Chapter

Advances in Graphene Platforms for Drug Delivery in Cancer and Its Biocompatibility

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Abstract

In the past decade, studies on the biomedical applications of graphene quantum dots (GQDs) have increased substantially, especially those related to cancer therapy. Experimental evidence has shown that GQD platforms do not merely serve for drug delivery but have multifunctional properties: their surface also allows several types of molecules to be joined and has photothermal properties that, when combined, make therapies more effective. Most studies have shown evidence of this specificity and therapeutic efficacy at the in vitro level. There is also evidence for potential use in the monitoring of cellular events given the high-quality bioimages that can be obtained with this type of nanomaterial. However, the application of this nanotechnology has stalled due to the lack of available biosafety and biocompatibility studies. This chapter addresses the advances in the use of GQD platforms for drug delivery and the biocompatibility studies reported so far.

Keywords: graphene, quantum dots, platforms, drug delivery, biocompatibility, cancer

1. Introduction

The major health problems currently afflicting the world population have spurred both research and the development of several medicines meant to treat historical diseases as well as more recent ones, such as the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The range of systems and approaches that can be used to deliver therapies is therefore growing and advancing at an accelerated rate. However, the development of any drug involves a research phase, during which several iterative tests and trials provide important information on the characteristics of the therapeutic target, the biological context, and possible physiological implications [1, 2]. These types of studies provide information on the formulation, efficacy, dosage, and safety of drugs. Products obtained from nanobiotechnology require very rigorous studies due to the great chemical diversity and toxicity said products can produce. These studies must be designed to provide detailed information on the biocompatibility of the nanomaterial and reveal any functional effect on the main

Model	Target	Result	References
***In vitro* models**			
MDAMB-231 cells	Genes	Suppression of gene expression and the reduction of the metastatic potential	Huang et al., [14]
MCF-7 and MDA-MB-231 cells	Genes	Induction of cell death	Imani et al., [15] Liyanage et al., [16]
MCF-7, MDA-MB-231 and MCF-10 cells	Genes	Induction of apoptosis and inhibition of the growth	Assali et al., [17]
MCF-7, MDA-MB-231 and MCF-10 cells	siRNA and pDNA	Protection of enzymatic degradation	Cheang et al., [18]
MCF-7 and MDA-MB-231 cells	P-gp/MDR-1	Reversal of multidrug resistance (MDR), anticancer drugs mediated by ATP-binding cassette (ABC) transporters	Luo et al., [19]
Huh-7 hepatocarcinoma cells	mRNA	Delivery intact mRNA	Liu et al., [20]
HeLa cells	miRNAs	Regulation of miRNAs	Dong et al., [21]
Myeloma cells and ovarian cancer cells	Enzymes	For the delivery of enzyme inhibitors to the nucleus for inducing cytotoxicity and cell death	Felix et al., [22]
4T1 cells, MFC7/ADR cells	miRNA-21	Reversal of multidrug resistance (MDR)	Tian et al., [23] Bukowski et al., [24]
Colorectal carcinoma cells	Mitochondria	Cellular stress and apoptosis	Ruan et al., [25]
Oral squamous cell carcinoma		Cytotoxic effect	Zhang et al., [26]
A549 cells	DNA	Cytotoxicity induced by doxorubicin	Iannazzo et al., [27]
Leukemia cells	DNA	Cytotoxicity induced by daunorubicin	Sinha et al., [28]
A549 cells	DNA	Cytotoxicity induced by doxorubicin	Ko et al., [29]
***In vivo* models**			
Mice/BALBc	DNA	Apoptosis of tumor cells and antitumoral effect induced by doxorubicin	Zhu et al., [30]
Breast tumor-bearing mice	Immune cells	Elimination of the tumor mass in a subcutaneous mammary tumor	Li et al., [31]
A549 tumor xenografts.	Tumor cells	Ablation of tumor	Gazzi et al., [32]
MDA-MB-231 triple-negative breast cancer (TNBC) model	miRNA-21i	Phototherapeutic efficiency of indocyanine green	Wu et al., [33]

Table 1.
Graphene quantum dots for cancer-targeted drug delivery.

physiological systems in order to decide whether a nanobiotechnological product should be tested in humans [3, 4].

