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GRAPHENE NANOMATERIALS IN NEUROLOGICAL DRUG DELIVERY: SYNTHESIS, CHARACTERIZATION, AND BIOMEDICAL APPLICATIONS

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ABSTRACT

Graphene nanomaterials (GNMs) offer significant potential in drug delivery and biomedical applications due to their unique properties. Graphene oxide (GO), a derivative with oxygen functional groups, is particularly promising for diagnosing and treating brain disorders due to its biocompatibility and low cytotoxicity. The rise in neurological diseases, necessitates sophisticated drug-delivery capable of traversing the Blood-Brain-Barrier (BBB). This review comprehensively addresses the synthesis, characterization, and properties of various GNMs, with an emphasis on their applications in targeted drug delivery, bioimaging, gene therapy, regenerative medicine, and antibacterial activity. It covers synthesis methods, including the modified Hummer's method, and characterization techniques such as Raman spectroscopy, FTIR, XPS, AFM, SEM, and TEM. Furthermore, the review explores the electrical and mechanical properties of GNMs and their potential to enhance drug delivery through both covalent and non-covalent modifications. This review provides an overview of GNMs' potential in biomedical applications, emphasizing their role as drug delivery vectors and therapeutic agents, with ongoing research aiming to optimize their properties for clinical use.

Keywords: Graphene nanomaterials (GNMs), Graphene oxide (GO), Blood-Brain-Barrier (BBB)

1. INTRODUCTION:

Graphene nanomaterials (GNMs) are convenient for drug and biological related applications, including the surface area, significant lateral dimensions, precise monolayer thickness, exceptional purity, and sophisticated surface chemistry. Notably, graphene's surface area, measuring 2600 m^2 (about the area of a large mansion) g^{-1} , surpasses the external area of various nanomaterials investigated for drug delivery by a remarkable fourfold, Graphene oxide (GO) is a monolayer derivative of graphene featuring oxygen functional groups, including epoxides, alcohol, and carboxylic acids. Its chemical assay reveals the carbon to oxygen ratio approximately equal to 3:1 [1]. The investigation of graphene-based compounds for their potential in diagnosing and treating diverse brain disorders is currently underway. Graphene quantum dots (graphene or rGO QDs) exhibit notable biocompatibility and low cytotoxicity, rendering them ideal for various medical uses. Among these applications are bioimaging, biosensing, drug delivery, and photothermal therapy [2].

The leading causes of illness, disability, and death worldwide predominantly arise from neurological or brain disorders. Conditions such as Alzheimer disease, Parkinson's disease, Huntington's disease etc, are the most

significant contributors to these outcomes. Over the past thirty years, there has been a significant rise in mortality rates associated with neurological diseases, and this upward trend is expected to persist due to the aging global population. Presently, patients encounter delays in diagnosis, avoidable deaths, and ineffective chemotherapy treatments, all of which impose a substantial financial burden on healthcare systems [2].

Brain-targeted drug delivery is a specialized approach aimed at enhancing the transport of therapeutic agents across the blood-brain barrier to effectively treat neurological disorders. This strategy addresses the challenges of limited drug access to the brain and seeks to improve drug delivery precision, potentially minimizing side effects on non-targeted tissues. Various technologies, such as nanoparticles and liposomes, are explored for their ability to optimize brain targeted drug delivery, opening new possibilities in treatment of brain-related conditions [3].

Rigidity plays an integral role by ensuring the robustness of the drug carriers' structure. However, excessive stiffness in the structure of graphene can potentially harm cells. Hence, it is crucial to lessen the stiffness of graphene and GO sheets to mitigate this adverse effect. Concerning the surface chemistry of the

graphene exhibits high hydrophobicity and poor dispersibility in water. Consequently, surface modifications are necessary for utilizing it in biological applications. On the other hand, GO possesses hydrophilic properties could be easily scatter in the water, forming lasting colloids.

The opted method for production of Graphene-based nanomaterials (GNMs) can introduce diverse contaminants, such as remnants from hydrazine, nitrates, sulfates, permanganates, peroxide, borohydride surfactants, and oxidative fragments of lower molecular weight. These contaminants carry the potential to induce adverse biological effects and profoundly influence toxicity levels [4].

