

Review

The Use of Crystalline Carbon-Based Nanomaterials (CBNs) in Various Biomedical Applications

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Abstract: This review study aims to present, in a condensed manner, the significance of the use of crystalline carbon-based nanomaterials in biomedical applications. Crystalline carbon-based nanomaterials, encompassing graphene, graphene oxide, reduced graphene oxide, carbon nanotubes, and graphene quantum dots, have emerged as promising materials for the development of medical devices in various biomedical applications. These materials possess inorganic semiconducting attributes combined with organic π - π stacking features, allowing them to efficiently interact with biomolecules and present enhanced light responses. By harnessing these unique properties, carbon-based nanomaterials offer promising opportunities for future advancements in biomedicine. Recent studies have focused on the development of these nanomaterials for targeted drug delivery, cancer treatment, and biosensors. The conjugation and modification of carbon-based nanomaterials have led to significant advancements in a plethora of therapies and have addressed limitations in preclinical biomedical applications. Furthermore, the wide-ranging therapeutic advantages of carbon nanotubes have been thoroughly examined in the context of biomedical applications.

Keywords: crystalline carbon-based nanomaterials; biomedical applications; cancer treatment; biosensors; drug delivery; cytotoxicity



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1. Introduction

Graphite, a natural material with a long history, holds the distinction of being one of the oldest and most widely utilized substances. While traditionally recognized as the primary component of pencil lead, giving rise to its name, graphite has found expanded application in various large-scale industrial sectors. These include its use in steelmaking for carbon raising, battery electrodes, and industrial-grade lubricants [1]. Notably, the demand for synthetic graphite has experienced a significant upsurge in recent years. Scientific investigations have extensively explored graphite, revealing that its distinctive combination of physical properties arise from its intricate macromolecular arrangement, which comprises successive hexagonal arrays of sp^2 carbon stacked together. Advancements and growing recognition in nanofabrication methods and nanomaterials over the past two decades have spurred the active utilization of graphite as a foundational material for engineering diverse types of carbon-based nanomaterials (CBNs) [2].

CBNs demonstrate remarkable mechanical strength, electrical and thermal conductivity, and optical characteristics [3]. Consequently, extensive research has been dedicated

to capitalizing on these favorable properties for diverse applications, including the development of high-strength composite materials and advancements in electronics. Notably, carbon materials encompass a wide range of forms within the carbon family, spanning from zero-dimensional (0D) to three-dimensional (3D) structures [4–8] (Figure 1). Carbon-based quantum dots and carbon nanotubes (CNTs) such as single or multi-walled nanotubes, nanoribbons, graphene, and substances derived from compounds like graphene oxide, biomass or polymer-derived carbon, fullerenes, and nanodiamonds are among the commonly employed crystalline carbon materials [9–12].

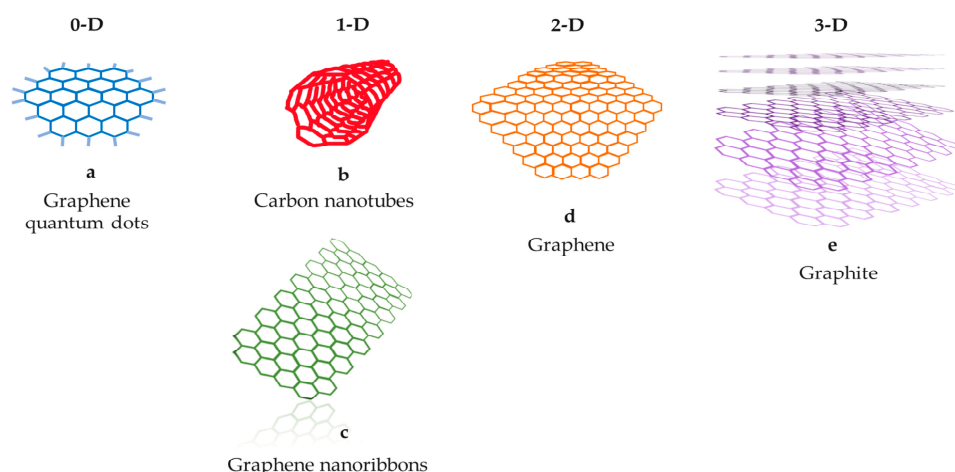


Figure 1. The main carbon-based nanomaterials. (a) Graphene quantum dots, (b) carbon nanotubes, (c) graphene nanoribbons, (d) graphene, (e) graphite.

In recent times, the field of biomedical engineering has warmly welcomed the growing popularity and influence of CBNs due to their crucial role in numerous applications, which heavily rely on biomaterial performance. Nanoscale biomaterials, in particular, are highly sought after due to their small size [13–18]. Among these materials, CBNs have gained considerable recognition as appealing biomaterials [19] because of their compelling characteristics, such as exceptional mechanical properties, enhanced specific surface area, environmentally friendly nature, and ease on surficial alterations [20–23]. Furthermore, they possess the highest distribution rate within the body [24]. Incorporating CBNs into existing biomaterials can enhance their functionalities, leading to their extensive utilization in various biomedical research areas [25], including drug delivery systems, the reinforcement of tissue scaffolds, and the development of cellular sensors [26].

This review paper provides an assessment of the recent advancements and presents uses of carbon-based materials across diverse biomedical fields and applications. Additionally, a condensed overview of this field is presented, along with a discussion of existing obstacles and projections for future development. The aim of the present review is for it to serve as a valuable guide for research focused on the advancement of carbon-based materials for biomedical purposes, fostering significant interest within the biomedical engineering and materials science community.

2. Carbon Nanotubes (CNTs)

Subsequent to their initial identification, carbon nanotubes (CNTs) have emerged as the prevalently employed CBNs [27,28]. CNTs are typically synthesized through processes like arc discharge or chemical vapor deposition of graphite. Characterized by their cylindrical structure, CNTs exhibit a broad array of both electrical and optical features. These characteristics do not solely stem from their sp^2 carbon structure but also from their customizable physical features, including their diameter, length, whether they are single-walled or multi-walled, their surface functionalization capacity, and even their chirality [28]. The CNTs' versatile and beneficial properties have the motivated exploration of

numerous industrialized purposes [29]. For instance, the exceptional mechanical strength constitutes one of the features comprising known CNTs, surpassing that of various widely employed robust materials, such as high-tensile steel and Kevlar. As a result, CNTs are employed as reinforcing constituents in composite materials, including plastics, as well as metal alloys, leading to the commercialization of several products [30]. Nevertheless, the potential for CNT composites to serve as ultra-high-strength load-bearing substances has not yielded adequate outcomes thus far. This is primarily attributed to the inadequate load transmission from the surrounding matrices to CNTs, stemming from the poor interaction between them [30].