The incorporation of nanomaterials into biological systems requires strategies for manipulating the ligands bound to the surface to make them more polar and biocompatible [5]. Nanomaterials must be soluble to have the biological application, and this is achieved by adding functional groups (functionalization). An ideal ligand must meet the following requirements: (1) provide stability and solubility to the nanomaterial in biological buffers; (2) maintain high resistance to photobleaching and other photophysical properties in aqueous media; (3) have functional groups that can conjugate biomolecules (conjugation), and (4) minimize the overall hydrodynamic

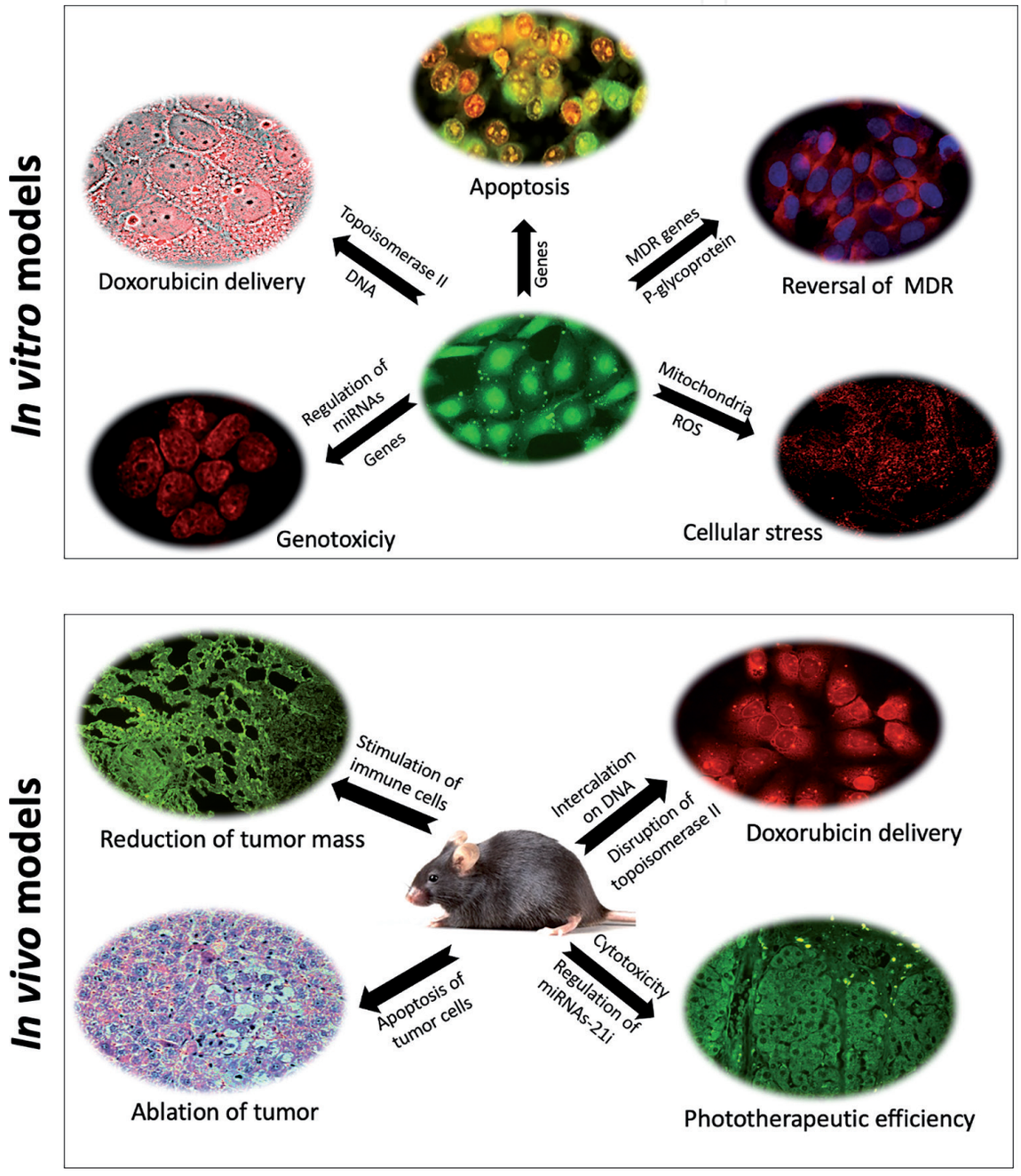

Figure 1.
Application of GQDs platforms for cancer treatment. Cellular targets and effects of GQDs platforms in cell lines and experimental animals.

size [6, 7]. Quantum dots (QDs) are among the most popular nanomaterials: they are semiconductor nanoparticles with photoluminescent properties and a wide variety of applications.