While several current drug delivery systems have demonstrated certain benefits, such as their capability to enhance drug solubilization and prolong the circulation of drugs in the bloodstream, their effectiveness is largely

hindered by their inability to accomplish high concentrations and effectiveness specifically the tumor sites. This issue arises because of their constrained loading capacity and limited ability to be functionalized. Furthermore, inadequate cellular absorption diminishes the curing efficacy of non-tumor medications. Simultaneously, non-specific pile in the unaffected tissues results in substantial adverse effects, thereby limiting the biological application. Hence, crucial for the development of the effective targeting systems capable of enhancing targeted cellular absorption of anti-cancer medications and facilitating smart controlled release [5]. Specifically, we explore their viability as drug nano-delivery vectors through chemical modification with pharmaceutical agents or other molecules. This approach aims to achieve precise targeting of diseased areas and controlled release of active compounds.

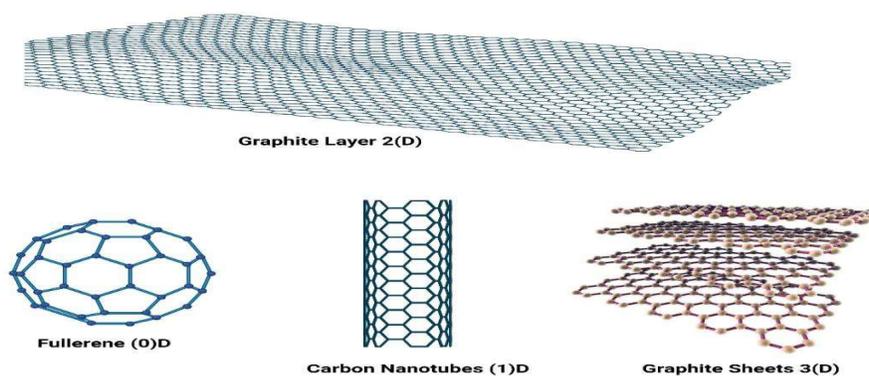


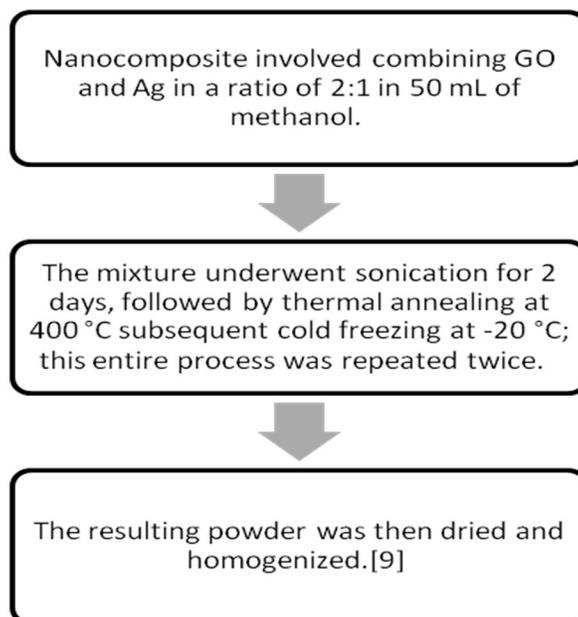
Figure 1: Carbon based nano particles

Nanoparticles like carbon nanotubes, silica, polybutylcyanoacrylate (PCBA), Polylactic-Co-Glycolic-Acid (PLGA), observed to traverse the Blood-Brain-Barrier (BBB) either in laboratory settings (in-vitro) or within living organisms (in-vivo). The methods by which these nanoparticles cross the BBB vary. Certain nanomaterials are paired with proteins that specifically target endothelial cells, allowing them to traverse the blood-brain barrier (BBB) and access the central nervous system (CNS). This process involves adsorptive transcytosis, which relies on the Electrostatic interactions between positively charged substances and negatively charged membranes, along with efflux transporters like proton pumps, contribute to the complex mechanism of transporting nanomaterials across the BBB [6].

