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Graphene Nanostructures: Pioneering Drug Delivery Vehicles for Targeting Etoposide to Cancer Cells

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Abstract

Etoposide (ETO) is a formidable chemotherapeutic agent that holds great promise for individuals grappling with various forms of cancer, including testicular, prostate, bladder, stomach, and lung malignancies. While traditional chemotherapy protocols are often accompanied by significant challenges and discomfort for patients, recent advancements in nanotechnology are revolutionizing the administration of etoposide by incorporating the groundbreaking properties of graphene and its derivatives. Extensive research has highlighted the extraordinary potential of graphene as a targeted delivery system for anticancer therapies. Graphene's unique structure and biocompatibility allow it to effectively home in on cancer cells, minimizing damage to healthy tissue. By harnessing this remarkable material, we can significantly enhance etoposide's therapeutic effectiveness while mitigating its cytotoxic side effects. This article delves into the exciting possibilities of employing graphene nanostructures as innovative vehicles for Etoposide delivery, illuminating a path toward more effective and patient-friendly cancer treatment options. The integration of advanced materials like graphene not only holds the promise of improving therapeutic outcomes but also signifies a transformative shift in the approach to cancer treatment, offering hope for a brighter future for patients and healthcare providers alike.

Keywords: Etoposide, Graphene oxide, Carbon-based nanostructures, Cancer, Drug delivery.

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1. Introduction

Cancer is the second leading cause of death in the world, considered by researchers due to its increasing growth. Chemotherapy and surgery are the primary treatment approaches that target cancer cells. Chemotherapy's drawbacks include inducing high levels of toxicity in the body (normal tissue and cancerous cells) by targeting cell essential biomolecules (1).

Chemotherapy-induced peripheral neuropathy is one of the most common complications of anti-neoplastic agents. It causes symptoms such as numbness, tingling, and pain, especially in the arms and legs, which make daily activities impossible. The burden of this complication is associated with increasing unemployment and decreasing annual incomes (2). Researchers have explored various

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drug delivery systems (DDS) to minimize the adverse effects of chemotherapeutic agents (3). The choice of delivery service significantly impacts the functionality of anticancer drugs through the cells and tissues. Targeted drug delivery systems (TDDS) typically consist of a transporter material, a targeting ligand, and an effective drug (4). Nanotechnology revolutionized drug delivery approaches and improved cancer treatment. Nanostructures with unique properties, such as increased surface-to-volume ratio, mechanical strength, photoelectric, active/passive targeting, and physicochemical features caused drug bioavailability, stability, solubility, and probable complications (5, 6).

One of the most important chemotherapeutic agents used to treat cancer is Etoposide (ETO). In this article, we will explore the potential of graphene nanostructures (nG) as highly effective nanocarriers for the delivery of ETO. First, we explain the properties of etoposide, its pharmacology, therapeutic efficacy and adverse effects. Then, we will delve into the unique properties of graphene, such as its exceptional surface area, mechanical strength, and biocompatibility, which make it an ideal candidate for drug delivery applications. Additionally, we will examine recent research findings on the interactions between graphene and ETO, highlighting how these interactions can enhance the drug's therapeutic efficacy while minimizing side effects. Throughout the discussion, we will also consider the implications of using graphene-based nanocarriers in clinical settings and the future directions for research in this promising field.

2. Pharmacology of Etoposide

ETO is a potent derivative of podophyllotoxin, a compound that has shown significant efficacy in the treatment of various types of cancer (Figure 1). Specifically, ETO is utilized in the management of acute lymphoblastic leukemia (ALL) and neuroblastoma, both of which primarily affect children. Additionally, it is effective in treating several solid tumors, including testicular, stomach, and ovarian cancers, as well as lymphoma and breast cancer. This versatility makes ETO an important therapeutic option in oncology. (7).

ETO specifically targets the enzyme topoisomerase II in cancer cells, playing a crucial role in regulating the cell cycle. This targeting is particularly effective during the synthesis phase (S) and the early gap 2 phase (G2), where DNA replication and preparation for cell division occur. By inhibiting the activity of topoisomerase II, ETO disrupts the normal process of DNA replication, effectively halting the proliferation of cancerous cells and ultimately leading to their growth inhibition. This mechanism makes ETO a valuable therapeutic agent in the fight against cancer (8). Also, ETO plus cisplatin is used in combination with cyclophosphamide, doxorubicin, and vincristine regimen (9). However, subsequent findings revealed that this medication does not enhance survival rates in prostate cancer patients. Consequently, its application is now limited to a few patients who lack alternative treatment options. The same fate has happened to etoposide phosphate (10).

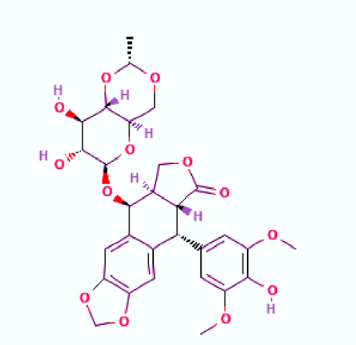

Figure 1. Chemical structure of etoposide.

Another application of ETO is as a DNA-damaging agent to confirm its effect on breast cancer type 1 (BRCA1) protein regulation. We can detect breast cancer by evaluating cytoplasmic BRCA1 protein levels with ETO induction. Increased sensitivity of BRCA1-deficient cells to ETO could be due to specific TOP2-linked DNA breaks (DSBs) caused by topoisomerase II (11).

The potential drawbacks of ETO include a range of uncomfortable and serious side effects. Patients may experience bone marrow suppression, leading to reduced blood cell production and increased risk of infections and anemia. Additionally, many individuals report nausea and vomiting, which can significantly impact their quality of life during treatment. Fever may also arise as a reaction to the medication, indicating that the body is responding to the treatment. Furthermore, hair loss is a common concern, as many patients notice thinning or complete hair loss while undergoing this therapy.

Furthermore, ETO encompasses hydrophobicity, limited solubility in water, drug deposition during dilution, variable bioavailability ranging from 25% to 74%, and a short half-life. Additives such as polysorbate 80 or benzyl alcohol are employed to resolve these challenges effectively. Benzyl alcohol serves as a preservative and solvent, while polysorbate 80 acts as an emulsifier, helping to improve the stability and consistency of formulations.; However, they can lead to low blood pressure and allergic reactions (8). Moreover, ETO and cisplatin are the leading standard cytotoxic drugs for small-cell lung cancer (SCLC). Their role is as the components of chemotherapy regimens affecting survival (12). Long-term exposure to ETO enhances toxicity in normal tissues; therefore, modified release is necessary to mitigate side effects and achieve the maximum therapeutic effect (13).

2.1. Drug delivery of ETO

ETO is a cytotoxic drug that increases the production of reactive oxygen species. However, drawbacks have been addressed before limiting its therapeutic efficacy. To solve this problem, nanocarriers can trap ETO and target cancerous cells of different organs. The usual goal of nanocarriers is to improve the pharmacological activity of therapeutic drugs and overcome problems such as limited solubility, drug accumulation, low bioavailability, poor biological distribution, lack of selectivity, or reduction of therapeutic drug side effects (14, 15).

Stimuli-responsive strategy: This strategy has been actively studied to achieve specific cancerous cell delivery and controlled secretion of their cargoes, where endogenous or exogenous agents can be used. The endogenous triggers include pH-sensitive, reactive oxygen species-sensitive, redox-sensitive, enzyme-sensitive, and temperature-sensitive delivery strategies toward some disease sites, such as tumors. Exogenous factors include light and stimulation temperature strategies induced by exogenous methods. Magnetic excitation and X-ray strategies have also been used to design stimulus-responsive systems. Ultrasound can also act as a stimulus to control the release of drugs deep into the body remotely (16).

Stimuli-responsive systems can deliver drugs to the target tissue and achieve the required drug release. This controlled drug release has an excellent therapeutic effect. The surface properties and structures of nanoparticles that respond to stimuli can also be modified through intrinsic or external stimuli to improve cell uptake and increase penetration (17).