Functionalized QDs are very useful in biomedicine because they can be modified with a great variety of molecules and small biological polymers, which help improve their bioactivity and reduce their toxic effects [8–10]. Thanks to these characteristics, QDs can bind effectively to cell membranes, meaning they can be employed as excellent probes for cell detection, diagnosis, imaging, and delivery of therapeutic agents. Due to the great coupling achieved between QDs and biomolecules, today these are used as a tool for biological goals, to improve the efficacy of drug release control and significantly reduce toxicity [11–13]. At present, a wide range of studies on GQD platforms are mainly focused on cancer treatment (**Table 1** and **Figure 1**). This chapter will review the advances in all these areas, as well as aspects related to the toxicity and biocompatibility of GQDs.

2. Application of graphene platforms for drug delivery

GQDs are carbon-based nanomaterials. Their structure consists of one or more graphene sheets with lateral dimensions of 10 nm [34]. GQDs have a large π-conjugated aromatic structure and a large surface area that allows them to be easily conjugated with various molecules to generate hybrid nanomaterials, but they can also be conjugated with antibodies, proteins, and nucleic acids due to their dimensional similarity with these molecules [35–38]. They also have a high capacity for loading drugs containing aromatic groups, such as camptothecin, paclitaxel, and doxorubicin through π -π stacking interactions between layers of GQDs and drug molecules. Currently, a variety of synthesis methods allow for size, structure, and optical profile design, depending on the intended application. Even green synthesis has been used to protect the environment [39]. Given the properties of GQDs, the biomedical sector has found several applications in the prevention, diagnosis, and treatment of diseases. Recent studies report that GQDs are less toxic, show greater biocompatibility than other nanomaterials, and also have stable and strong fluorescence. All these characteristics make these nanomaterials ideal for use in cancer treatment.

Targeted therapy is a cancer treatment employing drugs that target specific genes and proteins involved in the growth and survival of cancer cells. Targeted therapy can affect tissue conditions that help cancer grow and survive, or it can target cells related to cancer growth, such as cells in blood vessels. To develop targeted therapies, researchers first identify the genetic changes that contribute to a tumor's growth and change [40]. A possible target can be a protein present in cancer cells but not healthy ones. Specificity is required. Targeted therapies are a rapidly growing field of cancer research, and researchers are studying many new targets and drugs in clinical trials. Hence, multifunctional nanoparticles directed at specific targets of the tumor cell are also being developed in the field of nanobiotechnology. GQD platforms have been studied in gene-based therapies across various breast cell lines, where a variety of effects have been discovered. These include the suppression of gene expression and the reduction of the metastatic potential of MDAMB-231 cells [14]; induction of cell death in MCF-7 and MDA cells [15, 16]; the induction of apoptosis and inhibition of the growth of MCF-7, MDA-MB-231, and MCF-10 cells [17]; protection of small interference RNA (siRNA) and DNA plasmids (pDNA) from enzymatic

degradation [18]; and reversal of multidrug resistance (MDR) [19]. These same methodologies have been studied in animal models with good results. For example, in mice/BALBc, GQD platforms can induce apoptosis of tumor cells and have an antitumoral effect [30]. Furthermore, it has been observed that they can eliminate the tumor mass in a subcutaneous mammary tumor model [31].

Messenger ribonucleic acid (mRNA) delivery systems are another type of targeted therapy having a recent boom because of advantages such as biocompatibility and low genotoxicity. Stable graphene platforms functionalized with polyethyleneimine were used in one study, achieving successful delivery of intact mRNA to hepatocarcinoma cells [20]. Let us remember that mRNA has been widely used in the study of gene function and has become popular in the development of new therapeutic strategies for cancer immunotherapy and vaccines. GQDs have also been used as platforms for the delivery of nucleic acids for the regulation of microRNA (miRNAs), negative regulators of gene expression, with great therapeutic effectiveness in HeLa cells [21]. Various investigations indicate that the expression of some miRNAs is altered in some cancers; achieving their regulation would be useful in oncology. And while one would expect targeted cancer therapy to be less toxic than traditional chemotherapy drugs because tumor cells are more dependent on targets than normal cells, this is not the case. Clinical observation indicates that targeted therapies can also produce significant side effects.