In recent times, numerous research endeavors have focused on integrating CNTs into several substances to leverage their versatile properties. Instead of solely emphasizing composites' mechanical strength, attention has been directed towards utilizing CNTs' diverse attributes. For instance, the remarkable electrical features of CNTs, combined with their nano-dimensions, have garnered significant attention in electronics for constructing nano-electronic circuits [31,32]. Moreover, CNTs exhibit a decreased threshold for electric fields in field emission, surpassing that of the commonly utilized field emitters [33,34]. Consequently, there is ongoing dynamic research on CNTs for their exceptional performance in electron emission devices like electron microscopes, flat display panels, and gas-discharge tubes. Moreover, their ability to exhibit robust luminescence through field emission opens up promising avenues for their use in lighting components [32].

The utilization of CNTs in diverse biomedical applications has garnered significant attention, due to their aforementioned attributes, making them highly favorable contenders for the advancement of biomaterials [35]. Moreover, CNTs are able to be chemically modified to introduce distinct entities, such as functional groups, molecules, as well as polymers, to enhance their suitability for biomedical purposes. These modifications can enhance solubility, biocompatibility, and cellular responsiveness [36]. For this reason, CNTs are the most applied CBNs in biomedicine, particularly in sensing, drug delivery, cancer treatment, and tissue engineering. Their toxicity can be regulated through functionalization. Thus, they are considered ideal for biomedical applications compared to the other available CBNs.

The main biomedical applications of CNTs are presented in Figure 2 and are discussed in the following subsections.

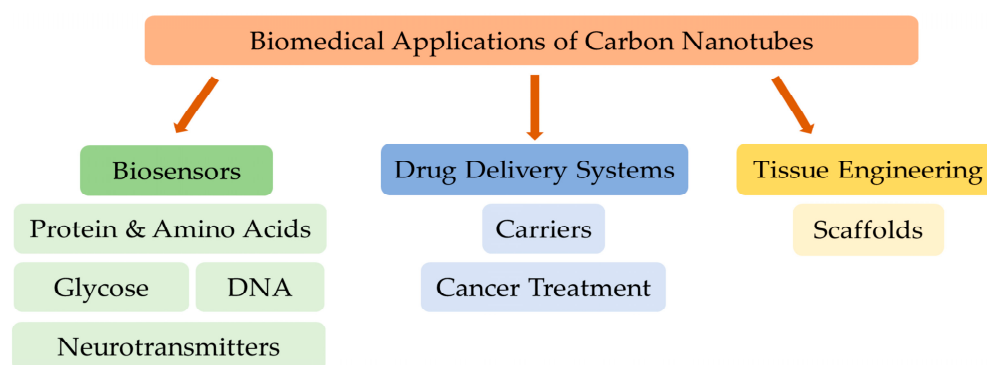


Figure 2. The main biomedical applications of carbon nanotubes.

2.1. CNTs as Biosensors

Given their remarkable attributes, CNTs have been recognized as innovative nanoprobess [37]. Their high aspect ratio, conductivity, chemical robustness, as well as sensitivity [38], along with their rapid electron-transfer rate [39], render them highly suitable for biosensing purposes. The fundamental aspect of CNTs-based biosensors involves the surficial immobilization of biomolecules, thereby enhancing recognition, as well as facilitating the signal transduction process. The biosensors are commonly classified as electrochemical and electronic CNTs-based biosensors, as well as optical ones, resting on

their target sensing and communication mechanisms. CNTs have gained significant acclaim as advantageous materials for enhancing electron transfer, making them well-suited for integrating electrochemical and electronic biosensors [40].

2.1.1. Sensing of Glucose

Several glucose biosensors utilizing CNTs in conjunction with glucose oxidase have been developed [26,41].

Compared to the first-generation sensor where the electrode and glucose oxidase were used [42], the second generation of glucose sensor incorporates an electron carrier within the enzyme electrode [43]. In this phase, the electron medium actively participates within the reaction, replacing the role of O_2 . The enhanced exclusivity and acuteness of the second-generation sensor have resulted in its commercial exploitation in glucose sensing. Enzyme-only sensors tend to be unsteady, as enzymes are susceptible to deactivation and detachment. In order to address this, enzyme immobilization has been identified as an effective approach to assure enzyme robustness and recyclability [44]. Additionally, leveraging the unique properties of the material can further enhance enzyme efficiency. For example, glucose oxidase can be anchored using polymers, as well as a vertical array of CNTs (VACNTs) [45]. Chemical vapor deposition (CVD) is utilized to modify the CNT wall with polyaniline (PANI) for fixing glucose oxidase (GOx), resulting in improved electrode stability and accelerated electron transfer. The sensor's range of linearity spans between 2 and 426 mM, having a detectable limit equal to 1.1 mM. Importantly, it is not affected by interference from uric acid and ascorbic acid. To overcome enzyme activity reduction or denaturation and to facilitate enzyme adsorption, metal or metal oxide nanoparticles are employed for enzyme immobilization. A $ZnFe_2O_4$ -CNTs-glucose oxidase composite has been fabricated for use in glucose sensing [46]. The as-mentioned sensor demonstrated stable catalytic efficiency for 20 days, as well as recyclability >5 times, benefiting from the $ZnFe_2O_4$'s-increased catalytic effectiveness, the enhanced surface area ratio of CNTs, and the elevated loading capacity of GOx.

An electrode consisting of Au nanoparticles/ MnO_x - VO_x /CNT/GCE was synthesized using electrochemical pulsed deposition by combining metal oxides [47]. MnO_x - VO_x was applied as a surficial modification of a glassy carbon electrode (CE) with CNT (CNT/GCE), while Au nanoparticles were deposited onto the oxide film. Glucose detection exhibited a linear range of 0.1–1.0 mM, and the detection limit was measured at 0.02 mM. Another approach involved the utilization of CNTs altered with platinum nanoparticles as electrodes for glucose detection [48]. The researchers initially formulated composite solutions comprising carboxyl functionalized MWCNTs and chemically derived graphene (CDG). These solutions were then deposited as thin films onto the gold electrode, followed by the deposition of Pt nanoparticles through an electrochemical process. The linear range for glucose detection was 0.5–13.5 mM, with a low detection limit (1.3 mM).

Ensuring long-lasting durability and optimal current density for continuous monitoring are crucial considerations for glucose sensors. The rate of alteration also impacts the efficiency of CNT materials. The presence of lignosulfonate can influence the composites' electrical effectiveness containing multi-walled CNTs (MWCNTs), subsequently affecting their electrocatalytic functionality [49]. The chemical alteration of glucose oxidase can enhance its efficiency and selectivity simultaneously. By combining glucose oxidase and MWCNTs modified with β -cyclodextrin, the sensor exhibited 95.8% stability in current retention over a span of 14 days [50].

In contrast to the conventional one-factor-at-a-time (OFAT) approach, several researchers have suggested the use of a design of experiments (DOE) approach to simultaneously maximize the current density and robustness [51].