Targeting polymeric drug delivery: Despite significant advances in tumor targeting technologies, one of the main limitations of current cancer treatments is the lack of special biomarkers for many metastatic cancerous cells. A new strategy developed for targeted drug delivery to cancer cells is to form

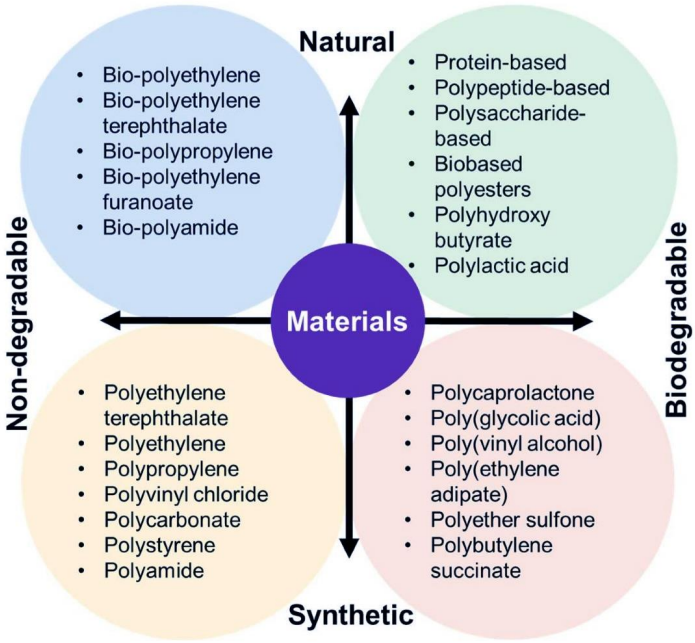

Figure 2. Biodegradable polymers with representative monomer units for polymeric drug delivery (18).

a physical conjugate between doxorubicin and the A10 RNA aptamer (Figure 2) (19). The dendrimer complex with ETO (CPX 5) shows the typical characteristics of a sound delivery system with a good drug loading (37%) and significantly improved solubility. When CPX 5 is combined with ETO, it exhibits a synergistic action, releasing the drug slowly over time and considerably improving and prolonging biological activity (20). To reduce ETO's prescribed dose and systemic side effects, targeted solid lipid nanoparticles containing etoposide hyaluronate are used, increasing ETO's cytotoxicity in the human ovarian cancer cell line (SK-OV-3) (21). As mentioned before, chemotherapy with cisplatin and ETO is a treatment regimen for small and non-small cell lung cancer. However, this diet has high toxicity characteristics. Therefore, monotherapy can be used. The challenge is that the formulation of cisplatin and ETO nanoparticles has a significant mismatch in chemical properties, so a hydrophobic platinum prodrug can be used, which is delivered using a nanoparticle along with ETO. Nanoparticles loaded with two drugs are significantly more effective than small-molecule chemotherapy in chemotherapy (22).

Another study discusses the production of methoxy poly(ethylene glycol)-poly(lactic-co-glycolic acid) (mPEG-PLGA) nanoparticles with optimal size to achieve co-delivery of ETO and Paclitaxel. To avoid the therapeutic limitations of ETO and paclitaxel, they are prepared by simultaneous loading using the nanoprecipitation method. mPEG-PLGA nanoparticles with ETO and paclitaxel were optimized using ANN, which confirmed the optimal physicochemically (23).

Also, solid lipid nanoparticles containing Quercetin showed significant potential in breast cancer treatment by improving the efficiency of ETO. These lipid nanoparticles are solid and spherical with a negative charge loaded with Quercetin. Combined Quercetin with ETO increases the inhibition of model late-stage breast cancer cell line (MDA-MB-231) proliferation compared to solid lipid nanoparticles of ETO or Quercetin alone. This combination treatment also significantly increased the apoptotic pathway. It increased the Bax/Bcl-2 gene ratio, p53 and p21 proteins,

and activated caspase 3 and 9 enzymes. These results indicate the potential of combination therapy as a strategy for the treatment of breast cancer, potentially overcoming the chemoresistance of ETO-resistant breast cancer (24).

Another article mentions that using PEGylated nanoliposomes as a carrier for the delivery of ETO leads to an increase in apoptotic cells compared to the control group (25).

3. Graphene derivatives (GDs)

Graphene is a single-layer allotrope of carbon hexagonal lattice in two-dimensional shape, nG, creating new exciting opportunities in the physics and materials of dense matter (26). They have outstanding properties; for example, they can be used to obtain sheets of thin atomic crystals that are stable (27). Geim and Novoselov published a simple method for making single-layer nG (28). nG layers are made from one or more layers of graphite, which are tightly bonded by van der Waals forces (7).

A brown color characterizes GO, while nG is identified as black. Recent experiments indicate that utilizing advanced technology allows for the effective peeling off of nG with the assistance of graphite oxide, resulting in the production of nG/GO nanosheets (Figure 3) (29).

Some researchers have used nG in drug and gene delivery, photothermal therapy, photodynamic therapy, imaging, and theranostics (30, 31). Folate receptor expression is used in targeting, drug delivery, and tumor cell counting. A quick and straightforward strategy for identifying cancer cells expressed based on selective folic acid/folate transplantation with FR-positive tumor cells is to use the folate-decorated Nitrogen-doped graphene as the target of choice (32). nG improves the toxicity of cancer cells and chemotherapeutic agent uptake. The high density of oxygen-rich functional groups facilitates functionalization, increased biocompatibility, and drug loading through π-π stacking, hydrogen bonding, and electrostatic interactions (33, 34). One application of nG is in the field of drug delivery. nG facilitates the transport of drugs and genes, either independently or in conjunction with other techniques in cancer treatment (31).

3.1. Design and preparation of nG/GO

nG is prepared by complete oxidation of pristine graphite, thermal exfoliation, and reduction of GO (35). One way of synthesis of nG:

A) The Scotch tape technique prepared a large nG crystal on an oxidized Si wafer.

B) Left panel: Suspended microcrystals from ultrasound graphite cutting in chloroform.

Right panel: Microcrystals printed on different layers. These films are very durable and retain their conductivity even if folded.

C) Availability of the first nG wafers (as 1 to 5-layer polycrystalline films) transferred to the Si wafer.

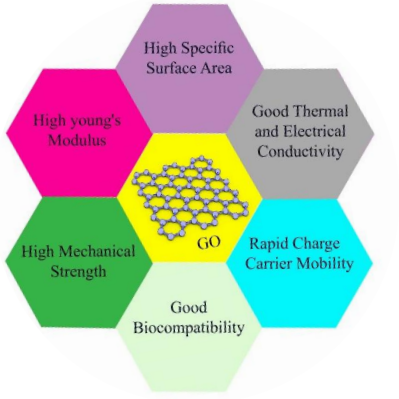

Figure 3. GO laminate is rigid, flexible, transparent, and insulating

D) Current SiC wafer with atomic terraces covered by a graphitic monolayer. Double and triple layers grow in stages (30).

Today, nG physical synthesis is performed at a lower cost with controlled graphite oxidation (1). GO layers are single sheets of ordinary graphite oxide synthesized from nG layers of graphite (1). Hummer's technique utilizes graphene oxide (GO) synthesis (Fig 4). This process involves graphite powder, concentrated sulfuric acid (H2SO4), sodium permanganate, sterile distilled water, hydrogen peroxide (H2O2) at a concentration of 35%, and hydrochloric acid (HCl) at 5%, and is carried out in six distinct steps (8).

3.2. Functionalization of nG/GDs

nG is peeled off with the help of graphite oxide and thus can be combined with GO. nG coatings have unique properties and are used as nanomaterials in industry (1, 36).

There are several structural models for GO nanosheets with different groups containing different oxygen, which are determined according to the raw materials and oxidation conditions (7, 37). These groups include epoxides (C-O-C) and hydroxyl phenols (-OH) located in the basal plane, carboxylic (-COOH). They are located at the edges, where the other carbon groups (C=O) create the property of polar surface and hydrophilicity (1, 36).