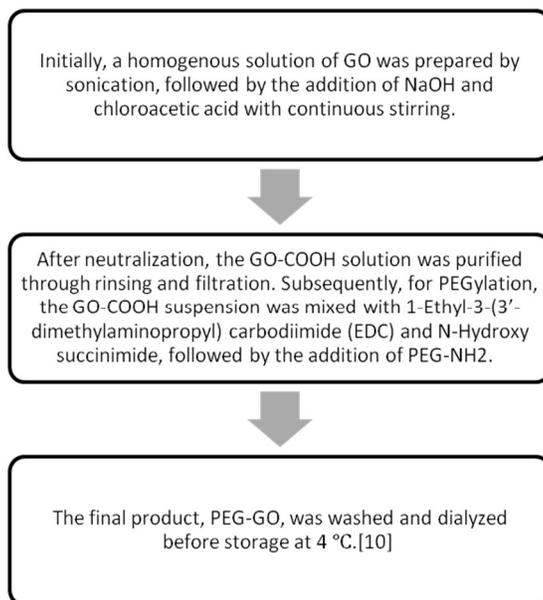
The range of graphene nanoparticles (GNPs) exhibits varying degrees of efficacy as therapeutic agents and carriers for drug delivery. In its pure form, graphene consists of a flat structure where each carbon atom forms bonds with three others in a hexagonal lattice. This hexagonal lattice, reminiscent of benzene, possesses inherent therapeutic properties. Key factors facilitating drug and molecule binding include the hydrophobic nature of graphene and the phenomenon of π - π stacking. However, extensive research on pure graphene has unveiled those surfactants and residual molecule adhering to its surface can induce toxicity, thereby prompting a shift away from its use in biomedical applications towards alternative graphene materials [7].

2. Synthesis

2.1. Synthesis of the GO-Ag:



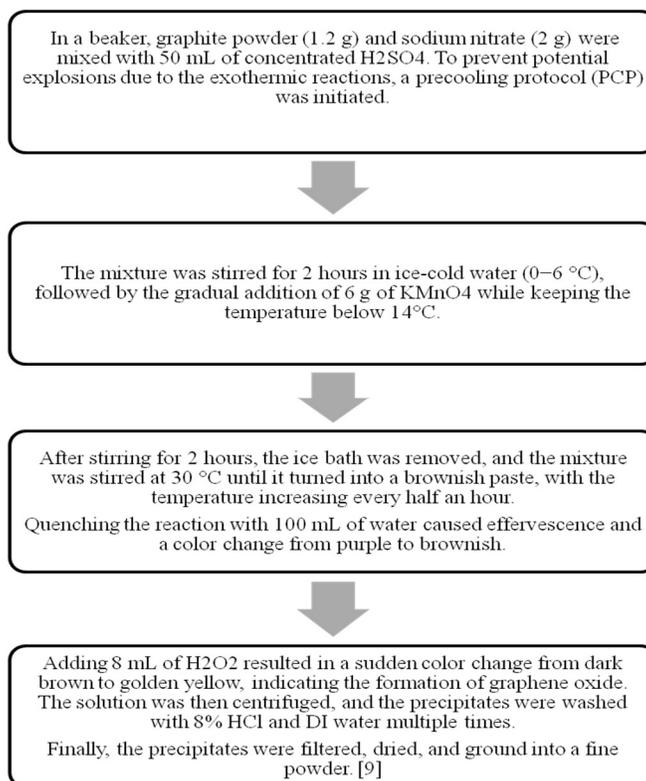
2.2. Synthesis of PEG-GO:



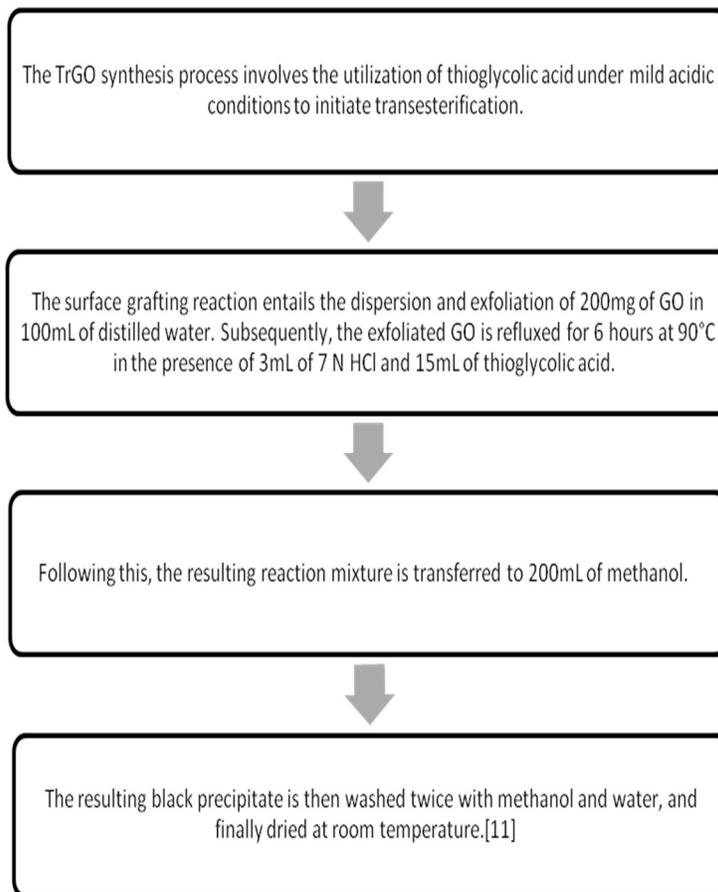
2.3. Synthesis of GO:

A modified Hummer's method was used to produce graphene oxide, involving the

oxidation of graphite with KMnO₄ and NaNO₃ in concentrated H₂SO₄ [8].



2.4. Synthesis of Thiolate reduced graphene oxide (TrGO):



3. Characterization:

- Characterization of GO involves both spectroscopic and microscopic techniques.
- Spectroscopic methods, including FTIR, Raman Spectroscopic and XPS, are applied to identify the chemical structure of Graphene Oxide.
- Microscopic tools, such as AFM, SEM, TEM, and STM, are employed to examine the

structure of GO across different vertical dimensions and lateral sizes.

3.1. Spectroscopic Method:

Raman Spectroscopy: Raman scattering is an effective technique for analyzing carbon-based compounds with sp² and sp³ hybridization, such as graphite. Graphite, composed of multiple layers of graphene sheets, exhibits distinct G band around 1580 cm⁻¹ representing planar vibrations of carbon atoms, and the D band at

approximately $1,350\text{ cm}^{-1}$ indicating structural defects. The $2D'$ band, an overtone of the D' mode, shows a weak signal at around $3,248\text{ cm}^{-1}$. In monolayer graphene, the $2D$ mode appears at a lower frequency with a narrower bandwidth compared to graphite, where the bandwidth range by 45 to 60 cm^{-1} . The potency ratio of the $2D$ and G bands (I_{2D}/I_G) is higher in monolayer graphene than in graphite. For graphene oxide (GO), the G band shifts to around $1,590\text{ cm}^{-1}$ and has a broader spectral profile.

Fourier transform infrared spectroscopy (FTIR): The mid-infrared region is essential for studying fundamental vibrations and rotational-vibrational structures of substances, particularly graphene oxide (GO). Infrared spectroscopy (IR) utilizes this region to analyze the vibrations of molecular bonds. During IR analysis, infrared radiation is transmitted through the sample, and the absorption at each wavelength is measured, revealing a unique "fingerprint" spectrum. For GO, notable IR peaks include a broad peak at $3,430\text{ cm}^{-1}$ for hydroxyl groups ($-OH$), a peak at $1,720\text{ cm}^{-1}$ for

carbonyl groups ($C=O$), a peak at $1,570\text{ cm}^{-1}$ for sp^2 carbon skeletal vibrations, and peaks at $1,225\text{ cm}^{-1}$ and around $1,100\text{ cm}^{-1}$ for $COOH$ and $C-O-C$ stretching vibrations, respectively.