Another approach to targeted therapy is for the delivery of enzyme inhibitors to the nucleus. For example, in one study, GQDs were conjugated to imatinib, successfully achieving cytotoxicity and apoptotic cell death in myeloma cells and ovarian cancer cells [22]; imatinib is an inhibitor of the protein tyrosine kinase, which potently and specifically inhibits breakpoint cluster region-Abelson (bcr-abl) tyrosine kinase. However, genetic manipulation and treatments directed at nuclear targets have numerous technical difficulties that are not yet fully resolved. Targeted therapy is complex and does not always work. One of the limitations of this type of therapy is that the drugs for some identified targets are difficult to formulate due to the structure of the target or the way its function is regulated in the cell. An example of this is Ras, a signaling protein that has mutations in up to a quarter of all cancers, but for this type of therapy to work, one would have to know what mutation the gene has [41]. In short, using nanotechnological platforms does not guarantee patient safety, given that side effects of drugs as well as those of the nanomaterial have yet to be assessed.

The lack of response to treatment and the recurrence of initially chemosensitive tumors are responsible for a significant number of deaths in cancer patients. Treatment options used as salvage, such as alternating chemotherapy, dose-escalation, or regional chemotherapy, have yet to yield the expected results. Most cancer patients who initially respond to chemotherapy have relapses because of the so-called acquired resistance to multiple antineoplastic drugs (MDR) [24]. Today, combination therapies seek to address different therapeutic targets using nanobiotechnology. GQD platforms can exhibit all the desirable characteristics of a combination therapy since, as previously mentioned, their surface can be conjugated with different molecules. Their physical, chemical, electrical, and optical properties, however, confer additional functions. As shown, GQDs have a high photothermal modification power under near-infrared radiation (NIR), which allows for their use as photothermal therapy [42–44]. Graphene platforms can also be employed for photodynamic therapy, the goal of which is to generate highly cytotoxic reactive oxygen species (ROS) [45]. A great variety of experimental studies involving different types of cancer have been carried out on animals, in most cases resulting in complete ablation of the tumor [32].

Both photothermic and photodynamic therapy show selectivity toward hyperthermic processes typical of cancer cells, but this is rare with normal cells. GQD platforms with more than one therapeutic effect have been used for the treatment of breast cancer; these include chemothermal therapy [46], chemogenic therapy [23, 47], chemo-photothermal therapy [33], and gene therapy [48]. With these platforms, it has been possible to induce greater cytotoxicity, apoptosis, and reverse drug resistance in breast cancer cells. Moreover, inhibition of tumor growth in an animal model of breast cancer MDA-MB-231 triple-negative has been achieved. Graphene platforms have also been employed as nano radiosensitizers to improve the effectiveness of radiotherapy. Oxidized GOQDs with high phototoxicity has been built to induce a cellular stress response via the production of the reactive oxygen species that would be generated during a tumor's exposure to radiation [49]. Important effects, such as mitochondrial damage and apoptotic death have been observed in colorectal carcinoma cells treated with graphene platforms and radiation therapy [25]. Based on this same principle and thanks to their photodynamic properties, GQDs have also been employed to induce phototoxicity and synergize the cytotoxic effect of radiation in oral squamous cell carcinoma [26].

In addition to these novel uses, GQD platforms are good for the delivery of multiple antineoplastic drugs. A multifunctional platform of GQDs for synergistic breast cancer therapy with controlled release of doxorubicin, methotrexate, and paclitaxel, showed a significant synergistic effect in killing tumor cells with improved efficacy [50]. The advantage of combination therapies is that a therapeutic effect is achieved while reducing drug resistance. On some occasions, however, and as happens in the clinic, the side effects could be considerable. Another method that has been tried for therapeutic efficacy is the conjugation of GQD with a ligand that directs it toward the therapeutic target while additionally carrying the antineoplastic drug. This methodology has been carried out in A549 cells treated with GQDs-biotin-doxorubicin and demonstrates GQDs may have multifunctional effects for cancer treatment [27].

As previously noted, graphene platforms can be built according to the needs of cancer therapy. The construction of ultra-small QDs makes them ideal for achieving not only cell penetration and drug delivery to target sites, but also visualization within the cell. Recently, a graphene platform was used in microspheres with daunorubicin. The small size allowed to monitor drug delivery and the intercalation of daunorubicin in DNA, exerting a better pharmacological effect [28]. Several studies have taken advantage of the fluorescence emitted by QDs to image neoplastic tissues so that, at the same time, drug delivery can be tracked and controlled [51]. In this sense, GQD platforms have become ideal candidates for such purposes due to the high quality of image formation obtained thanks to their fluorescence emission [52]. Additionally, drug/gene delivery in tumor cells has been achieved with greater efficiency both *in vitro* and *in vivo* [53]. For example, GQDs have proved an optimal multifunctional nanocarrier for delivering doxorubicin to specific cancer cells, allowing for the monitoring of intracellular anticancer drug release via imaging and therapeutic efficacy [29, 54, 55]. Ge et al. employed these properties for imaging and the application of dynamic phototherapy for the treatment of breast cancer and induced melanoma in female BALB/c nude mice with favorable results [56]. Other groups have performed functionalization studies of GQDs with silica, hypocrelin A, and porphyria derivatives, managing to obtain multi-color images and antitumor effects in cervical, lung, and breast cancer [57–59]. The results obtained to date appear promising, though they usually depend on the biological variability of the experimental animals.