Oxidation of nG layers of graphite causes defects. One is oxidation in these defect sites, mainly ketone and quinone-type groups. The oxidation process also creates by-products, such as oxidative debris, which affect the stability of the nanosheets (1). Several structural models exist for the functionalization of GO. One is the Lerf-Klinowski model, the most cited model for GO, with problems (38). Carboxyl groups are shown at the edges. The hydroxyl groups are close to each other and cause electrical instability in the structure (1).

3.3. Carbon-Based Nanostructures for Delivering Etoposide

Carbon-based nanostructures (CBNS) are an intriguing class of materials known for their remarkable diversity and unique properties. They encompass a wide range of forms, including amorphous carbon, which exhibits a non-crystalline structure; diamond, prized for its hardness and optical clarity; graphite, characterized by its layered structure and excellent conductivity; carbon nanotubes, celebrated for their remarkable strength and electrical properties; graphene oxide, a versatile compound with potential for numerous applications; fullerenes, known for their spherical structures; and carbon dots, which are getting attention for their fluorescence and biocompatibility.

These materials are pivotal in cutting-edge applications across various domains, including drug delivery systems that enhance the targeted transport of medications, advanced imaging techniques that improve diagnostics, and innovative tissue engineering approaches that support cellular growth and repair. In the realm of cancer therapy, CBNS are being explored for their potential to deliver therapeutics directly to tumors. Additionally, their sensitivity and selectivity make them invaluable in biosensing applications, allowing for highly precise detection of biological markers. Carbon-based nanostructures represent a thrilling frontier in scientific and technological innovation (39).

Their extraordinary mechanical properties and rigidity enable them to effectively penetrate the tissues and extracellular matrix. Moreover, the nontoxic characteristics of these materials, coupled with their compatibility for intravenous administration, position them as effective carriers for a broad range of medications and imaging agents. However, several challenges need to be addressed, including their limited solubility in water, decreased biodegradability, and potential safety issues. Despite these hurdles, the unique properties of these materials foster ongoing research and

innovation, inspiring scientists to explore new pathways for advancement in the field (40).

CBNS showcases extraordinary biological and physicochemical characteristics, highlighting its remarkable ability to absorb functional groups and elevate the stacking of carbon sheets (41). These structures can be precisely customized for specific applications by employing surface functionalization techniques. This process not only enhances their ability to attract and retain water but also controls their physical and chemical properties. Through these adaptations, the structures can be optimized for a wide range of uses, making them more effective in various environments and conditions (42). This promises a bright future for their use in transformative technologies.

3.4. Carbon nanotubes

Carbon nanotubes (CNTs) embody remarkable properties that position them as innovative carriers for drug delivery (42). With the ability to enhance their water solubility through modification, chitosan (CS), a naturally occurring, biodegradable, and biocompatible polysaccharide, emerges as a powerful ally (43, 44). Researchers have explored the potential of epidermal growth factor as a specialized ligand, which has proven to greatly enhance the delivery and accumulation of therapeutic drugs in tumor sites. CNTs are usually released drugs with a pH range of 5.5-7.5 to decrease tumor cell viability, yet we and others. However, CNT functionalization affects PH responsiveness and chemotherapeutic release. This innovative approach increases the concentration of medication at tumor sites while minimizing exposure to healthy surrounding tissues, thereby improving treatment efficacy and reducing side effects (45).

The groundbreaking TDDS using a complex system including epidermal growth factor, CS, along with single-walled carbon nanotubes, exemplifies this innovation, with EGF as the guiding molecule and SWNTs as cargo for delivering ETO (46). This innovative TDDS boasts a remarkably small diameter of only 20 nm, allowing for precise and efficient targeting of cancer cells. In vitro studies have demonstrated exceptional anticancer efficacy, achieving an impressive 2.7 times greater mortality of cancer cells compared to the administration of free drugs. These figures underscore the effectiveness of the three distinct treatment strategies employed in this system. Moreover, the release profile of the drug is particularly promising. Within just 60 minutes, the CS/SWNT with ETO formulation released 32.4% of the encapsulated drug in a neutral pH environment (7.4), while in a more acidic environment (pH 5.0), which mimics tumor conditions, the release surged to 59.3%. This differential release underscores the potential for optimized therapeutic effects in varied physiological environments. After 720 minutes, these release rates soared to 57.5% and 95.9%, reflecting the potential for targeted impact. Compared to healthy tissue, the faster release of ETO-loaded SWNTs in malignant cells represents a leap forward in precision medicine. For the EGF/CHI/SWNT-COOHs/ETO complex, the active targeting ligand initially released 31.9% at pH 7.4 and 60.2% at pH 5.0, with final release rates reaching 62.6% and 95.0% after 720 minutes, all while preserving the integrity of the complex.

In a forward-thinking follow-up study, scientists explored the loading potential of oxidized MWCNTs for this potent chemotherapeutic agent, employing electrochemical forces (47). The remarkable discovery that this pegylated multi-walled carbon nanotube exhibited a significantly superior binding potential than conventional MWCNTs-PEG emphasizes the continuous quest for improvement.

3.5. Diamond nanostructures (DNPs)

Diamond nanostructures (DNPs) are fascinating carbon-based materials characterized by their unique properties and small size,

typically measuring just a few nanometers in diameter. These nanoparticles have attracted significant interest across a range of industries due to their remarkable qualities, including exceptional strength, biocompatibility, and high surface area. As a result, DNPs are being explored for innovative applications in engineering, where their durability can enhance composite materials; in biology and biotechnology, where they hold promise for targeted drug delivery and imaging; in medicine, where they can potentially improve therapeutic outcomes; and in cosmetics, where they may be used to create advanced formulations that enhance skin health and appearance (48). Their unique properties and nanoscale size unlock innovative solutions and enhancements for various applications. Recent advancements in nanotechnology empower the modification of DNPs, amplifying their functionality and versatility (49).

One of the most remarkable traits of DNPs is their ability to induce cell necrosis and apoptosis, positioning them as invaluable tools in diagnostic and therapeutic fields. However, using DNPs at high concentrations calls for careful consideration of their biocompatibility, an issue researchers passionately address (50). Modifying DNPs often begins with introducing hydroxyl (-OH) groups, which paves the way for further functionalization and allows diverse additional chemical groups to attach. The Fenton reaction is a widely embraced method for this transformative process, generating reactive species that enhance their capabilities. In their study, Solarska et al. provide compelling evidence of the substantial effects that functionalized DNPs have on human-derived non-small cell lung cancer (NSCLC) cells. Their research reveals how these nanoparticles interact with cancerous cells, demonstrating potential pathways for therapeutic applications and advancements in cancer treatment strategies (51). Their innovative experiments involved utilizing ETO and hydroxyl groups as key modifiers to elevate the properties of the DNPs. In their experimental process, NSCLC cells were cultured in DMEM medium and treated with five concentrations of ETO, alongside the introduction of nanopowders at concentrations between 2 and 100 µg/mL. The striking findings highlighted a significant decrease in cell viability and increased production of reactive oxygen and nitrogen species during incubation. Furthermore, the modified detonation nanoparticles meaningfully impacted glutathione levels within the cells, rehabilitated the antioxidant effectiveness, and brought oxidative stress, emphasizing the profound interactions between the nanoparticles and the biological environment of the target cells (51).

4. Delivery of etoposide using GO

Advanced DDS utilizing graphene derivatives have been developed to address significant treatment challenges. GOs hold significant promise in the field of medicine, particularly for their ability to facilitate TDDS. By using GOs, the release of the drug ETO can be controlled over an extended period, which ensures that it remains effective without requiring frequent dosing. Additionally, GOs can improve the absorption, distribution, metabolism, excretion and toxicity (ADMET) of ETO, meaning they enhance how the body absorbs, distributes, metabolizes, and excretes the drug. This dual function not only amplifies the beneficial effects of ETO but also reduces its potential adverse effects, making treatment safer and more effective for patients (52). GOs represent a promising solution for effectively delivering chemotherapeutic agents while protecting them from degradation and immune system clearance (52, 53).