The enzymes' chemical features are composed of proteinic molecules that are prone to denaturation when exposed to pH value modifications, temperature, and various environmental factors. This leads to decreased sensor stability and affects the effectiveness of detection. While physical immobilization helps preserve the enzymes, it does not

guarantee a reduction in activity. Enzyme leakage occurs when enzymes detach and access the solution, rendering them ineligible for involvement in the reaction. To address the issue of poor stability, a nGOx/N-CNTs-Chi/GCE (Chitosan/Glassy CE) sensor was developed [52]. A polymerization approach was suggested to enclose glucose oxidase in single-molecule enzyme nanocapsules (SMENs) in situ. The synthesized SMENs of GOx-substituted traditional glucose oxidase, improving enzyme activity preservation. In comparison to traditional CNTs, n-doped CNTs (NCNTs) were employed to enhance the catalytic conductivity, specific surface area, and biocompatibility. Another approach utilized glucose oxidase micro-particles instead of independent enzymes [53]. The relevant sensitivity persisted >86% for 9 days. Additionally, the issue of enzyme leakage was effectively addressed through the implementation of a hollow fiber membrane with a gradient structure. The range of linear detection spanned from 0 to 24 mM. The enzymes' catalytic efficiency was intimately linked to temperature. Microencapsulated phase change materials (MEPCMs) were employed to inhibit enzyme leakage and attain temperature control, thereby enhancing the enzyme-sensing performance at increased temperatures [54].

Several crucial aspects are considered during the development of enzymatic sensors to enhance robustness and sensitivity. Firstly, effective screening is carried out to identify enzymes with high activity. Secondly, efforts are made to maintain the activity and stability of the enzymes. Lastly, reliable fixation of the enzymes to the semiconductor is ensured. To address the aforementioned challenges, a third generation of glucose sensors resting on direct electron transfer (DET) has emerged. These sensors operate without the need for media or chemical reactions, enabling a direct transfer of electrons from glucose to the electrode. In one study, a GOx-Chit-CNT/ITO electrode was created by modifying the composite chitosan-CNTs onto an indium tin oxide (ITO) electrode [55]. Various solutions with diverse mass ratios of CNTs were prepared, and it was found that chitosan-CNT85 exhibited the optimal performance. In the long run, glucose detection biosensors are expected to be transformed into wearable devices for convenient in vitro sensing [56]. Another approach involved utilizing a dual enzyme system consisting of glucose oxidase and horseradish peroxidase in a CNTs-EVA composite membrane [57]. The glucose oxidase/horseradish peroxidase/CNT-EVA electrode was instantly employed for glucose detection in sweat. A non-intrusive glucose sensor utilizing two electrodes demonstrated superior performance compared to existing invasive glucose meters, presenting sensitivity equal to $14.45 \pm 2.97 \text{ mA mM}^{-1} \text{ cm}^{-2}$ [58].

Zhu and his team [59] employed non-woven fabrics made of CNTs (CNTFs) to detect glucose by incorporating glucose oxidase into a polyvinyl alcohol solution. In addition, Gaitán and co-researchers focused on the impact of surficial chemistry, as well as glucose oxidase-coated MWCNTs' structure in electrochemical glucose sensing [60].

Non-enzymatic sensors possess several advantageous features, such as long-lasting durability, low-cost, enhanced sensitivity, effective electron transfer, and strong electrocatalytic efficiency [61,62]. These benefits overcome the constraints of enzyme-based sensors, which are prone to instability and storage difficulties. Consequently, numerous research studies have been conducted [63–65], encouraging advancements in glucose sensor technology. Ideal glucose sensors should exhibit an extensive linear span, decreased limit of detection, and increased sensitivity. While enzyme-based sensors offer a broad linear range and sensitivity, their fixation and preparation present challenges. Moreover, enzyme stability issues can lead to discrepancies. Consequently, extensive studies are dedicated to the fabrication of non-enzymatic sensors to enhance their sensing capabilities. Non-enzymatic sensors typically involve the modification of electrodes with metal nanoparticles. For instance, an electrode composed of stacked cup CNTs (SCCNT) modified with nickel oxide (NiO) was utilized [66]. Another approach involved a bimetallic nanoparticle-altered electrode (Fe, Ni/CNTs/GCE) that harnessed the advantageous electrochemical features of Fe and Ni [67]. The impact of an NiO layer's thickness on sensing has been also investigated. Pt nanoparticles have been employed to modify MWCNTs, with the functionalization of 3-mercaptophenylboronic acid (3MPBA) attached to the platinum particle surface [68].

The linear range of sensing was 0–10 mM, presenting a detection limit equal to 4.5 mM. Moreover, PdNi@f-MWCNT/GCE was produced by modifying functionalized MWCNTs with Pd and Ni nanoparticles [69]. This particular sensor presented a linear range of 0.01–1.4 mM, and an exceptionally decreased LOD of 0.026 mM.

Transition metal oxide/carbon hybrid electrodes offer enhanced sensitivity and improved electron transfer efficiency. A fluorescent nanosensor utilizing a hybrid nanocomplex of cobalt and CNTs (Co-CNT) was documented [70]. This sensor employs unlabeled and enzyme-free fluorescence ratio approaches for the simultaneous sensing of H₂O₂ and glucose. The detection sensitivity for H₂O₂ reached 150 nM, while for glucose, it was also 150 nM. A straightforward approach was proposed for the preparation of a cobalt-containing electrode using melamine foam [71]. The glucose sensor employing the CoeCo₃O₄/CNT/CF/GCE electrode exhibited a detection range of 1.2 mM to 2.29 mM. The catalytic properties of binary metal composites outperformed those of metal oxides, making Ni and Co-based alloys with functionalized MWCNTs (f-MWCNTs) highly effective in glucose detection [72]. The combination of cobalt phthalocyanines (CoPc) with single-walled CNTs (SWCNTs) and reduced graphene oxide (rGO) allowed for a rapid response, responding within 1.2 s to changes in glucose concentrations in saliva [73].

With a focus on nanomaterials, researchers have become increasingly dedicated to nanostructured design and novel approaches [74]. An electrode composed of Co₃O₄/NCNTs, based on a metal–organic skeleton, was created for detecting glucose and H₂O₂ [75]. A unique dodecahedral CNT array was achieved through the carbonization of a specific zeolitic imidazolate framework material (ZIF-67), with Co₃O₄ nanoparticles encapsulated within the CNTs ends. Furthermore, to enhance the robustness and catalytic effectiveness of binary metal composites, porous carbon materials were coupled with a CoCu organic framework catalyst, resulting in the development of nitrogen-doped CNTs@MOF@CoCu biosensors [76].