The growth of solid tumors is characterized not only by the uncontrolled proliferation of cells but also by changes in the tumoral microenvironment. In solid tumors, hypoxic areas generally have a low pH. There may be low levels of glucose and other nutrients, as well as changes in temperature, all associated with various alterations in tumor cell metabolism [60]. While the heterogeneity of the tumor microenvironment sometimes makes it difficult to adequately characterize tumors [61], this has spawned interest in developing new nanotechnology therapeutic strategies to improve not only drug delivery conditions and directly destroy tumor cells, but also alter the balance between neoplastic cells and their microenvironment. Therefore, intelligent systems have been developed for the administration of drugs that respond to stimuli, and therapeutic agents can be activated by endogenous or exogenous stimuli [62, 63]. Platforms based on graphene have proven excellent due to their physicochemical properties since, according to the functional groups that are attached to them, they can be sensitive to changes in the tumor microenvironment or to intracellular signals in response to physical stimulus factors. Graphene platforms have been conjugated with functional chemical groups that allow the drug to be released when there are changes in pH and temperature [64]. For example, it has been observed that when pH-sensitive functional groups (COOH, $-NH_2$, and SO_3H) are added to graphene platforms, controlled drug release can be achieved in tumor areas [65]. The functionalization allows the pH of the platform to change in the bloodstream and, with this, remain in circulation for longer and favor the delivery and effectiveness of the treatment. This same effect has been achieved by changes in the loading of the platform. This was the case with the construction of the graphene platform with polymers such as polyethylene glycol and doxorubicin, where it was observed that the release of the drug is accelerated in an acidic environment [66]. Or with the construction of graphene microspheres conjugated with a dendrimer and maltose (Fe_3O_4@C@TDG) as a potential transporter to promote the release of doxorubicin and improve its therapeutic efficacy at specific pH [67]. Polymer aggregation has also served to make photoluminescence more stable at different pH for imaging tumor cells, which, as already mentioned, is part of the multifunctionality of the graphene platform.

3. Evidence regarding the biocompatibility and toxicity of graphene platforms

The available literature indicates that research on GQDs has grown widely in relation to their uses, and that is why we now know their biomedical applications include the elimination of bacteria, the administration of drugs, the development of nanocarriers, cancer therapy, and tissue engineering [35–37, 68]. The therapeutic applications of nanomaterials remain quite limited, and there is no safe and effective formulation yet that can be administered in humans [69–71]. While QDs produce a series of morphological and functional alterations that lead to tumor cell death, what happens to healthy cells is unknown [72]. Therefore, the toxicological profile of each nanomaterial is needed to make decisions regarding potential risks vs. benefits. However, what is known about the biocompatibility of GQDs and what evidence is there of the toxicity of drug delivery platforms?

GQDs and their derivatives have variable toxicity in biological systems ranging from prokaryotic to eukaryotic, depending on the dose and the functional groups with which they are coated [34]. They have also been evaluated in a series of human cell lines. For example, studies carried out on leukocytes showed that there was

significant uptake of GQDs in monocytic and granulocytic cells, suggesting that phagocytic cells can incorporate GQDs. The toxicity observed in this study was relatively low (10%) after a 36-hour exposure period at concentrations of 500 μg/mL [73]. In another study using GQDs functionalized with NH_2, COOH, and $CO{-}N(CH_3)_2$ it was observed that A549 and C6 cells showed a slight increase in their proliferation at concentrations of 200 μg/mL, but no death due to apoptosis [74]. GQDs have also produced toxic effects on mesenchymal stem cell self-renewal and differentiation [75]. Several studies have pointed to the toxic effects of graphene derivatives [76–81]. These functionalized QDs can produce a variety of toxic effects at the cellular level and in vivo due to the series of impurities produced during the oxidation process. The same happens in the coating process with other molecules [82]. However, when GQDs are coated with polyethylene glycol at concentrations of 320 μg/mL, they do not affect the viability and differentiation capacity of neural stem/progenitor cells (NSPCs) [83]. Also, reduced toxicity, absence of ROS production, absence of apoptosis, and lack of morphological changes have been observed in HeLa and A549 tumor cells under concentrations of 100 μg/mL [84, 85].