Using GOs as a drug delivery system transforms conventional administration methods. Directly delivering ETO to tumor sites through GO decreases damage to normal tissues and significantly lowers side effects. These innovative systems showed a sustained release profile over an approximately long pe-

riod, ensuring consistent serum levels of ETO and decreasing the regularity of dosages required.

Moreover, plate-like structures known as GOs can significantly facilitate ETO absorption and ensure a more efficient distribution. This enhanced solubility makes ETO more readily accessible for uptake, leading to improved therapeutic effects as it circulates through various tissues and organs. This increases the effectiveness of ETO while lowering its poisonousness. Combining ETO and GOs signals a new era in developing reliable and efficient chemotherapy (8, 54).

Etoposide is primarily used to fight various malignancies, including hepatocellular carcinoma (Hep-G2). To elevate the therapeutic applications of ETO against Hep-G2 cells while safeguarding the integrity of its apoptotic pathways, researchers Gholami and their team embarked on an innovative journey to explore the potential of carboxylated graphene oxide (GO-COOH) as a groundbreaking drug delivery carrier (8).

The research team harnessed a range of advanced characterization techniques to deeply evaluate the properties of ETO-loaded graphene oxide. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) provided high-resolution images that unveiled the material's surface morphology and structural integrity. Ultraviolet-visible spectroscopy (UV-Vis) revealed the material's light absorption properties, essential for grasping its optical characteristics. Fourier-transform infrared spectroscopy (FT-IR) identified specific functional groups within the composite, deepening the understanding of its chemical nature. Further analysis with dynamic light scattering (DLS) explored particle size distribution, while Raman spectroscopy illuminated molecular vibrations, offering a profound insight into the molecular structure of the compounds.

Initial findings demonstrated that the average size of GO was approximately 134 nanometers (nm). Following the transformative carboxylation process aimed at enhancing its functional capabilities, this average size increased to 178 nm, affirming the successful modification of the material's properties. The investigations illustrated a significant relationship between the concentration of GO-COOH and its capability to load ETO. As the GO-COOH concentration rose from 50 µg/mL to 1000 µg/mL, the encapsulation percentage of ETO soared from 13.6% to an astounding 83.20%, showcasing the remarkable potential of higher concentrations of GO-COOH in drug delivery formulations.

To evaluate the cytotoxic potential of these formulations, the researchers conducted MTT assays, yielding half-maximal inhibitory concentration (IC50) values that reflect their remarkable achievements. The IC50 for ETO alone was determined to be 61.73 µg/mL. In comparison, the IC50 for the ETO-GO-COOH complex significantly decreased to 40.11 µg/mL, illustrating the enhanced potency of this innovative formulation. Moreover, real-time polymerase chain reaction (PCR) revealed that ETO-loaded carboxylated GO effectively stimulated the gene expression of apoptotic proteins linked to cellular impairment. Remarkably, the ETO-loaded carboxylated GO complex exhibited far greater efficacy in inducing apoptosis in Hep-G2 cells than ETO alone, reinforcing the transformative impact of this research. This significant finding highlights the incredible potential of GO-COOH as a therapeutic agent in cancer treatment, inspiring hope for enhancing the delivery and effectiveness of etoposide against hepatocellular carcinoma.

4.1. ETO targeting cancer cells using GO

Antibody-conjugated GO is a novel approach to targeting tumor cells in cancer therapy (55). This method leverages the unique properties of graphene oxide, which can be modified to enhance its targeting capabilities. One significant advancement in this

area is GO-TRC105, a specialized monoclonal antibody that selectively binds to CD105. By attaching this antibody to graphene oxide, researchers can improve the accuracy of tumor targeting, which may lead to more effective treatments and reduced side effects (56).

Another strategy to further increase the targeting effectiveness involves functionalizing sulfonic acid groups with graphene oxide (57). This modification can improve the GO's chemical properties and stability, potentially enhancing its ability to interact with tumor cells. These innovations represent promising strategies for developing targeted cancer therapies that improve patient outcomes and minimize collateral damage to healthy tissues.

4.2. Why is GO superior to other nGs?

One issue associated with nG is its potential to alter biological environments. Numerous studies have demonstrated that the physicochemical properties of nG, including the size of the sheets, whether they are single-layer or multi-layer, and the various synthesis methods, do not significantly influence their biological behaviors (58).

The second challenge is solubility and stability. nG is soluble and stable in most common solvents. However, the dispersion of GO changed after its reduction, providing better interaction with solvents such as dichlorobenzene and 1-chlorophthalene (7).

Several studies show that the solubility and stability of nG have increased due to covalent and non-covalent surface functionalization. Colloidal stability of nG has been achieved using water-soluble materials (59).

GDs have unique properties such as high surface-to-volume area, mechanical strength, and flexibility, making them suitable for multifunctional drug systems (60). In the pharmaceutical sciences, GDs load low-soluble drugs while maintaining their potency and efficacy due to their high specific surface area,π-π accumulation, and electrostatic or hydrophobic interactions (7).

Due to the unique physical and chemical properties of nG and its derivatives, such as GO, reduced GO, carboxylated GO, and GO-nano composites, many sciences have attracted much attention (7, 61). Therefore, one of the critical derivatives of nG is GO, which we will discuss later.

4.3. Is GO safe for ETO's drug delivery?

Augment biocompatibility, biodegradation, and other properties of GO can help enhance the medicine on its surface without compromising the potency and efficacy of drugs (8, 62). To increase the biocompatibility of GO, its surface needs to be functionalized with oxygenated groups, such as carboxyl. Carboxylated GO (GO-COOH) has higher biocompatibility and safety for biological applications than GO (8, 36). Although ETO-loaded carboxylated GO is significantly more efficient than naked drug, both ETO-loaded carboxylated GO and naked drug cause toxicity by inducing the gene expression of several apoptotic proteins. The cytotoxicity of GO and carboxylated GO is dose-dependent. Carboxylated GO enhances ETO's cytotoxic effect without affecting ETO's apoptosis pathway (8).

Carboxylated GO increases the solubility of ETO and its half-life, thus, the time of exposure to cancer cells, which causes its toxicity. This means carboxylated GO improves the pharmacokinetic properties of ETO (8). Polyethylene glycol can be used to increase the biocompatibility of nG or GO (31).

4.4. Is ETO-loaded GO of low systemic toxicity?

GO-based nanomaterials have less toxic function than inactive substances in vivo. However, inhalation of nG revealed they were inflammatory in the lung and pleural space (63). It has previously been said that the toxicity of ETO complexed with GO-COOH is due to ETO itself. GO-COOH has no detrimental effect on ETO's anticancer mechanism

and function. Therefore, GO-COOH can be used as a suitable carrier for ETO. It should be noted that the toxicity of the ETO-GO-COOH complex is concentration-dependent. This complex was more toxic to cancer cells at low concentrations than free ETO.

In contrast, at higher concentrations, the toxicity was independent of the concentration factor (8). Cells exposed to ETO-GO-COOH showed higher apoptosis than cells exposed to free ETO. There may not be a substantial alteration in the extent of necrosis, as the generation of reactive oxygen species and oxidative stress contributes to the persistence of necrotic conditions (8).

5. Experimental studies

In an experiment, three different sizes of nG and GO were prepared. Both small and large sizes suggestively diminished the viability of the normal cells and augmented the impairment of nucleic acids (64). In terms of size, large nG and GO had a more significant effect than medium size and more impact than small size (65). The toxicity of nG and GO is based on different physical characteristics, especially the size, oxidation state, and concentration (66). However, the effects of nG are higher than those of GO (67). The toxicity of GO is controversial, and some have suggested that GO is more toxic than nG. However, there is no doubt that nG and GO are more harmful than their other derivatives. nG showed a more vital ability to reduce survival and induce acute poisoning, while GO showed apparent toxicity in terms of DNA damage and abnormal gene expression (65).