X-Ray photoelectron spectroscopy

(XPS): Uses photoionization to analyze the elemental composition, chemical state, and electronic state of materials. Under ultra-high vacuum (UHV), a material is exposed to X-rays, exciting core electrons to higher energy levels. By examining the kinetic energy of emitted photoelectrons, researchers can deduce surface chemistry and electronic structure. XPS is essential in materials science, chemistry, and physics for characterizing surfaces, interfaces, and thin films. It is particularly useful for identifying carbon nanomaterials by revealing elemental ratios and surface functional groups, which influence their properties.

Microscopic approaches:

Microscopes have been essential in the investigation of nano-structured materials. A range of techniques are employed to gain insights into the

surface characteristics of nanomaterials, including optical microscopy, atomic force microscopy (AFM), scanning electron microscopy (SEM), and high-resolution transmission electron spectroscopy. These methods enable researchers to examine and analyze the intricate details of nanomaterials, facilitating a deeper understanding of their properties and behavior.

Atomic force microscopy (AFM):

AFM is an advanced scanning probe microscopy technique that provides high-resolution imaging of surface topography at the nanometer scale. It uses a mechanical probe on a cantilever to physically interact with the sample surface, scanning it in a raster pattern. The cantilever's deflection, influenced by van der Waals forces, is precisely controlled by piezoelectric elements for accurate imaging. A laser beam detection system monitors the cantilever's movements by reflecting a laser off its back toward spatially aware sensor. Its functions in the three of the main modes: Tapping, surface-approach, and Non-Contact, each suited to different imaging needs, allowing

researchers to study surface characteristics and properties at the nanoscale.

Optical microscopy: Optical microscopy continues for the comprehensive assessment of graphene and GO sheets, serving as a cost-effective and efficient imaging technique. Its ability to provide high throughput imaging makes it an indispensable tool in the evaluation process. Notably, when examining individual graphene sheets, it is observed that they absorb approximately 2.3% of visible light, highlighting their unique optical properties [9].

Scanning electron microscopy (SEM):

SEM is extensively used for sample examination owing to its high clarity, comfortable operation, considerable integrity of focus, and exceptional amplification capabilities. It utilizes an electron microscope to capture images by scanning the sample with a focused beam of high-energy electrons. An electron gun emits this beam in a vacuum chamber, which is then focused and accelerated by magnetic lenses. The electrons interact with the sample, generating

various signals, including secondary electrons. These signals are collected by a positively charged secondary electron detector, pass through a Faraday cage, and are transformed into two-dimensional grayscale images for detailed sample analysis [9].

4. Properties:

Electrical properties: Graphene, due to its unique Fermi energy levels and linear Dirac-like spectrum, is classified as an intrinsic semiconductor. Double-layer graphene, with a small parabolic spectrum of 1.6 meV, behaves like a metal with a single type of exciton. In contrast, graphene with three or more layers has complex band structures with multiple charge carriers and overlapping conduction and valence bands. On a nonconductive substrate, graphene's charge mobility is about 6×10^4 cm²/Vs, with values for electrons and holes around 1.5×10^4 cm²/Vs at the temperatures of 4 K and 300 K, and a maximum concentration of 10^{13} cm⁻². Its exceptional conductivity results in a low sheet resistance of 30 Ω/sq cm [10].

4.1. Mechanical properties: Graphene is renowned for its impressive mechanical strength of around 1100 GPa,

making it a highly resilient material. In contrast, GO may have lower mechanical properties than graphene, but its strength can be enhanced by linking individual particles together. When graphene is mixed with polymers, it not only improves the material's module but also enhances its durability, making it well-suited for a variety of biological applications [1].

Activity of Graphene oxides: Activating graphene sheets is an effective method to improve their dispersion and stabilization within polymer framework. These are the two main strategies to activating graphene.