Cu₂O presents itself as a favorable option for a metal oxide electrode. Along with material selection, the utilization of various nanostructures can enhance the performance of detection. For instance, a non-enzymatic sensor employed a copper nanowire-bilayer (CuNW-CNT-BL) configuration [77]. The sensor presented a linear range of 10–2000 mM, characterized by a limit of quantification equal to 1.1 nM and a low detection limit equal to 0.33 nM. Moreover, this sensor demonstrated selectivity towards uric acid, dopamine, and various molecules. In the Cu₂O-MSs/S-MWCNTs/GCE composite electrode, the Cu₂O material exhibited a spherical design [78]. By incorporating sulfur atoms, the electrocatalytic performance of CNTs was enhanced. The linear range spanned from 4.95 mM to 7 mM, with a comparatively decreased detection limit (1.46 mM). In order to enhance manufacturing accessibility, researchers have explored novel approaches, like microfluidic technology, which enables the regulation and sensing of complex fluids at the micro-scale. A decreased-fluid-consuming sensor built upon Pt-Ni NPs-MWCNTs/screen-printed CE was also introduced [79]. Additionally, microfluidic cloth-based analytical devices (mCADs) with multiple empty channels have been synthesized for enzyme-free glucose sensing [80]. The utilization of a method named photoelectrochemical (PEC) monitoring involved CdS quantum dots and poly (dimethyl diadly ammonium chloride)-functionalized MWCNTs, enabling the efficient and straightforward detection of glucose in saliva possessing a decreased detection limit (15.99 nM).

2.1.2. Sensing of DNA

Deoxyribonucleic acid (DNA) plays a crucial role as a genetic information carrier in organisms, and its analysis can aid in the timely identification and management of diseases [81]. Cancer, a major cause of mortality, benefits greatly from early detection, increasing the chances of successful treatment. For instance, urokinase plasminogen activator (uPA) serves as a biomarker for distinct categories of prostate cancer [82], while alpha-fetoprotein (AFP) is associated with liver cancer [83]. Colorectal cancer diagnosis could be facilitated through detecting the glycoprotein CEACAM5 [84]. However, conventional

methods, like polymerase chain reaction (PCR), for the clinical detection of diseases have limitations, including the risk of contamination [85]. Consequently, there is a requirement for economical and exceptionally sensitive methods for biomolecule sensing in medical applications. CNT-based biosensors have emerged as promising candidates [86]. Over time, notable strides have been taken in the evolution of DNA sensors based on CNTs, employing both optical [87] and electrochemical techniques [88] in the medical field.

For instance, HIV is a contagious illness that devastates the immune system and carries a high fatality rate. Detecting the infection at an early stage can impede its progression. A wearable device utilizing a paper electrode MOF-based sensor exhibited remarkable tensile attributes and demonstrated promising prospects. Furthermore, it showcased excellent detection capabilities [89]. The detection range spanned from 10 nM to 1 mM, with an impressively low limit of detection (0.13 nM). The unexpected emergence of COVID-19 [90] and the escalating infection incidents have presented significant difficulties in swiftly identifying nucleic acids. By utilizing ss-DNA-modified SWCNTs, its binding with the RNA of the virus target becomes possible. Moreover, the ss-DNA can be substituted to capture other viruses, making it a versatile and adaptable solution [91].

CNTs were modified with extremely spinning Fe atoms to perform label-free cancer gene testing repeatedly [85,92]. A Pt-interdigitated electrode surface was coated with Au/Fe₂O₃ magnetic nanoparticles and CNTs prepared from two different types of nanoparticles for influenza virus detection using an altered DNA probe approach [93]. A label-free DNA sensor with a 3D spatial framework, consisting of four DNA types, was developed [94]. Furthermore, CNTs selectively integrate with DNA that was immobilized on an ITO electrode, and the detection of DNA was achieved using the electrochemical luminescence approach [95]. In this procedure, CNTs had a vital role in suppressing electrochemical reactions and augmenting electro-chemiluminescence signals. In order to surpass the complexity of fabricating CNTs electrodes on supporting matrixes, like polymer-based and glassy CE, self-standing three-dimensional CNTs structures have been introduced. Prakash and his team recently developed a standalone CNTs aerogel electrode for exclusive DNA sensing, achieving a detection limit equal to 1 pM [89]. The as-mentioned electrode, imposed with a multi-directional CNT network, exhibited favorable electrical features, enabling increased sensitivity in DNA sensing. A sensor using AuNPs/Chit-MWCNTs/GCE was prepared with methylene blue as a procedure indicator [96]. CNTs/chitosan, Au nanoparticle thin films, and ss-DNA probes were successively altered on the surface of a glassy CE. Additionally, polymer- and MWCNT-modified electrodes were used in DNA, drugs, and interaction sensing by modifying double-stranded DNA (ds-DNA) [97].

Nano-biosensors utilizing a field-effect transistor (FET) offer remarkable advantages, including high label-free detection specificity. In this approach, the probe-ss-DNA is immobilized within the interval between the nanotube and the Au-gate, forming a double-stranded DNA structure when coupled with the target ss-DNA [98]. However, the substrate used in traditional FETs can hinder the sensing performance. To overcome this limitation, a suspended CNT FET was proposed, which separates the CNTs from the substrate and exhibits a decreased detection limit [99]. Liang and his team further enhanced the sensitivity by optimizing the FET structure, introducing a Y₂O₃ thin film between the Au nanoparticles and the CNT layers [100]. Additionally, nitrogen-containing CNTs with a chiral structure can detect DNA damage, which is associated with aging, gene mutation, and carcinogenesis, providing insights into pathogenesis [101]. Nanobridges developed by wrapping MWCNTs with elongated DNA strands can detect DNA damage resulting from various free radicals [102]. Another innovative approach is the CNT thin-film-transistor (CNT-TFT) sensor, which incorporates peptide electrodes at the ends of CNTs' channels and functionalizes CNTs. This enables the quantitative detection of cancer cell genes, expanding the range of sensing and reducing the detection limit [103].

2.1.3. Sensing of Proteins and Amino Acids

Proteins, which are involved in various physiological activities, play a crucial role in body metabolism [104]. Despite the use of multiple techniques for protein detection, there is still a need for rapid and simple sensing methods [105,106]. Recently, CNTs have been utilized in point-of-care protein sensing, with the aim of increasing sensitivity and reducing the response time. Janssen and co-researchers designed a label-free biosensor based on CNTs to detect bovine serum albumin, which demonstrated a detection limit of 2.89 ng/mL [107]. This biosensor incorporates cellulose paper, SWCNTs, and BSA-specific antibodies, utilizing the principle of electrical percolation for the simplified and rapid quantification of BSA. Additionally, Huang et al. introduced an Fe₃O₄ nanoparticle-CNT-based biosensor for the visual detection of Rabbit IgG without complicated sample processing [108]. The biosensor employs Fe₃O₄ nanoparticles decorated on the surface of magnetized CNTs, which are altered with specific antibodies. This approach favored the immediate sensing of target proteins in whole blood possessing a low detection limit (10 ng/mL). Such low-cost and visual protein detection methods hold promise for clinical diagnosis. Furthermore, a redox probe-free electrochemical biosensor was developed by incorporating copper nanoparticle-doped CNTs as the electroactive film and a plastic antibody used as the bio-recognition constituent [109]. The biosensor eliminated the need for a redox probe-reading stage in conventional electrochemical detection. It exhibited a detection limit equal to 1.0 ng/mL for protein detection, and its application in serum samples suggests its potential for direct sample readings during in situ biomedical analysis.