In another real-time PCR implemented experiment, the result revealed that etoposide-loaded carboxylated GO and the free drug have induced cellular toxicity by inducing the gene expression of apoptotic proteins. However, etoposide-loaded carboxylated GO is more efficient compared to free drugs in inducing apoptosis. Carboxylated GO enhanced the anticancer efficiency of ETO, while this nano-drug did not impact the ETO apoptotic path. Etoposide-loaded carboxylated GO increased the expression of five genes: APAF1, BAX, BCL-2, Caspase 3, and P53—these proteins, together with Caspase 9, induced by ETO in the apoptosis pathway (8).

In one experiment, GO was injected intravenously into mice. As a result of this injection, GO accumulates in the lungs, resulting in pulmonary edema and granulomas (68). After oral administration, the organs cannot absorb PEGylated GO derivatives and are rapidly excreted (69). Despite the long-term preservation of intraperitoneal injection of GO and PEGylated GO into the mouse body, no significant toxicity was observed in the systematic serum biochemistry, complete blood panel examination, or histological analysis. Granuloma formation, which could be induced by intraperitoneal prolonged injected long multi-walled carbon nanotubes, was not observed in mice injected with high doses of GO or PEGylated GO.

The results of this experiment showed that the in vivo behaviors and toxicology of nanomaterials, including nG, are closely related to surface coatings, size, and, most importantly, how they are used (69).

6. Conclusion

This article presents an inspiring analysis of the latest articles for delivering ETO using carbon-based nanostructures and their transformative potential in medical treatment. It delves into the pros and cons of ETO-DDS, which utilizes carbon-based nanostructures and showcases remarkable advancements in this dynamic field. We reviewed the promising nature of GO-based technologies for effectively delivering ETO. For instance, polymeric-based GO functionalization of ETO demonstrates suitable loading efficiency and slow-release profile during a certain period, offering hope for breast cancer patients. Cationic biological nanopolymers, such as chitosan, significantly prolong the circulation time

of ETO, reduce toxicity, and elevate antitumor activity (70).

Furthermore, the potential of partially esterifying the peripheral hydroxyl groups of GO with specific compounds, like folic acid, promises to enhance the efficacy of chemotherapeutic agents (71). However, in vivo and clinical trials are essential for evaluating safety profile and effectiveness. Carbon-based structures may suggest enhanced anticancer effectiveness with an optimized ADMET profile, desirable release rate, and reduced adverse effects. Nevertheless, challenges such as instability, manufacturing scalability, and optimal targeting remain to be addressed.

Carbon-based delivery methods—including EGF, chitosan, and single-walled carbon nanotube-carboxylic acids (SWNT-COOHs)—hold immense capacity to amplify the efficiency of this anticancer agent. While concerns about the biocompatibility and environmental hazards of GOs linger, the journey to achieving predictable drug loading and release profiles fosters hope for the future of cancer treatment (72, 73). These groundbreaking nanosheets encapsulate the compounds and specifically bond to transformed cells by several methods. Active attachment, which implicates attaching specific receptors at the exterior of the GO, works hand in hand with passive targeting that leverages the enhanced permeability and retention (EPR) effect commonly seen in tumors (74, 75). GO-based drug delivery systems present a powerful opportunity to boost therapeutic efficacy by enhancing the accumulation of compounds in the microenvironment of transformed cells while lessening injuries to normal cells (76).

PEGylated GO-containing anticancer drugs have been shown to advance pharmacokinetics and antitumor activity in mouse lung cancer models (77). However, the journey of innovation is not without its hurdles. Variations in micelle size, shape, and drug release kinetics present several challenges that must be navigated to achieve optimal efficacy and safety. The EPR effect, coupled with micelle size, supports passive targeting of tumor tissue, enhancing accumulation in tumors while minimizing damage to healthy tissues. Maintaining micelle stability and managing drug release rates are vital components of this evolving landscape. Polymeric GO-based delivery methods hold tremendous promise in amplifying the potency and selectivity of ETO. These biocompatible and biodegradable polymeric GOs facilitate the effective encapsulation of pharmaceuticals, bringing hope through improved drug delivery to tumor tissues. The small size of polymeric GO allows for passive targeting via the EPR effect, further increasing drug accumulation in tumors and sparing healthy tissues.

In conclusion, the tailored administration of ETO using GO heralds a new dawn of therapeutic potential, promising improved outcomes for cancer patients. These pathways can direct drugs to tumor tissues precisely, amplifying concentrations within cancer cells while protecting healthy tissues from harm. Targeted delivery with GO alleviates the frequency and severity of side effects and enhances the overall treatment experience. By extending the half-life and reducing the clearance rate of ETO, GO-based strategies can afford a steady and extended beneficial efficiency. Moreover, these delivery methods can potentially overcome drug resistance, directing ETO to tumor cells and circumventing challenges posed by drug efflux pumps. While the path forward may have challenges, the promise of targeted administration of ETO nanomedicine inspires a commitment to advancing safety and efficacy data, paving the way for regulatory approval and transformative clinical use.

Producing GO-based medication delivery systems may be complex and costly, yet these challenges open the door to groundbreaking advancements. While tumor heterogeneity poses obstacles in effectively delivering drugs to all tumor cells, the potential to harness the targeted distribution of ETO in

diverse therapeutic scenarios is promising. GO-based drug delivery systems empower the synergy of multiple medications, enhancing efficacy while minimizing toxicity. By tailoring these systems to the unique characteristics of each patient's tumor, we pave the way for precise targeted delivery. Moreover, releasing imaging agents or biomarker compounds alongside the drugs can revolutionize real-time disease monitoring. These innovative systems combat drug resistance and offer opportunities to repurpose previously approved drugs in a novel way. The TDDS for ETO could significantly elevate cancer treatment effectiveness. We addressed clinical challenges head-on while exploring these strategies' benefits and limitations. The future of ETO nanomedicine is bright and filled with the potential for personalized chemotherapy. The current experiment's efforts to enhance nanocarriers' directing and diffusion, optimize the release profile, and develop industrial synthesis aim to encapsulate diverse chemotherapeutic agents in an individualized medicine paradigm.

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Authors' Contributions

BZ and AG carried out the literature research, wrote the manuscript parts, and KA prepared the figures and tables. KA and AG participated in editing the manuscript. AG, BZ, and KA helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Aliyev E, Filiz V, Khan MM, Lee YJ, Abetz C, Abetz V. Structural Characterization of Graphene Oxide: Surface Functional Groups and Fractionated Oxidative Debris. *Nanomaterials (Basel)*. 2019 Aug 18;9(8):1180. doi: 10.3390/nano9081180. PMID: 31426617; PMCID: PMC6724119.

2. Chan A, Hertz DL, Morales M, Adams EJ, Gordon S, Tan CJ, et al. Biological predictors of chemotherapy-induced peripheral neuropathy (CIPN): MASCC neurological complications working group overview. *Support Care Cancer.* 2019 Oct;27(10):3729-3737. doi: 10.1007/s00520-019-04987-8. Epub 2019 Jul 30. PMID: 31363906; PMCID: PMC6728179.

3. Samiraninezhad N, Asadi K, Rezazadeh H, Gholami A. Using chitosan, hyaluronic acid, alginate, and gelatin-based smart biological hydrogels for drug delivery in oral mucosal lesions: A review. *Int J Biol Macromol.* 2023 Dec 1;252:126573. doi: 10.1016/j.ijbiomac.2023.126573. Epub 2023 Aug 28. PMID: 37648126.

4. Mousavi SM, Hashemi SA, Mazraedoost S, Yousefi K, Gholami A, Behbudi G, et al. Multifunctional Gold Nanorod for Therapeutic Applications and Pharmaceutical Delivery Considering Cellular Metabolic Responses, Oxidative Stress and Cellular Longevity. *Nanomaterials (Basel)*. 2021 Jul 20;11(7):1868. doi: 10.3390/nano11071868. PMID: 34361251; PMCID: PMC8308363.

5. Asadi K, Samiraninezhad N, Akbarizadeh AR, Amini A, Gholami A. Stimuli-responsive hydrogel based on natural polymers for breast cancer. *Front Chem.* 2024 Jan 18;12:1325204. doi: 10.3389/fchem.2024.1325204. PMID: 38304867; PMCID: PMC10830687.

