temperature using DC reactive magnetron sputter deposition technique. Pure zirconium (50 mm diameter and 3 mm thick) was used as sputter target for deposition of films. Magnetron sputter deposition system with sputter down configuration was employed for preparation of ZrO<sub>2</sub> films. Sputter chamber was evacuated using conventional diffusion pump and rotary pump combination. After attaining the base pressure of 5x10<sup>-6</sup> Torr, required quantities of oxygen and argon gases were admitted in to sputter chamber individually by fine controlled needle valves. ZrO<sub>2</sub> films were formed at an oxygen partial pressure of 3x10<sup>-4</sup> Torr and sputter pressure of 3x10<sup>-3</sup> Torr. DC power fed to the sputter target was 60 W. Energy dispersive X-ray analyser (Oxford Instruments Inca Penta FETX3) attached to scanning electron microscope was used to determine the chemical composition of the films. X-ray diffractometer with copper radiation wavelength of 0.15406 nm was used to determine the crystallographic structure and crystallite size of the films. Fourier transform infrared spectrophotometer (Thermo Nicolet 6700) was used to ascertain the chemical binding present in the deposited films. Optical band gap and refractive index of the films deposited on quartz substrates was recorded using UV-Vis-NIR spectrophotometer (Hitachi modelU-3400) in the wavelength range from 200 nm to 800 nm.

## Discussion

The introduction of substituted amines groups in GO, is one of the most common methods of covalent functionalization, and the

final products have been investigated for various applications in biotechnology and nanomedicine applications. Thus, many researchers have developed functionalization processes these nanostructures adopted strategies based on carboxylation reactions. This reaction has a great advantage because the carboxylated graphene can be derivatized to ester or amide. The amine groups and hydroxyl groups on the basal plane of graphene oxide have also been used to attach polymers through either grafting-onto or grafting-from approaches.<sup>14</sup> One possible functionalization route is through reaction of the carboxylated groups (COOH) with thionyl chloride (SOCl<sub>2</sub>),<sup>5-15</sup> followed by an additional reaction with amine groups.<sup>16</sup> The functionalization process of GO can be achieved by different chemical species or groups, such as amine/amino acids,<sup>17</sup> 4-aminobenzenesulfonic acid, 4,4'-diaminodiphenyl ether,<sup>18</sup> 4-bromophenyl,<sup>19</sup> octadecylamine (ODA),<sup>15</sup> isocyanates,<sup>20</sup> diisocyanate,<sup>21</sup> polyethylene glycol (for delivery of water-insoluble cancer drugs),<sup>22</sup> amine-functionalized porphyrin,<sup>23</sup> polyethylene glycol (PEG),<sup>22</sup> among others. Stankovich et al.,<sup>20</sup> functionalized covalently the GNS with amide and carbamate groups via methylene chloride. The isocyanate was ligated to carboxylic by hydroxyl groups and formation of amide and carbamate respectively which produced stable suspensions in all polar aprotic solvents.<sup>20</sup>

Chitosan modified graphene nanosheets (GNS-CS) were prepared under microwave irradiation in N,N-dimethylformamide medium by Hu et al.,<sup>24</sup> which involved the reaction between the carboxyl groups of GNS and the amine groups of chitosan followed by the reduction of GO in hydrazine hydrate. Chitosan, is a natural polymer biomaterial, is a high molecular weight poly-saccharide composed mainly of 2-amino-2-deoxy (1,4)- $\beta$ -D-glucopyranose residue (or D-glucosamine units) and is derived from the extensive deacetylation of chitin. Due to its nontoxicity, biocompatibility, biodegradability, bioactivity and solubility in aqueous medium, the chitosan and its derivatives, have become increasingly important biomaterials in biotechnology.<sup>25</sup> The specific structure and properties of GNS-CS has attracted significant interest in a broad range of applications such as biomedicine, agriculture, food package film, water treatment, bone substitutes, among other applications.<sup>26</sup>

Shan et al.,<sup>27</sup> functionalized GNS with polyethyleneimine (PEI) that is a water-soluble polymer with amine groups in the molecular backbone, which provides a positive charged structure in the acid solution. Due to its active amine groups, PEI can react with other biomaterials with some certain active groups, such as carboxyl or epoxy groups. These properties of PEI make it an ideal candidate for the modification of graphene and further extend its application. PEI was grafted covalently onto GNS via the nucleophilic ring-opening reaction between the amine groups of PEI and epoxy groups of GO. Since it was positively charged, this biocomposite had an excellent dispersibility in water.<sup>27</sup> Due to the excellent dispersibility of PEI-graphene with active amino groups, this biomaterial becomes feasible for constructing of nanocomposites with attractive biotechnology properties.

Among other important applications, we can highlight the use of GNS in conjunction with polyethylene glycol, (PEG) a reagent of great biological potential. The combination between GNS and PEG changes their surfaces properties increasing the biocompatibility of the final compound.<sup>28-29</sup> In view of such premises, also with the aim of making its use more efficient and specific, conjugations between r-GO and other substances are necessary and challenging.

## Conclusion

In all cases previously mentioned, the modified surface of GNS and

its derivatives prevents their agglomeration and facilitates the formation of stable dispersions in different solvents, thus, facilitating their interaction in several biological systems. The modified GNS might be expected as potential application in several bio-devices in drugs delivery, biosensors, biocatalyst, biological robots in nanomedicine. In this way, the chemical modification process of graphene is an important subject that must be considered.

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## Conflicts of interest

The authors declare no conflict of interest.

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