4.2. Covalent Activity: Covalent activation can be achieved through various methods, such as Nucleophilic Substitution, Electrophilic Addition, Condensation, and Addition. In case of nucleophilic substitution, the primary reactive sites are the epoxy groups on graphene oxide (GO). These sites can bond with groups possessing amino functionalities (-NH₂), which have lone electron pairs capable of attacking the epoxy groups. This reaction is straightforward, occurring at ambient temperature in an aqueous medium, rendering it an appealing method for graphene modification on a large scale. It

allows the incorporation of a diverse array of molecules, encompassing different Amines, Polymers, Biomolecules, Enzymes and Nanoparticles [11][12]. Covalent activation involves chemical bonding with surface moieties on graphene oxide (GO) through acidic treatment. These severe acidic conditions can create structural imperfections, consequently boosting physical and chemical properties of GO [13]. Drug delivery systems utilizing covalently functionalized graphene oxide (GO) derivatives featuring suitable functional groups have emerged as promising tools are extensively investigated for systemic targeting platforms. The modified Graphene Oxide-PEG-PCT model demonstrated a heavy loading ratio and excellent constability under biological conditions. The modification of graphene oxide can also be achieved by attaching small molecules on it. The use of Graphene oxide and Folic Acid together introduces a new approach for molecular recognition, enabling targeted delivery of anticancer drugs to malignant cells that express folate receptors. This innovation paves the way for advanced smart drug delivery systems [14][15].

4.3. Non-Covalent Activity:

Noncovalent modifications rely on highly hydrophobic moieties. Graphene sheets themselves are stabilized by π - π stacking and Van Der Waals interaction, highlighting the significance of surface modifications with these components [16-18]. Typically, noncovalent functionalization of graphene oxide surfaces involves either wrapping polymers and biomacromolecules around it or absorbing such molecules onto its surface. The adsorption of polymers onto the surface of graphene oxide through noncovalent means is weaker compared to covalent bonding and is susceptible to fluctuations in the external environment. This instability can compromise the stability of drug delivery systems in biological settings, whether in laboratory settings or within living organisms. Moreover, non-covalently modified graphene oxide may support a smaller number of aromatic drugs compared to covalently modified graphene oxide, as many conjugation locations on the graphene oxide layer of sheets are partially occupied by the polymer coating [15].

5. Targeting Strategies of Graphene Oxide:

The physical and chemical attributes of the methods for delivering molecules with GO-based nanocarriers are shaped by the physiological properties and local surroundings of the target site. This section investigates how the features of the carrier and the target site's environment influence the selection between the active and passive targeting strategies.

5.1. Active Targeting: Active targeting plays a crucial role in the delivery of the drugs, genes, therapy and diagnostics to specific sites, while minimizing exposure to normal tissues, thereby enhancing therapeutic effectiveness and reducing side effects. Compared to free drugs or passively targeted nano systems, active targeting significantly increases the amount of drug delivered to target cells. Following accumulation in the tumor region, active targeting further boosts drug efficiency by decorating nano-carrier surface with the ligand that bind to the receptors amplifying on the affected cells. This approach enhances the nanocarriers' affinity for cancer cell surfaces, facilitating deeper penetration

of drug. Additionally to the enhanced permeability and retention (EPR) effect, active targeting provides an alternative strategy to enhance tumor uptake by attaching or conjugating affinity ligands to nano systems for specific recognition of tumor cells [18][19].

5.2. Passive Targeting: Passive targeting leverages the distinctive physiological traits of tumor blood vessels to facilitate the accumulation of nanodrugs within tumor tissues. This approach is appealing for drug delivery as it does not require complex functional modifications, and graphene has been utilized in exploiting these mechanisms [20]. Passive targeting entails the conveyance of nanocarriers through porous tumor vasculature, allowing them to penetrate tumor cells via passive movement [21]. In this process, molecules move through fluids, selectively gathering drugs and nanocarriers as a result of the enhanced permeability and retention (EPR) effect [22].