6. Asadi K, Heidari R, Hamidi M, Ommati MM, Yousefzadeh-Chabok S, Samiraninezhad N, et al. Trinitroglycerin-loaded chitosan nanogels: Shedding light on cytotoxicity, antioxidativity, and antibacterial activities. Int J Biol Macromol. 2024 Apr;265(Pt 2):130654. doi: 10.1016/j.ijbiomac.2024.130654. Epub 2024 Mar 29. PMID: 38553395.

7. Emadi F, Emadi A, Gholami A. A Comprehensive Insight Towards Pharmaceutical Aspects of Graphene Nanosheets. *Curr Pharm Biotechnol.* 2020;21(11):1016-1027. doi: 10.2174/1389201021666200318131422. PMID: 32188383.

8. Gholami A, Emadi F, Nazem M, Aghayi R, Khalvati B, Amini A, et al. Expression of key apoptotic genes in hepatocellular carcinoma cell

line treated with etoposide-loaded graphene oxide. *J Drug Deliv Technol.* 2020;57:101725.
9. Shimizu E, Yamamoto A, Takahashi Y, Maniwa K, Yoshida S, Mukai J, et al. Effect of alternating combination chemotherapy consisting of cyclophosphamide, doxorubicin, vincristine, cisplatin, and etoposide for small cell lung cancer on hematopoietic progenitors in the peripheral blood. *Br J Cancer.* 1993 Apr;67(4):798-800. doi: 10.1038/bjc.1993.145. PMID: 8385979; PMCID: PMC1968335.
10. Cattrini C, Capaia M, Boccardo F, Barboro P. Etoposide and topoisomerase II inhibition for aggressive prostate cancer: Data from a translational study. *Cancer Treat Res Commun*. 2020;25:100221. doi: 10.1016/j.ctarc.2020.100221. Epub 2020 Oct 13. PMID: 33091733.
11. Zhang X, Hofmann S, Harbeck N, Jeschke U, Sixou S. Impact of Etoposide on BRCA1 Expression in Various Breast Cancer Cell Lines. *Drugs R D*. 2017 Dec;17(4):569-583. doi: 10.1007/s40268-017-0208-6. PMID: 28879638; PMCID: PMC5694421.
12. Mascaux C, Paesmans M, Berghmans T, Branle F, Lafitte JJ, Lemaitre F, et al. A systematic review of the role of etoposide and cisplatin in the chemotherapy of small cell lung cancer with methodology assessment and meta-analysis. *Lung Cancer*. 2000 Oct;30(1):23-36. doi: 10.1016/s0169-5002(00)00127-6. PMID: 11008007.
13. Zhang W, Gou P, Dupret JM, Chomienne C, Rodrigues-Lima F. Etoposide, an anticancer drug involved in therapy-related secondary leukemia: Enzymes at play. *Transl Oncol.* 2021 Oct;14(10):101169. doi: 10.1016/j.tranon.2021.101169. Epub 2021 Jul 6. PMID: 34243013; PMCID: PMC8273223.
14. Tu Y, Gong J, Mou J, Jiang H, Zhao H, Gao J. Strategies for the development of stimuli-responsive small molecule prodrugs for cancer treatment. *Front Pharmacol*. 2024 Jul 31;15:1434137. doi: 10.3389/fphar.2024.1434137. PMID: 39144632; PMCID: PMC11322083.
15. Zhou J, Li K, Qin H, Xie B, Liao H, Su X, et al. Programmed-stimuli responsive carrier-free multidrug delivery system for highly efficient trimodal combination therapy. *J Colloid Interface Sci*. 2023 May;637:453-464. doi: 10.1016/j.jcis.2023.01.091. Epub 2023 Jan 23. PMID: 36716669.
16. Ashrafizadeh M, Delfi M, Zarrabi A, Bigham A, Sharifi E, Rabiee N, et al. Stimuli-responsive liposomal nanoformulations in cancer therapy: Pre-clinical & clinical approaches. *J Control Release.* 2022 Nov;351:50-80. doi: 10.1016/j.jconrel.2022.08.001. Epub 2022 Sep 20. PMID: 35934254.
17. Li C, Wang J, Wang Y, Gao H, Wei G, Huang Y, et al. Recent progress in drug delivery. *Acta Pharm Sin B*. 2019 Nov;9(6):1145-1162. doi: 10.1016/j.apsb.2019.08.003. Epub 2019 Aug 19. PMID: 31867161; PMCID: PMC6900554.
18. Shahriar SMS, Andrabi SM, Islam F, An JM, Schindler SJ, Matis MP, et al. Next-Generation 3D Scaffolds for Nano-Based Chemotherapeutics Delivery and Cancer Treatment. *Pharmaceutics*. 2022 Dec 3;14(12):2712. doi: 10.3390/pharmaceutics14122712. PMID: 36559206; PMCID: PMC9784306.
19. Sung YK, Kim SW. Recent advances in polymeric drug delivery systems. *Biomater Res.* 2020 Jun 6;24:12. doi: 10.1186/s40824-020-00190-7. PMID: 32537239; PMCID: PMC7285724.
20. Alfei S, Marengo B, Domenicotti C. Polyester-Based Dendrimer Nanoparticles Combined with Etoposide Have an Improved Cytotoxic and Pro-Oxidant Effect on Human Neuroblastoma Cells. *Antioxidants (Basel).* 2020 Jan 6;9(1):50. doi: 10.3390/antiox9010050. PMID: 31935872; PMCID: PMC7022520.
21. Mohammadi Ghalaei P, Varshosaz J, Sadeghi Aliabadi H. Evaluating Cytotoxicity of Hyaluronate Targeted Solid Lipid Nanoparticles of Etoposide on SK-OV-3 Cells. *J Drug Deliv.* 2014;2014:746325. doi: 10.1155/2014/746325. Epub 2014 Apr 24. PMID: 24868467; PMCID: PMC4020396.
22. Zhang M, Hagan CT 4th, Foley H, Tian X, Yang F, Au KM, et al. Co-delivery of etoposide and cisplatin in dual-drug loaded nanoparticles synergistically improves chemoradiotherapy in non-small cell lung cancer models. *Acta Biomater.* 2021 Apr 1;124:327-335. doi: 10.1016/j.actbio.2021.02.001. Epub 2021 Feb 5. PMID: 33556606; PMCID: PMC9083847.
23. Maleki H, Naghibzadeh M, Amani A, Adabi M, Khosravani M. Preparation of paclitaxel and etoposide co-loaded mPEG-PLGA nanoparticles: an investigation with artificial neural network. *J Pharm Innov*. 2021;16:11-25.