The cellular and nuclear effects that GQDs produce are due to their high permeability in biological membranes. It is known that the uptake and localization of GQDs are highly dependent on size, shape, coating, and pH, among other factors. Previous studies have shown that GQDs use membrane lipid rafts for their transport across the cell membrane. This process is better, the smaller the QDs are [86]. However, protein-coated GQDs enter mainly by phagocytosis and with smaller coatings by clathrin-mediated endocytosis [87, 88]. GQDs with amide groups enter the cell through energy-dependent mechanisms by endocytosis, mediated by caveolae and phagocytosis [89]. Within the cell, GQDs are distributed in different organelles producing a variety of cellular effects. They are later distributed through endosomal trafficking and reach lysosomes, mitochondria, and the nucleus, and can produce autophagy, apoptosis, and DNA damage [90–92]. At the nuclear level, the NPC Kap2 and Nup98 genes can participate in the uptake of GQDs and can produce morphological and functional alterations associated with genotoxicity, including oxidative stress and DNA damage [93, 94].

There are many reports in the literature regarding the toxic effects of both GQDs and their derivatives in a variety of human cell lines and it is impossible to mention them all in this chapter. What is evident is the ease with which they penetrate cells, position themselves and participate in strategic cellular processes, thus potentially affecting cell functionality and leading to cell death. However, of the studies reviewed so far, most were done in tumor cell lines where physiological processes are altered and there are specific survival and adaptation mechanisms. To date, there are no studies carried out on cell lines from healthy tissue, so we cannot rule out the fact that GQDs could produce morphological and functional modifications associated with toxicity in healthy cells.

What effects do they produce in higher organisms and experimental animals? What is known about the processes of absorption, distribution, metabolism, and excretion (ADME) of GQDs? The information so far is limited. Previous studies in nematodes have shown that nitrogen-bound GQDs (N-GQDs) produce degeneration of dopaminergic and glutamatergic neurons at concentrations of 100 μg/mL [95]. A series of studies on the biocompatibility and biodistribution of GQDs in adult and embryonic zebrafish have been reported and provide important information on embryos' developmental delays, pigmentation inhibition, pericardial edema, and delayed hatching among other things. In adults, GQDs showed high biocompatibility

and accumulation in the digestive tract [96]. Apparently, the accumulation of QDs depends on the stage of development of the zebrafish (embryo, larva, adult). Studies in adult zebrafish using GQDs at different concentrations (0.1 ng/mL to 100 μg/mL) and exposure times (8 h to 6 days) showed distribution in the heart, blood vessels, brain, intestine, head, and tail [97–101]. The effects that have been found in zebrafish are morphological and functional alterations, while mortality is attributed to the generation of ROS, oxidative stress, and, finally, apoptosis [102]. On the other hand, studies carried out in chicken embryos have also shown evidence of GQDs-induced toxicity. It was found this affected survival but did not produce morphological or biochemical alterations in the embryo [103]. However, another study found morphological alterations and hemolysis of erythrocytes [104], as well as ultrastructural alterations of the brain, suggesting neurotoxicity [105]. These results suggest that GQDs can alter key processes, not only in adulthood but also during embryonic development.

Biodistribution studies in rodents have shown that GQDs are distributed in various tissues and produce certain toxic effects as well. For example, in mice that received GQDs in a single dose of 10 mg/kg intravenously, it was found that 6 hours after inoculation the QDs were distributed in several organs. Clearance began after 3 days and, at 14 days, the QDs had been completely removed. Histological and biochemical studies did not reveal alterations, only weight loss [106]. However, in another biodistribution study carried out in rodents treated with a single dose of 5 and 15 mg/kg of GQDs intravenously, they produced morphological alterations compatible with inflammation and biochemical damage in the lungs after 7 days of exposure [107]. Additionally, yet another study using repeated doses of 5, 10, and 15 mg/kg every third day for 30 days, showed a reduction in blood cells, morphological alterations in the liver, lipofuscin deposits in the kidney, and the presence of inflammatory infiltrate in the lungs. These alterations were dose-dependent [108]. Taken together, these data suggest that GQDs produce acute toxicity at both single and repeated doses in mammals.