CNT-FET biosensors employing the chemo-dosimetric detection principle have been fabricated for cysteine's detection by [110]. The molecular interaction among the selective chemo-dosimeter and cysteine induces a chemical alteration, affecting the surficial distribution of charge of CNTs and triggering electrical responses in the CNT-FET. The acquired data demonstrate that the as-mentioned biosensor enabled label-free, selective, and highly sensitive cysteine sensing (LOD = 0.45 fM). Additionally, the interaction between serine amino acid and CNTs was investigated [111]. The chirality of serine amino acid significantly influences CNTs' dielectric features, proposing the CNT-based sensors' potential to discriminate chiral molecules. Furthermore, a dual-signal electrochemical biosensor was constructed using chiral CNTs coupled with platinum nanoparticles and cyclodextrin [112]. This biosensor exhibited the ability to differentiate among amino acids, specifically between tyrosine and tyrosine, presenting the limits of detection equal to 0.107 nM and 0.133 nM, respectively.

2.1.4. Sensing of Neurotransmitters

Neurotransmitters like dopamine, acetylcholine, and norepinephrine are crucial chemical components involved in cell-to-cell communication [113]. Acetylcholine, for instance, is related to the nervous system and is linked to the increased threat of Alzheimer's disease [114]. Epinephrine contributes to regulating blood pressure and heart function [115]. Dopamine serves as a hormone and neurotransmitter, and insufficient levels of it can cause conditions like Parkinson's disease and mental dysfunctions. Typically, neurotransmitters are absent in isolation within the body but exist alongside other biomolecules like ascorbic acid, uric acid, and tyrosine [116], which are crucial for preserving normal physiological function. To enable the simultaneous sensing of dopamine, ascorbic acid, and uric acid without interference, a green reduction process was employed using Pelargonium graveolens extract to reduce hydroxylated MWCNTs [117], followed by hybridization with Pb nanotubes. The detection platform involved modifying a graphite electrode through the hybridization of reduced carboxylated MWCNTs and Pd nanotubes.

Guan and his team conducted a hydroxylation process on both SWCNTs and MWCNTs in order to acquire the MWCNT-COOH/SWCNT-OH-modified glassy CE sensor [118]. The arrangement of the two types of nanotubes in a staggered distribution created a simple grid structure, which facilitated electron mobility. Metal-based electrodes incorporating CNTs are commonly used in electrochemical sensors. For instance, GC/Ni-CNT electrodes

have been employed to sense dopamine and adrenaline [119]. PPy has been extensively utilized in biosensor fabrication, and in this case, sodium dodecyl sulfate was used to modify the sensor, resulting in the overoxidized PPy/sodium dodecyl sulfate-modified MWCNT electrode [120]. This sensor demonstrated high sensitivity in detecting DA while minimizing interference from glucose and other substances. Another innovative approach involved the development of a molecularly imprinted polymer (MIP)/MWCNT/graphene aerogel composite electrode (MIP/MWCNT/GAs/GCE), where the MIP was created through electro-polymerization of pyrrole [121]. Initially, MWCNT/graphene aerogels were synthesized, while the resulting mixture was applied onto the glassy CE surface using drop casting. Furthermore, a novel type of graphene quantum dots was fabricated from glucose [122], and these dots were integrated with modified MWCNTs to enable the sensing of dopamine. The linear detection range was 250 nM–250 mM, with a detection limit equal to 95 nM. Gupta et al. suggested a CNT array-based method for dopamine detection, utilizing increasingly densified CNTs fibers [123]. The range of detection of this approach was 1 nM–100 mM, with an LOD of 32 pM.

Uric acid and dopamine are present alongside ascorbic acid in living organisms, making their detection crucial. Electrochemical techniques are extensively employed in neurotransmitter detection because of their benefits, including heightened sensitivity, rapid response, and cost effectiveness [124]. To detect dopamine and uric acid, a sensitive and selective biosensor was developed using Au nanoparticle-loaded MWCNTs altered with PCA [125]. Serotonin, comprising an essential gastrointestinal neurotransmitter, has also garnered significant attention in medical and life sciences. A remarkably sensitive electrochemical sensor for serotonin sensing was developed by using Cu₂O encapsulated in CNTs with platinum nanoparticles [126]. The as-described sensor exhibited exceptional electro-catalytic performance towards serotonin oxidation, achieving a low detection limit (3 nM). Furthermore, MWCNTs modified with capsaicin were employed to alternate glassy CEs towards the quantitative sensing of xanthurenic acid, dopamine, epinephrine, ascorbic acid, and uric acid at a micromolar level [127]. A ternary nanocomposite of N-doped CQDs@Fe₂O₃/MWCNTs enabled the sensing of uric acid and xanthine, characterized by detection limits equal to 0.106 mM and 0.092 mM, respectively [128]. The practical applicability of this nanocomposite was demonstrated in a human urine sample.

2.2. CNTs for Drug Delivery

CNTs have gained tremendous attention as an efficient means of transferring several drug molecules into vital cells due to their unique structure, which facilitates non-intrusive permeation across biological membranes [129]. Typically, drug molecules are anchored to CNT sidewalls either through covalent or non-covalent bonds with functionalized CNTs [130]. Nevertheless, each of these methods comes with its own set of benefits and limitations. Covalent interactions provide stability to the drug-loaded CNTs both outside and inside cells, but they lack controlled drug release within the cancer cells' cellular micro-environment that comprises a limitation of the drug delivery system. On the other hand, non-covalent interactions allow for gradual drug release in the acidic tumor sites' conditions but suffer from robustness issues at extracellular pH values [131]. Therefore, utilizing the inner hollow cavity of CNTs for drug loading offers an optimal solution by providing drug isolation from the physiological environment. To overcome these challenges related to drug release, researchers have investigated various external stimuli, either individually or in combination, including temperature, electric field, and light. For instance, a chitosan-modified CNT with a thermosensitive polymer (NIPAAm) and NIPAAm-co-Vim was developed and encapsulated in bovine serum albumin at body temperature (37 °C) to evaluate the temperature-responsive discharge of biomolecules. Bovine serum albumin was released just above the lower critical solution temperature of polyVIm (38–40 °C) [18,132].

Shi and co-researchers [133] employed an electric field to release ibuprofen from a hybrid hydrogel that consisted of sodium alginate, bacterial cellulose, and MWCNTs.

Estrada and his team [134] investigated the temperature and NIR light-responsive discharge of methylene blue from a hydrogel composed of MWCNTs and k-carrageenan. CNTs have been utilized as carriers for a range of drugs, such as doxorubicin, paclitaxel, docetaxel, and oxaliplatin, in both in vitro and in vivo cancer therapy. Modified CNTs for drug delivery purposes have been thoroughly researched, developing strategies to enhance water solubility and entrap drug molecules [135]. Jain and Mehra, in 2015, compared the cancer-targeting abilities of doxorubicin-loaded folic acid and estrone-anchored PEG-modified MWCNTs in tumor-bearing mice, showing improved survival with the use of DOX/ES-PEG-MWCNTs [136]. Calcium phosphate-crowned MWCNTs as nanocapsules for intracellular drug transfer have been also explored, demonstrating pH-stimulated dissolution and controlled discharge within lysosomes [137]. Xu and co-researchers [138] developed an amine-PEG-modified polydopamine-CNT nanosystem for drug transfer to enhance the biocompatibility of CNTs. Finally, Picaud et al. [139] carried out theoretical investigations on the loading and discharge of cisplatin from CNTs.