24. Afarin R, Ahmadpour F, Hatami M, Monjezi S, Igder S. Combination of Etoposide and quercetin-loaded solid lipid nanoparticles Potentiates apoptotic effects on MDA-MB-231 breast cancer cells. *Heliyon*. 2024 May 24;10(11):e31925. doi: 10.1016/j.heliyon.2024.e31925. PMID: 38841445; PMCID: PMC11152947.
25. Zare Kazemabadi F, Heydarinasab A, Akbarzadeh A, Ardjmand M. Preparation, characterization and in vitro evaluation of PEGylated nanoliposomal containing etoposide on lung cancer. Artif *Cells Nanomed Biotechnol.* 2019 Dec;47(1):3222-3230. doi: 10.1080/21691401.2019.1646265. PMID: 31373225.
26. Emadi F, Amini A, Ghasemi Y, Gholami A. Graphene: recent advances in engineering, medical and biological sciences, and future prospective. *Trends Pharm Sci.* 2018;4(3):131-8.
27. Ares P, Novoselov KS. Recent advances in graphene and other 2D materials. *Nano Mater Sci*. 2022;4(1):3-9.
28. Dresselhaus MS, Araujo PT. Perspectives on the 2010 Nobel Prize in physics for graphene. *ACS Nano.* 2010 Nov 23;4(11):6297-302. doi: 10.1021/nn1029789. PMID: 21090813.
29. Tian J, Wu S, Yin X, Wu W. Novel preparation of hydrophilic graphene/graphene oxide nanosheets for supercapacitor electrode. *Appl Surf Sci*. 2019;496:143696.
30. Geim AK. Graphene: status and prospects. *Science*. 2009;324(5934):1530-4.
31. Orecchioni M, Cabizza R, Bianco A, Delogu LG. Graphene as cancer theranostic tool: progress and future challenges. *Theranostics*. 2015 Mar 28;5(7):710-23. doi: 10.7150/thno.11387. PMID: 25897336; PMCID: PMC4402495.
32. Soleymani J, Hasanzadeh M, Somi MH, Ozkan SA, Jouyban A. Targeting and sensing of some cancer cells using folate bioreceptor functionalized nitrogen-doped graphene quantum dots. *Int J Biol Macromol*. 2018 Oct 15;118(Pt A):1021-1034. doi: 10.1016/j.ijbiomac.2018.06.183. Epub 2018 Jul 2. PMID: 30001595.
33. Chung S, Revia RA, Zhang M. Graphene Quantum Dots and Their Applications in Bioimaging, Biosensing, and Therapy. *Adv Mater.* 2021 Jun;33(22):e1904362. doi: 10.1002/adma.201904362. Epub 2019 Dec 12. PMID: 31833101; PMCID: PMC7289657.
34. Mousavi SM, Kalashgrani MY, Javanmardi N, Riazi M, Akmal MH, Rahmanian V, et al. Recent breakthroughs in graphene quantum dot-enhanced sonodynamic and photodynamic therapy. *J Mater Chem B*. 2024;12(29):7041-62.
35. Yang L, Zhang S, Chen Z, Guo Y, Luan J, Geng Z, et al. Design and preparation of graphene/poly (ether ether ketone) composites with excellent electrical conductivity. *J Mater Sci.* 2014;49(5):2372-82.
36. Gholami A, Emadi F, Amini A, Shokripour M, Chashmpoosh M, Omidifar N. Functionalization of graphene oxide nanosheets can reduce their cytotoxicity to dental pulp stem cells. *J Nanomater.* 2020;2020(1):6942707.
37. Krishnamoorthy K, Veerapandian M, Yun K, Kim SJ. The chemical and structural analysis of graphene oxide with different degrees of oxidation. *Carbon*. 2013 Mar 1;53:38-49.
38. Siklitskaya A, Gacka E, Larowska D, Mazurkiewicz-Pawlicka M, Malolepszy A, Stobiński L, et al. Lerf-Klinowski-type models of graphene oxide and reduced graphene oxide are robust in analyzing non-covalent functionalization with porphyrins. *Sci Rep*. 2021 Apr 12;11(1):7977. doi: 10.1038/s41598-021-86880-1. PMID: 33846412; PMCID: PMC8041773.
39. Mousavi SM, Nezhad FF, Ghahramani Y, Binazadeh M, Javidi Z, Azhdari R, et al. Recent Advances in Bioactive Carbon Nanotubes Based on Polymer Composites for Biosensor Applications. *Chem Biodivers*. 2024 Jul;21(7):e202301288. doi: 10.1002/cbdv.202301288. Epub 2024 Jun 11. PMID: 38697942.
40. Gholami A, Mousavi SM, Masoumzadeh R, Binazadeh M, Bagheri Lankarani K, Omidifar N, et al. Advanced Theranostic Strategies for Viral Hepatitis Using Carbon Nanostructures. *Micromachines*. 2023;14(6):1185.
41. Mousavi SM, Yousefi K, Hashemi SA, Afsa M, BahranI S, Gholami A, et al. Renewable Carbon Nanomaterials: Novel Resources for Dental Tissue Engineering. *Nanomaterials (Basel)*. 2021 Oct 22;11(11):2800. doi: 10.3390/nano11112800. PMID: 34835565; PMCID: PMC8622722.
42. Gholami A, Farjami F, Ghasemi Y. The Development of an Amperometric Enzyme Biosensor Based on a Polyaniline-Multiwalled Carbon Nanocomposite for the Detection of a Chemotherapeutic Agent in Serum Samples from Patients. *J Sens*. 2021;2021(1):5515728.

43. Rezaei Abbas Abad F, Pourmadadi M, Abdouss M, Behzadmehr R, Rahdar A, Pandey S. Chitosan-Carbon nanotube Composite: An approach for controlled release of Quercetin, Modified with carboxymethyl Cellulose, for potential Anticancer therapy. *Inorg Chem Commun*. 2023;158:111621.
44. Kavousi M, Chavoshi MS. Effect of coated carbon nanotubes with chitosan and cover of flaxseed in the induction of MDA-MB-231 apoptosis by analyzing the expression of Bax and Bcl-2. *Meta Gene*. 2020;26:100807.
45. Son KH, Hong JH, Lee JW. Carbon nanotubes as cancer therapeutic carriers and mediators. *Int J Nanomedicine.* 2016 Oct 7;11:5163-5185. doi: 10.2147/IJN.S112660. PMID: 27785021; PMCID: PMC5066859.
46. Chen C, Xie XX, Zhou Q, Zhang FY, Wang QL, Liu YQ, et al. EGF-functionalized single-walled carbon nanotubes for targeting delivery of etoposide. *Nanotechnology.* 2012 Feb 3;23(4):045104. doi: 10.1088/0957-4484/23/4/045104. Epub 2012 Jan 6. PMID: 22222202.
47. Heger Z, Moulick A, Nguyen HV, Kremplova M, Kopel P, Hynek D, et al. Characterization of Multi-Walled Carbon Nanotubes Double-Functionalization with Cytostatic Drug Etoposide and Phosphorothioate Oligodeoxynucleotides. *Int J Electrochem Sci.* 2015;10(9):7707-19.
48. Pan F, Khan M, Ragab AH, Javed E, Alsalmah HA, Khan I, et al. Recent advances in the structure and biomedical applications of nanodiamonds and their future perspectives. *Mater Des.* 2023;233:112179.
49. Nunn N, Torelli M, McGuire G, Shenderova O. Nanodiamond: A high impact nanomaterial. *Curr. Opin Solid State Mater Sci.* 2017;21(1):1-9.
50. Chauhan S, Jain N, Nagaich U. Nanodiamonds with powerful ability for drug delivery and biomedical applications: Recent updates on in vivo study and patents. *J Pharm Anal*. 2020 Feb;10(1):1-12. doi: 10.1016/j.jpha.2019.09.003. Epub 2019 Oct 1. PMID: 32123595; PMCID: PMC7037532.
51. Solarska K, Gajewska A, Skolimowski J, Woś R, Bartosz G, Mitura K. Effect of non-modified and modified nanodiamond particles by Fenton reaction on human endothelial cells. *Int J Manuf Eng.* 2010;43(2):603-7.
52. Gholami A, Habibi B, Hosseini SA, Matin AA, Samadi N. Controlled release of anticancer drugs via the magnetic magnesium iron nanoparticles modified by graphene oxide and polyvinyl alcohol: Paclitaxel and docetaxel. *Nanomed J*. 2021;8:200-10.
53. Hashemi SA, Mousavi SM, Bahrani S, Gholami A, Chiang W-H, Yousefi K, et al. Bio-enhanced polyrhodanine/graphene Oxide/Fe3O4 nanocomposite with kombucha solvent supernatant as ultra-sensitive biosensor for detection of doxorubicin hydrochloride in biological fluids. *Mater Chem Phys*. 2022;279:125743.
54. Emadi F, Amini A, Gholami A, Ghasemi Y. Functionalized Graphene Oxide with Chitosan for Protein Nanocarriers to Protect against Enzymatic Cleavage and Retain Collagenase Activity. *Sci Rep.* 2017 Feb 10;7:42258. doi: 10.1038/srep42258. PMID: 28186169; PMCID: PMC5301474.
55. Hong H, Yang K, Zhang Y, Engle JW, Feng L, Yang Y, et al. In vivo targeting and imaging of tumor vasculature with radiolabeled, antibody-conjugated nanographene. *ACS Nano.* 2012 Mar 27;6(3):2361-70. doi: 10.1021/nn204625e. Epub 2012 Feb 16. PMID: 22339280; PMCID: PMC3314116.
56. Shi S, Yang K, Hong H, Valdovinos HF, Nayak TR, Zhang Y, Theuer CP, Barnhart TE, Liu Z, Cai W. Tumor vasculature targeting and imaging in living mice with reduced graphene oxide. *Biomaterials*. 2013 Apr;34(12):3002-9. doi: 10.1016/j.biomaterials.2013.01.047. Epub 2013 Jan 29. PMID: 23374706; PMCID: PMC3570619.
57. Ayyaru S, Ahn Y-H. Application of sulfonic acid group functionalized graphene oxide to improve hydrophilicity, permeability, and antifouling of PVDF nanocomposite ultrafiltration membranes. *J Memb Sci.* 2017;525:210-9.
58. Reina G, González-Domínguez JM, Criado A, Vázquez E, Bianco A, Prato M. Promises, facts and challenges for graphene in biomedical applications. *Chem Soc Rev*. 2017;46(15):4400-16.
59. Bottari G, Herranz MÁ, Wibmer L, Volland M, Rodríguez-Pérez L, Guldi DM, et al. Chemical functionalization and characterization of graphene-based materials. *Chem Soc Rev*. 2017;46(15):4464-500.
60. Eskandari F, Abbaszadegan A, Gholami A, Ghahramani Y. The antimicrobial efficacy of graphene oxide, double antibiotic paste, and their