6. Challenges to cross BBB

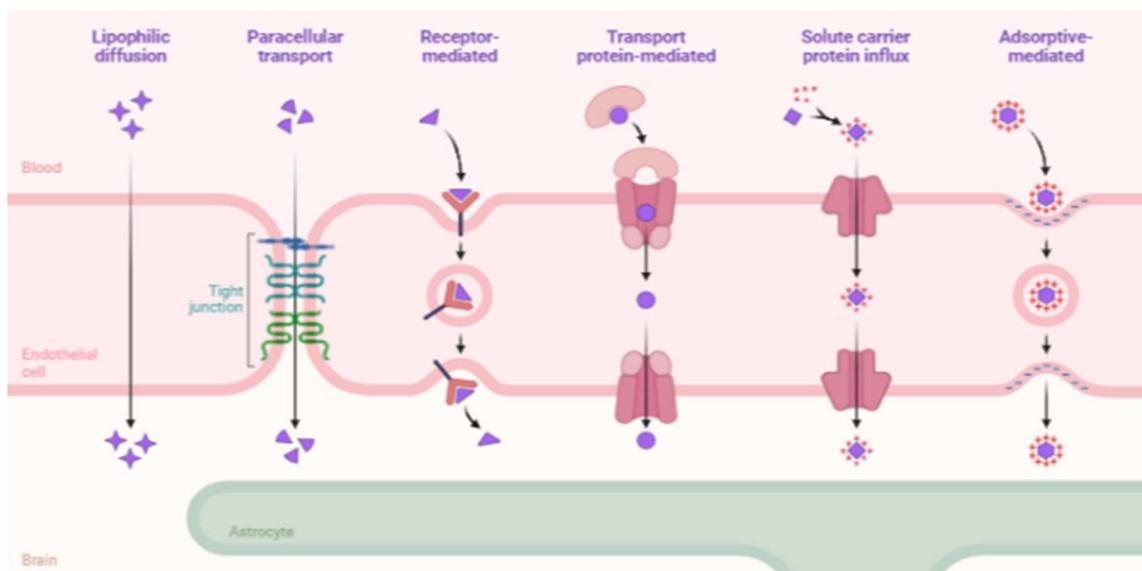


Figure 2: Blood Brain Barrier (BBB)

The passage through the BBB is challenging, greatly restricting drug delivery. It blocks nearly 100% of large molecule neuro-therapeutics and over 98% of all small-molecule drugs [23]. The precise chemical makeup of the extracellular environment in the CNS, maintained by the BBB, is crucial for proper neural function [24]. In fact, many diseases are linked to local disruptions of the BBB.

To overcome the blood-brain barrier (BBB) and improve treatments for brain disorders, scientists are creating new materials and technologies [25]. They use both passive and active methods to cross the BBB safely and effectively, allowing drugs directly target to the brain [26]. These

sophisticated drug delivery systems are engineered to transport various treatments like small-molecule drugs, proteins, and genes [27]. To maximize efficiency and safety, most of these systems are in nanoscale, with specific surface properties and chemicals [28]. Carbon materials are being used as a biocompatible carrier for the delivery of drugs directly to the brain. Their adjustable properties, stability under light, small size, and ease of production make them ideal for this purpose. Drugs can be easily loaded onto these graphene-based systems through multiple non-covalent interactions such as π - π stacking, hydrogen bonding, and electrostatic interactions and hydrophobic

interactions [29]. Graphene oxide (GO) and reduced graphene oxide (rGO) have functional groups like -OH and -COOH that make it easy to attach targeted molecules and integrate with other materials. Interestingly, these graphene products also have potential therapeutic results in the therapy of central nervous system conditions (rGO) [30]. For instance, Graphene quantum dots (GQDs) ranging in size from 2 to 4 nm demonstrate greater blood-brain barrier (BBB) permeability than GO particles sized between 5 and 20 nm, emphasizing the influential importance of particle size in the delivery of substances to the brain [31]. Because of their diminutive dimensions, GQDs can be enclosed within mesoporous nanostructures to facilitate the theragnostic delivery of drugs and photolytic agents [32]. Moreover, the incorporation of engineered cell membranes onto these hybrid particles extends their circulation time in the bloodstream, thereby promoting increased accumulation in brain tumors [33].

7. Applications

7.1. Carrier for Gene therapy:

Assignable to exceptional surface area of the Graphene-based nanomaterials (GBNMs), they are widely utilized in the development of effective drug delivery systems. GBNMs find application in biological contexts because they display fluorescence suitable for biosensing and possess high reactivity due to functional groups.[34] Its surface can be functionalized through covalent or non-covalent bonding to attach anti-cancer drugs. Its hydrophobic nature provides an optimal platform for anchoring amphiphilic functional groups, enabling effective targeting of cancer cells. Various anti-cancer drugs such as doxorubicin, camptothecin, lucanthone, and paclitaxel are commonly functionalized onto GBNMs [35][36].