2.3. CNTs for Cancer Treatment

Since cancer remains a major health issue, several studies have focused on increasing the understanding of the molecular mechanisms that mediate tumorigenesis [140], oxidative-stress [141], and cellular senescence and aging [142], and others have tried to provide alternative therapeutic approaches [15–18].

CNTs possess versatile properties that make them highly valuable in biomedical applications. They are extensively utilized as carriers for anticancer drugs, genes, and proteins in chemotherapy, making them a promising option [143]. Additionally, their enhanced absorption of NIR light enables effective photothermal therapy. Su and his team [144] synthesized iRGD-PEI modified MWCNTs, which were then conjugated with candesartan. This functionalized nanocomposite targeted $\alpha v\beta 3$ -integrin and AT1R receptors on tumor endothelium and lung cancer cells, respectively. The combination of candesartan with [pAT (2)] resulted in the downregulation of VEGF and the effective inhibition of angiogenesis [144].

Another study by Zhang and his team [19] involved the design of a nanocomposite consisting of DOX-loaded MWCNTs and magneto-fluorescent CQDs for combined chemo- and photothermal therapy [19]. The negatively charged GdN@CQDs-MWCNTs enabled the bonding with positively charged DOX molecules. This nanocomposite exhibited strong NIR irradiation absorption. In in vivo photothermal therapy experiments, laser irradiation of tumor sites led to a temperature increase of 51.8 °C in the mice provided with GdN@CQDs-MWCNTs/DOX-EGFR, while the control group showed no significant change in temperature. The localized heating effect facilitated the release of DOX and led to successful photothermal therapy, as demonstrated by a reduction in the tumor volume.

In a recent study, a nanosystem consisting of TAT-chitosan functionalized MWCNTs loaded with DOX was utilized by Dong et al. [145] to combine chemo and photothermal therapy. To promote apoptosis in cancer cells, a PEG-coated CNT-ABT737 nanodrug was employed, which targeted the mitochondria [146]. The discharge of the nanodrug in the cytosol induced apoptosis in lung cancer cells by disrupting the mitochondrial membrane. In vivo, this approach showed impressive therapeutic effectiveness, further enhanced by the localized heating effect triggered by NIR light irradiation. Another development involved an Au nanoparticle-coated CNT ring that exhibited exceptional Raman and optical signal characteristics, leading to enhanced photoacoustic signal and photothermal conversion behavior [147]. The as-mentioned composite indicated promising results in image-guided cancer therapy. Additionally, the photothermal cancer-killing efficacy was improved by the surface plasmon resonance absorption of Au in SWNT-Au-PEG-FA nanomaterials [148].

2.4. CNTs for Reinforcing Tissue Engineering Scaffolds

The utilization of CNTs in composite reinforcements of tissue engineering scaffolds has predominantly emphasized increasing their mechanical attributes [149]. Traditional scaffold materials, like hydrogels and fibrous scaffolds, are commonly pliable to emulate the inherent rigidity of tissues, resulting in decreased structural robustness and support. However, by introducing CNTs into the as-mentioned materials, their mechanical properties can be significantly improved. For instance, Shin et al. conducted a study where they incorporated CNTs into gelatin hydrogels, resulting in notable enhancement of tensile strength [150]. Similarly, Sen and his team illustrated the enhanced tensile strength of CNTs-reinforced polystyrene and polyurethane fibrous membranes [151]. Lately, researchers have explored the versatile capabilities of CNTs in tissue scaffold engineering, particularly their ability to confer electrical conductivity. Most biomaterials utilized in tissue engineering are electrically insulating due to their composition of non-conductive polymers [152–154]. Nevertheless, specific applications, like neural and cardiac tissues, require conductive scaffolds to efficiently transfer electrical signals and support optimal electro-physiological operations. For instance, Kam and co-researchers conducted experiments where they employed electrical stimulation to neural stem cells cultured on CNT–laminin composite thin films, resulting in increased activity potential and the functional neural networks' differentiation [153]. Similarly, Shin and his team cultured cardiomyocytes on CNT-reinforced gelatin hydrogels, which exhibited increased electro-physiological efficiency and eventually produced functional cardiac tissue [155]. The aforementioned studies demonstrate the capability of CNTs to successfully supply electrical conductivity to previously non-conductive biomaterials.

2.5. Cytotoxicity of CNTs

Although CNTs have been successfully applied in biomedical engineering, there is a growing apprehension regarding their harmlessness. Several contemporary *in vitro* studies have documented the elevated cytotoxicity of CNTs, which can be attributed to factors such as cellular uptake, aggregation, and induced oxidative stress [156–158]. The contradictory results regarding the CNTs' biocompatibility primarily arise from variations in their properties (such as size, surficial characteristics, and functionalization), as well as the different testing protocols employed (including *in vitro* and *in vivo* studies, cell type, tissues, and animals tested). Furthermore, the insufficient elimination of metal catalysts utilized during CNT preparation has been associated with increased cytotoxicity [157]. The majority of *in vivo* studies have indicated the minimal toxicity of CNTs and their clearance through renal pathways, although some accumulation in organs like lungs, liver, and spleen has been observed, potentially leading to inflammation [156–158]. Nevertheless, cytotoxicity appears to demonstrate increased fluctuation and significance at the cellular level, as indicated by numerous *in vitro* cell culture studies [159,160]. Given the expanding use of CNTs in diverse biomedical fields, it is crucial to conduct comprehensive biological evaluations considering the various chemical and physical properties of CNTs to ascertain their pharmacokinetics, cytotoxicity, and optimized dosages. Nonetheless, a plethora of research studies have demonstrated that functionalizing CNTs utilizing biocompatible polymers or surfactants can effectively minimize toxicity and prevent aggregation [161,162].

3. Graphene and Graphene Oxide

Graphene, a novel nanomaterial, has gained significant attention in recent years. Geim and Novoselov's groundbreaking research introduced a facile technique for isolating graphene from graphite through exfoliation and uncovered its remarkable electrical attributes [163,164]. While graphene and CNTs share comparable electrical, optical, and thermal characteristics, the unique 2D graphene's atomic structure allows for more wide-ranging electronic properties. Notably, the presence of the quantum Hall effect and massless Dirac fermions accounts for its low-energy charge excitation in ambient conditions, as well as the optical transparency across the NIR and visible light spectrum. Furthermore,

graphene exhibits structural strength combined with exceptional flexibility, making it an appealing choice for producing thin and flexible materials [165,166].

It was expected that those characteristics would be exploited in biomedical applications, and thus graphene and graphene oxide are used in the sensing of nucleic acids, as a basic component of drug delivery systems, focusing on cancer treatment. Their use in biomedicine is not as wide as that of CNTs, and concerns about their toxicity still limit their biomedical applications.