combination against Enterococcus faecalis in the root canal treatment. *BMC Oral Health.* 2023 Jan 13;23(1):20. doi: 10.1186/s12903-023-02718-4. PMID: 36639767; PMCID: PMC9840282.

61. Mousavi SM, Hashemi SA, Ghahramani Y, Azhdari R, Yousefi K, Gholami A, et al. Anti-proliferative and Apoptotic Effects of Graphene Oxide @AlFu MOF Based Saponin Natural Product on OSCC Line. *Pharmaceuticals (Basel).* 2022 Sep 12;15(9):1137. doi: 10.3390/ph15091137. PMID: 36145358; PMCID: PMC9504826.

62. Rezaei A, Aligholi H, Zeraatpisheh Z, Gholami A, Mirzaei E. Collagen/chitosan-functionalized graphene oxide hydrogel provide a 3D matrix for neural stem/precursor cells survival, adhesion, infiltration and migration. *J Bioact Compat Pol*. 2021;36(4):296-313.

63. Tonelli FM, Goulart VA, Gomes KN, Ladeira MS, Santos AK, Lorençon E, Ladeira LO, Resende RR. Graphene-based nanomaterials: biological and medical applications and toxicity. *Nanomedicine (Lond)*. 2015;10(15):2423-50. doi: 10.2217/nnm.15.65. Epub 2015 Aug 5. PMID: 26244905.

64. Chakraborty A, Das A, Raha S, Barui A. Size-dependent apoptotic activity of gold nanoparticles on osteosarcoma cells correlated with SERS signal. *J Photochem Photobiol B*. 2020 Jan;203:111778. doi: 10.1016/j.jphotobiol.2020.111778. Epub 2020 Jan 7. PMID: 31931389.

65. Jia PP, Sun T, Junaid M, Yang L, Ma YB, Cui ZS, et al. Nanotoxicity of different sizes of graphene (G) and graphene oxide (GO) in vitro and in vivo. *Environ Pollut*. 2019 Apr;247:595-606. doi: 10.1016/j.envpol.2019.01.072. Epub 2019 Jan 24. PMID: 30708322.

66. Gurunathan S, Kang MH, Jeyaraj M, Kim JH. Differential Cytotoxicity of Different Sizes of Graphene Oxide Nanoparticles in Leydig (TM3) and Sertoli (TM4) Cells. *Nanomaterials (Basel).* 2019 Jan 22;9(2):139. doi: 10.3390/nano9020139. PMID: 30678270; PMCID: PMC6410280.

67. Gurunathan S, Arsalan Iqbal M, Qasim M, Park CH, Yoo H, Hwang JH, Uhm SJ, Song H, Park C, Do JT, Choi Y, Kim JH, Hong K. Evaluation of Graphene Oxide Induced Cellular Toxicity and Transcriptome Analysis in Human Embryonic Kidney Cells. *Nanomaterials (Basel)*. 2019 Jul 2;9(7):969. doi: 10.3390/nano9070969. PMID: 31269699; PMCID: PMC6669460.

68. Zhang L, Ouyang S, Zhang H, Qiu M, Dai Y, Wang S, et al. Graphene oxide induces dose-dependent lung injury in rats by regulating autophagy. *Exp Ther Med.* 2021 May;21(5):462. doi: 10.3892/etm.2021.9893. Epub 2021 Mar 5. PMID: 33747194; PMCID: PMC7967850.

69. Yang K, Gong H, Shi X, Wan J, Zhang Y, Liu Z. In vivo biodistribution and toxicology of functionalized nano-graphene oxide in mice after oral and intraperitoneal administration. *Biomaterials*. 2013 Apr;34(11):2787-95. doi: 10.1016/j.biomaterials.2013.01.001. Epub 2013 Jan 20. PMID: 23340196.

70. Elkomy MH, Ali AA, Eid HM. Chitosan on the surface of nanoparticles for enhanced drug delivery: A comprehensive review. *J Control Release*. 2022 Nov;351:923-940. doi: 10.1016/j.jconrel.2022.10.005. Epub 2022 Oct 11. PMID: 36216174.

71. Mauro N, Scialabba C, Agnello S, Cavallaro G, Giammona G. Folic acid-functionalized graphene oxide nanosheets via plasma etching as a platform to combine NIR anticancer phototherapy and targeted drug delivery. *Mater Sci Eng C Mater Biol Appl.* 2020 Feb;107:110201. doi: 10.1016/j.msec.2019.110201. Epub 2019 Sep 13. PMID: 31761243.

72. Fathi-Karkan S, Arshad R, Rahdar A, Ramezani A, Behzadmehr R, Ghotekar S, Pandey S. Recent advancements in the targeted delivery of etoposide nanomedicine for cancer therapy: A comprehensive review. *Eur J Med Chem.* 2023 Nov 5;259:115676. doi: 10.1016/j.ejmech.2023.115676. Epub 2023 Jul 23. PMID: 37499287.

73. Yaghoubi F, Motlagh NSH, Naghib SM, Haghiralsadat F, Jaliani HZ, Moradi A. A functionalized graphene oxide with improved cytocompatibility for stimuli-responsive co-delivery of curcumin and doxorubicin in cancer treatment. *Sci Rep.* 2022 Feb 4;12(1):1959. doi: 10.1038/s41598-022-05793-9. PMID: 35121783; PMCID: PMC8816945.

74. Mariadoss AVA, Saravanakumar K, Sathiyaseelan A, Wang MH. Preparation, characterization and anti-cancer activity of graphene oxide--silver nanocomposite. *J Photochem Photobiol B*. 2020 Sep;210:111984. doi: 10.1016/j.jphotobiol.2020.111984. Epub 2020 Jul 30. PMID:

32771914.
75. Li R, Wang Y, Du J, Wang X, Duan A, Gao R, et al. Graphene oxide loaded with tumor-targeted peptide and anti-cancer drugs for cancer target therapy. *Sci Rep.* 2021 Jan 18;11(1):1725. doi: 10.1038/s41598-021-81218-3. PMID: 33462277; PMCID: PMC7813822.
76. Miao W, Shim G, Lee S, Lee S, Choe YS, Oh YK. Safety and tumor tissue accumulation of pegylated graphene oxide nanosheets for co-delivery of anticancer drug and photosensitizer. *Biomaterials.* 2013 Apr;34(13):3402-10. doi: 10.1016/j.biomaterials.2013.01.010. Epub 2013 Feb 4. PMID: 23380350.
77. Pei X, Zhu Z, Gan Z, Chen J, Zhang X, Cheng X, et al. PEGylated nano-graphene oxide as a nanocarrier for delivering mixed anticancer drugs to improve anticancer activity. Scientific Reports. 2020;10(1):2717.