Today there are no reports of long-term studies (chronic toxicity), studies on reproduction and development, or of any other type that allow a general overview of the toxicological profile of GQDs. However, there is experimental evidence showing that other materials derived from graphene can produce a series of toxic effects that must be considered. For example, studies of the distribution of graphene and its derivatives after aerial exposure showed toxic effects in the lungs of rodents [109, 110]. In a chronic inhalation toxicity study of graphene nanoplates, deposits of the nanomaterial were observed in the lungs and pulmonary lymph nodes in mice [111]. In a distribution study in rats using doses of 10, 20, and 40 mg/kg of graphene oxide orally, it was found that it produced nephrotoxic effects due to oxidative stress [112]. While in another study, the administration of multiple doses of oxidized graphene (4 mg/kg) for 4 weeks showed deposits of the material in different tissues in rats [113]. Mutagenic effects have been observed in rats when exposed to graphene oxide at a dose of 4 mg/kg for 4 weeks [114]. Likewise, toxic effects on the reproductive capacity and development of offspring have also been reported after the administration of oxidized graphene to mice with doses from 6.25 mg/kg [115]. Unfortunately, when reviewing the subject, we noted there are no toxicity studies regarding the GQDs platforms employed for drug delivery in cancer research. In fact, all the studies have focused on evaluating its efficiency and specificity toward the tumor cell. That is, what has mattered so far is to demonstrate their possible therapeutic applications in cancer, but not the possible toxic effects they may produce. Therefore, we could say that biosafety studies on GQDs platforms are null.

To date, GQDs have been widely studied as carriers with a large surface area favoring drug transport and particular interest has been placed on characterizing their therapeutic bio properties *in vitro*. However, the preclinical studies carried out so far are hardly enough. Most of the studies in cells and animals have focused on evaluating the efficiency of drug/gene delivery at the site of interest. The dosage of the treatments used in animals has been empirical, since no study has demonstrated the real drug/QD concentration within the body, and it is not known if there could be pharmacological interactions between these platforms and other therapies used in the clinic. One aspect that has been completely neglected is the bioavailability of GQDs. What will be the appropriate route of administration? Do they bind to plasma proteins? Do they accumulate? Where do they metabolize? In the route of excretion? There are many questions that remain unanswered. In addition, long-term toxicity studies are required in different species of animals to test the effects on reproduction, carcinogenicity, and teratogenicity, among others. Many preclinical studies are still needed if GQDs are to be used for diagnosis and treatment in humans.

Concern regarding the toxicity of graphene not only stems from the findings mentioned above, but also from the long-standing concern about environmental and occupational exposure to graphene [116]. Inhalation toxicity data of graphene analyzed in experimental animals suggest that acute exposure by repeated inhalation to graphene-derived materials could induce inflammatory/fibrotic reactions, suggesting that it could also induce fibrotic disease in humans [117, 118]. Hence the importance of conducting preclinical biosafety studies of graphene nanomaterials and their derivatives using specific criteria, for these are not necessarily the same as those used for chemical products. The toxicological evaluation must be extrapolated with special care due to the size of the nanomaterials and the chemical groups they contain. If there is no complete toxicological profile that meets the standards required by the guidelines of administrative agencies such as the US Food and Drug Administration (FDA), the European Medicines Agency (EMA) or the European Medicines Evaluation Agency (EMEA), and the Japanese Agency for Pharmaceutical and Medical Devices Agency (PMDA), the research will not leave the laboratory.

4. Disadvantages of using graphene platforms

Drug delivery through nanocarriers has been used successfully in recent years; however, there are still certain challenges that must be addressed to achieve successful drug delivery to target sites. Each of these nanocarrier drug systems has its own chemical, physical and morphological characteristics, and may have an affinity for the different polarities of drugs through chemical or physical interactions, in addition to its own toxicity [119–123]. One of the goals of using GQDs platforms is to transport and deliver ligands to specific tumor targets and improve antitumor therapy by taking advantage of the supposedly low toxicity of this nanomaterial. However, and as was discussed above, one of the main problems with GQDs and GQD platforms is the lack of toxicological studies that effectively demonstrate their safety and biocompatibility. We have nothing to indicate that they have low toxicity, if there is no evidence to prove this. Additionally, there are several issues inherent to GQDs, the therapeutic targets to be reached and the drugs to be delivered that we must take into consideration.