3.1. Graphene and Graphene Oxide as Biosensors

Graphene oxide exhibits dynamic interactions with the probe and can transduce specific responses towards target molecules. This transmission procedure utilizes fluorescence, Raman scattering, as well as electrochemical reactions. As a result, graphene oxide is widely employed as a biosensor [146]. Graphene-based nanomaterials have been intensively utilized towards the electrochemical detection of single- and double-stranded DNA with high selectivity [167]. The superior graphene's electrochemical features, coupled with the robust ionic interaction among the negatively charged -COOH groups and positively charged nucleobases, as well as the sturdy π - π stacking among nucleobases and the carbon structure, contribute to its exceptional sensitivity. Reduced graphene nanowires have been introduced as biosensors for detecting DNA bases through the oxidation signals of individual nucleotide bases [168]. The as-mentioned nanowires exhibited remarkable robustness, presenting only a 15% variation in oxidation signals during the increment of the differential pulse voltammetry up to 100 scans. Lately, Cheng and colleagues developed -COOH-modified graphene oxide and polyaniline-altered graphene oxide to effectively detect DNA through differential pulse voltammetry within the range of 1×10^{-6} – 1×10^{-14} [169]. Moreover, Ping and colleagues developed a label-free DNA biosensor by employing graphene field-effect transistors that were modified with single-stranded probe DNA. The as-described ultra-sensitive biosensor demonstrated an extensive analytical range possessing a limit of detection equal to 1 fM for 60-mer DNA oligonucleotides [170]. In another study, researchers fabricated a device using physical vapor deposition, incorporating Au nanoparticle-decorated graphene field-effect transistors. Thiol-modified Au NP-Gr-FETs successfully detected DNA (LOD = 1 nM) and presented increased specificity of non-complementary DNA [171]. Furthermore, a single-layer graphene-based FET biosensor achieved the detection of extremely low DNA concentrations (10 fM) [172]. Kim and his team [173] synthesized a graphene surface-modified vertically aligned Si nanowire for the sensitive and selective detection of oligonucleotides.

They initiated the surficial attachment of oligonucleotides onto the silicon nanowire arrays, which then underwent hybridization with the probe, leading to an amplification of the biosensor's response. The biosensor current was observed to increase from 19% to 120% as the DNA concentration ranged from 0.1 nM to 500 nM [173]. The process of single- and double-stranded DNA being absorbed and desorbed on graphene oxide was investigated by Park and co-researchers [174]. It was found that single-stranded DNA exhibited a higher affinity for graphene oxide, while double-stranded DNA showed lower affinity. Additionally, recent studies have indicated that the adsorption of DNA on graphene oxide depends on the length of the DNA molecules [175]. Prabowoa and his team [176] proposed a novel approach for the sensing of Mycobacterium tuberculosis DNA hybridization utilizing graphene integrated onto a surface plasmon resonance-sensing chip. The utilization of graphene oxide-based nanomaterials towards glucose detection has been rapidly advancing [177]. A device incorporating graphene electrodes with glucose oxidase demonstrated excellent selectivity and increased glucose sensitivity, characterized by a low limit of detection (0.5 mM) [178]. Reduced graphene oxide with $\text{C}_6\text{H}_5(\text{CH}_2)_3\text{COOH}$ as a glucose-binding linker has been also developed. The as-proposed sensor device exhibited the capability to detect glucose levels ranging from 1 to 4 mM [179]. In addition, researchers have successfully developed a more durable and reusable graphene-bismuth composite device that demonstrated impressive glucose detection capabilities. It covered

a broad linear range (1–12 mM) with an enhanced sensitivity and a reduced limit of detection (0.35 mM) [180]. A carbon-functionalized graphene/fullerene C60 composite was successfully fabricated for glucose detection (0.1–12.5 mM), with an LOD = 35 μ M and enhanced sensitivity [181]. Hydroxyapatite 1D nanorods on a graphene nanosheet-altered glassy CE were fabricated, demonstrating excellent sensing capabilities across a broad concentration range (0.1–11.5 mM), with an LOD = 0.03 mM and increased sensitivity [182].

3.2. Graphene and Graphene Oxide for Drug Delivery

Graphene, with its enhanced specific surface area and abundant π electrons, has proven to be a suitable carrier for drug delivery. Wang and his team [183] grafted a substantial amount of doxorubicin onto graphene coated with a phospholipid monolayer and observed sustained release of DOX, particularly at extremely low pH values as compared to a basic pH [184]. Another approach involved loading DOX onto a graphene sheet through physisorption, accompanied by surficial alteration with PEG-NH₂ to increase robustness and compatibility within a biological environment [185]. Nandi and co-researchers successfully loaded both DOX and indomethacin onto PNIPAM (PolyN-isopropylacrylamide)-grafted graphene oxide using π - π interactions, H-bonding, and hydrophobic interactions [186]. PNIPAM was covalently grafted onto graphene oxide through free radical polymerization to achieve the controlled discharge of DOX, particularly in an acidic pH environment, where increased hydrophilicity, higher DOX solubility, and minimized hydrogen bonding interactions were observed between the DOX and the grafted graphene oxide surface. Xu and his team [187] loaded paclitaxel onto graphene oxide-PEG through π - π stacking and hydrophobic interactions, achieving a loading efficiency of 11.2 wt%.

Zhao and co-researchers [188] developed well-defined PMMA-coated polyethylene glycol-functionalized graphene oxide nanoparticles, which displayed outstanding dispersion in PBS solution and acted as a proficient drug delivery system. The PMMA coating enabled the controlled release of DOX, with reduced release in healthy tissues and accelerated discharge in tumor tissues upon the presence of glutathione as a reducing agent. In another study, DOX-loaded graphene oxide was synthesized, followed by alteration with hyaluronic acid to enhance stability and serve as a targeting agent for the HAGO-DOX nanohybrid [189]. Recently, Mahdavi et al. [190] conducted simulation research on the loading and release of DOX in graphene oxide at several pH values. A DOX-loaded PEI, biotin, β -Cyclodextrin-conjugated, reduced-graphene oxide nanosystem was also developed, where the PEI and biotin contributed to increased robustness and targeting effectiveness, respectively. The β -Cyclodextrin served as a host molecule to accommodate guest molecules, including water-insoluble anticancer drugs [191].

3.3. Graphene and Graphene Oxide for Cancer Treatment

The unique features of graphene and graphene oxide also render them ideal candidates for various cancer therapy applications [177]. Yu and his team developed GO functionalized with α v β 6-targeting peptide and coated with the photosensitizer HPPH. This functionalized graphene oxide triggered dendritic cells and efficiently hindered tumor growth and lung metastasis via promoting the infiltration of cytotoxic CD8⁺ T lymphocytes into tumors [192]. A combination of reduced graphene oxide and gold nanorods loaded with doxorubicin (DOX) was engineered as a vehicle for simultaneous photothermal therapy and chemotherapy. The discharge of DOX was significantly enhanced by the photothermal heating effect induced by NIR light and the acidic conditions within the micro-environment of the tumor [193]. The compact arrangement of Au nanoparticles on graphene oxide resulted in an increased absorption peak. Under laser light, the Au nanoparticle-graphene oxide system exhibited a maximum temperature enhancement equal to 23.2 $^{\circ}$ C [132]. Cheon and his team [194] proposed a novel approach for combined chemo- and photothermal therapy for brain tumors using a DOX-loaded bovine serum albumin-modified graphene sheet.