7.2. Bioimaging: The knowledge intricate biological systems involve studying how individual components within living cells come together, organize, interact, to provide the structure and the function. The need to observe and assess these processes in living cells, tissues, and organisms has spurred the advancement of

sophisticated bioimaging techniques. Bioimaging not only enhances our comprehension of normal biological processes but also facilitates the monitoring of abnormal processes linked to diseases like cancer, highlighting its crucial role in medicine. At its core, the techniques for visualizing of biological processes in live cells or animals using specific imaging agents. However, these biological applications are often limited by challenges such as insufficient sensitivity, specificity, and targeting, necessitating continual advancements in bioimaging probes and modalities. In this regard, hybrid materials combining graphene and nanoparticles show significant potential as the next generation of imaging probes. This briefly explores their impact in three primary imaging modalities: MRI, Raman spectroscopy, and fluorescence imaging [15][37].

7.3. Gene Therapy: Graphene-based nanomaterials (GBNs) exhibit interactions not only with drugs but also with biomolecules such as nucleic acids, encompassing DNA and RNA. This characteristic endows them with

utility as carriers and for nucleic acid identification, leveraging their expansive sp²-hybridized carbon surface. Gene therapy has recently emerged as a focal point in regenerative medicine for disease treatment. Graphene oxide (GO) has demonstrated the capability to adsorb nucleobases via pi-pi interactions and effectively shield nucleotides from enzymatic degradation, underscoring its potential in gene therapy applications. Effective gene delivery vectors must meet specific criteria, such as safeguarding DNA from degradation and achieving high transfection efficacy. Beyond graphene oxide (GO), extensive research has focused on both viral and non-viral vectors for gene delivery applications. Investigations have indicated that GO complexes with the vascular endothelial growth factor-165 (VEGF) gene enhance delivery efficiency for myocardial therapy. Additionally, graphene oxide nanosheets have emerged as promising vectors owing to their efficient cellular uptake capabilities [38].

7.4. Regenerative Medicine: Tissue engineering represents an advanced discipline within the life sciences, dedicated to developing biological substitutes that can repair and maintain tissue function. Known as scaffolds, these constructs are fabricated from biodegradable materials designed to mimic natural tissue structures, thereby effectively guiding tissue growth and facilitating healing processes [5]. Graphene oxide (GO), reduced graphene oxide (RGO), and comparable graphene-based composites possess easily modifiable surface functional groups. These groups facilitate interactions with a range of biological molecules, including DNA, proteins, peptides, and enzymes. In tissue engineering, RGO and other GO composites are valued for their versatile fabrication capabilities. Biomaterials such as GO can activate specific cellular functions, direct cell differentiation, and influence interactions between cells. Various strategies involve integrating graphene with biopolymers, proteins, peptides, DNA, and polysaccharides to achieve desired outcomes in fabrication [39]. The

capacity of graphene-based nanomaterials (GBNs) to sustain prolonged cellular viability post-differentiation is crucial for applications in regenerative medicine.

7.5. Antibacterial Activity:

Excessive use of conventional antibiotics has resulted in the emergence of antibiotic resistance. Over the past two decades, there has been a concerted effort to develop new drugs to combat multi-drug resistant pathogens. To address antibiotic resistance, various anti-bacterial treatments are created, including nanoparticles made from metals and metal oxides [40]. Graphene-based nanomaterials (GBNs) have demonstrated antibiotic properties due to their distinctive physicochemical characteristics. Researchers have created diverse nanocomposites based on GBNs by modifying their surfaces with biomolecules, polymers, and inorganic nanostructures to enhance antibacterial effectiveness and reduce toxicity. This section summarizes the antibacterial capabilities of GBNs and elucidates their mechanisms of action. The versatility of GBNs, supported by numerous studies, suggests their

potential utility as antimicrobial agents [41]. Graphene-based nanomaterials (GBNs) show promising applications as antibacterial agents in a range of fields. It has been extensively documented that graphene-based nanomaterials (GBNs) exhibit antibacterial properties due to their sharp edges, which interact with bacterial membranes, resulting in the disruption of lipid biomolecules and oxidative stress [42].

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