One of the main problems with small nanomaterials, including GQDs, is the tendency toward aggregation. The lack of dispersion of a nanomaterial can result in

transportation problems through the blood, the binding to the plasma protein corona, and the deposition of QDs in biological fluids and tissues [124]. Due to their size, they can go undetected by the immune system and, if they are not biocompatible, could induce toxicity. The dispersion of these QDs has been achieved with the use of some polymers. However, this can sometimes make the QDs larger and thus recognizable by the immune system [125]. Covalent functionalization of GQDs platforms is easy and simple, given their properties and the high surface area for their functionalization. On the other hand, the binding of non-covalent GQDs is more complicated and unstable and can lead to loss of important functional groups that can, in turn, lead to loss of electronic properties. It is also possible to obtain a wide area of functionalization [126] but the presence of a large, functionalized surface area can have adverse consequences, especially if it is a biologically active ligand that can impact cellular physiological processes. There are currently no studies on real-time monitoring and distribution of GQDs in animal models, so the effect of these platforms remains unknown.

If we want to direct GQD platforms toward specific tumor targets, we must know the molecular biology of the tumor. That is, where they need to be directed and with what do we intend them to interact. To achieve this, we require platforms that can specifically locate and access the tumor and not reach healthy tissue. Unfortunately, as we saw in the previous section, very few of the studies on animal models provide any information on this, since the studies only focus on the effects of GQD platforms at the tumor site but do not mention whether neighboring or distant tissues were affected, if systemic toxic effects were observed, or if there was mortality. The great disadvantage of most nanomaterial platforms, including GQDs, animal models have not yielded enough information about them. All nanomaterials are widely known to be cytotoxic, and so not a single one has been identified as harmless. Therefore, it is important that we obtain detailed information regarding the effects they produce *in vivo*. Additionally, we must remember that inter-individual biological variability is considerable, and it is not always possible to extrapolate data obtained directly from experimental animals to human beings.

Furthermore, all drugs used in clinical oncology are in themselves toxic and produce a variety of adverse effects. While GQD platforms have been used to target specific cells and molecules, most of the studies have been carried out using cells cultured *in vitro*, where the conditions and cellular response are more controlled. Also, only tumor cell lines have been used. There are currently no studies using cell lines from healthy tissue to determine the effect GQDs platforms may have on healthy tissue, either that adjacent to the tumor or healthy cells at a distance. The response of the tumor cell can vary greatly, as well as sensitivity to the GQDs platform and to the delivered drug. One of the big problems when extrapolating these findings to animal models is the dosage and exposure time, since we need to consider the different compartments where the platform will be distributed and the nanomaterial that will be lost during the ADME processes. Another important problem is the scaling of the product: it is not the same to produce the amounts to be used in *in vitro* models, than those needed to treat a laboratory animal, which is generally more complex and expensive. One of the characteristics of GQDs platforms is their large surface area for drug loading. However, more than an advantage, this can have adverse consequences given the large amounts of a certain drug that will be delivered to the cells. One of the great problems of nanobiotechnology is that it has not been possible to determine the exact amount of drug that can be attached to the QDs, nor how much of this actually reaches the target site. We could say that GQDs platforms have a great advantage

insofar as they could have high therapeutic efficacy, but what about safety and specificity? Are they so efficient that they will only target tumor tissue? During the ADME processes, will they not affect other healthy tissues? At most, only five drugs have been used in the production of antitumor drug delivery platforms. Why? Can they not be viably employed with any type of antitumor drug? There are still questions that need to be clarified. If this information is not available, the lack of answers will remain one of the main limitations to these platforms vis-à-vis other nanomaterials.

5. Conclusions and future perspectives

GQDs hold great promise as a platform for multifunctional drug/gene delivery as well as an excellent tool for quality bioimaging. Current studies of drug delivery systems based on nanotechnology are expected to facilitate advanced forms of this kind of delivery. However, they are currently limited by the lack of preclinical pharmacological and toxicological studies, and their unknown biosafety and biocompatibility. A detailed understanding of how GQDs interact with blood components, the immune system, and aspects related to ADME processes is of vital importance. If the regulatory requirements requested by pharmacovigilance agencies are not addressed and resolved, the biotechnological and biomedical potential of GQDs cannot be employed in clinical studies. There is no doubt that, in the past decade, there have been great advances in drug delivery methods. GQD platforms have advantages over other platforms, including their surface area, size variability, their ability to functionalize with different ligands, and their photothermal and photodynamic properties. All these features make these platforms into ideal tools, not only as intelligent and multifunctional platforms for cancer therapy but also to monitor drug delivery and therapeutic effectiveness via their fluorescent emission. All these qualities could open up new pathways toward improved technological knowledge on nanoparticle-based therapies, particularly those aimed at a variety of cancers currently affecting the human population.

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Conflict of interest

The authors declare no conflict of interest.

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