Su and co-researchers [195] devised a novel material comprising sponge-like carbon grafted with dual chemotherapeutics and supported lipid bilayers on graphene nanosheets altered with a tumor-targeting protein. The as-described composite exhibited remarkable accumulation at the tumor site, resulting in the effective suppression of xenograft tumors within 16 days.

Shao and his team [196] fabricated a polydopamine-functionalized reduced graphene oxide coated with mesoporous silica and subsequently modified with hyaluronic acid to facilitate DOX loading. This system demonstrated pH-dependent and NIR-triggered release of DOX, making it an efficient chemo-photothermal agent. In a study by Dai and co-researchers [197], a smart material composed of TiO_2 - MnO_x -conjugated graphene composite was synthesized for tumor elimination purposes.

3.4. Cytotoxicity of Graphene and Graphene Oxide

Graphene and graphene oxide, similar to CNTs, have limitations in their biomedical application given their potential toxicity. Ou and his team [198] extensively discussed graphene's toxicity in various organs. Numerous studies have examined graphene's toxicity in both animals and cells [199]. Several factors, including the concentration, size, surface properties, and functional groups, have been identified as crucial in influencing its toxicity in biological systems [200]. Li and co-researchers [201] reported that when GLC-82 cells were incubated with graphene oxide (100 mg/L) for 24 h, it induced the development of reactive oxygen species (ROS) and resulted in toxicity. To mitigate the noxious effects of graphene oxide in distinct biomedical applications, researchers have explored the modification of graphene sheets by coating them with biological molecules, as demonstrated in a study on reducing the toxic effects of graphene through a blood protein coating [202].

4. Graphene Quantum Dots (GQDs)

Quantum dots (QDs) are semiconducting nanoparticles with unique properties, and there is increasing interest for their various applications [203]. Graphene quantum dots (GQDs) are a very promising material in nanomedicine since they combine high biocompatibility and low cytotoxicity in parallel with fluorescent potential. Thus, they are ideal in diagnostics and therapeutics, and recently, in theragnostics.

4.1. GQDs as Biosensors

In recent times, considerable advancements have been made in the field of biosensors, particularly in the utilization of graphene quantum dots (GQDs), which can detect biomacromolecules with high selectivity and sensitivity. GQDs exhibit outstanding chemical and optical properties, such as photoluminescence and electrochemiluminescence, which are key properties for biosensors used in clinical analysis and diagnosis [203]. Researchers [204] have developed a DNA detection approach relying on the fluorescence sensing technique called Fourier resonance energy transfer (FRET), using reduced graphene quantum dots (GQDs) functionalized with DNA probes. They created a conjugate of phosphorylated peptide-GQD coordinated with Zr^{4+} , which was capable of detecting casein kinase II (CK2) at concentration as low as 0.03 mL^{-1} [205]. Another group [19] developed a glucose sensor by fabricating graphene quantum dots (GQDs) with pyrene-1-boronic acid. They observed a shift in Dirac voltage as they increased the concentration of pyrene-1-boronic acid, which indicated there was a strong relation between the PBA concentration and glucose sensitivity. Another reason that this functionalization proved to be successful was the increase in the relative capacitance with higher glucose concentrations. In a separate study, an electro-chemi-fluorescent nanofiber composed of polyvinyl alcohol (PVA) and GQDs was created for the highly sensitive and selective detection of both H_2O_2 and glucose [178]. The mechanism relies on the incorporation of glucose oxidase into the composite, leading to the generation, which can be detected by the fluorescent GQD. Xi and colleagues designed a hybrid material based on N-doped GQDs and palladium nanoparticles for the detection of

many different cancer cells [206]. These types of sensors can become instrumental in the early detection of cancer.

4.2. GQDs for Drug Delivery

Very often, drug developers face challenges when designing new drug molecules due to their bad stability in the body and also the need for more targeted delivery. Graphene quantum dots are being investigated as potential drug delivery systems thanks to their unique properties, such as extremely small size and an oxygen-rich surface. Also, GQDs are fluorescent, which allows for monitoring the delivery of the drug to the targeted tissue [207]. As a result, there are numerous studies using GQDs for drug delivery. For instance, Tian and his team created a zeolite imidazolate framework embedded with graphene quantum dots as a delivery system for doxorubicin. The system released the drug only in acidic conditions, which enabled the targeted release of this anticancer drug to the low pH environment of tumor sites [208]. In another method, intracellular drug delivery and the real-time monitoring of drug release were achieved through DOX-loaded aptamer/GQD-capped fluorescent mesoporous silica nanoparticles. The system can be triggered by the adenosine triphosphate molecule, which is present in high doses in cancer cells and releases the loaded anticancer drug, providing targeted release and monitoring [178]. Leveraging the notable physicochemical properties of GQDs, Wei and coworkers developed a graphene quantum dot system conjugated with Cy5.5 dye using a cathepsin D-responsive peptide for the delivery of doxorubicin [209]. They reported improved therapeutic performance both in laboratory tests (in vitro) and live subjects. The result was attributed to better tissue penetration and high cellular uptake. In their study, they used confocal laser scanning microscopy (CLSM). They detected blue fluorescence from the cells that were treated with the conjugate, while the absence of visible fluorescence from Cy5.5 was promising for the biocompatibility of the conjugate. The release of the therapeutic molecule was detected thanks to its green fluorescence. This signal can be used in vivo to monitor the release of the anticancer drug into the cancer tissue. Nigam and his team (2014) designed a targeted drug delivery nanocarrier, which included GQD-conjugated gemcitabine-loaded human serum albumin (HSA). Albumin facilitated the transportation of gemcitabine to tumor cells using the gp60 pathway [210]. In another study, Iannazzo and his team [211] designed a different nano-system for targeted anticancer drug delivery using biotin-conjugated graphene quantum dots and loaded them with doxorubicin. Sui and coworkers created a nanoconjugate of cisplatin and GQD for cancer treatment. GQDs that facilitated cellular uptake and cisplatin that interacted with DNA, improving nuclear uptake, have also been used [212]. Wang and his team designed a GQD-folic acid nanocomposite loaded with doxorubicin. They reported successful targeting of the cancer tissue, high cellular uptake, and easy monitoring of the process [213].

4.3. GQDs for Cancer Treatment

Graphene quantum dots exhibit many properties that make them ideal for use in nanomedicine. They are stable in the environment of the body and non-toxic. They can help improve the hydrophilicity of other molecules and have surface functional groups that facilitate interactions with other molecules. Their strong fluorescence is another important factor that makes them very promising for many therapeutic applications, especially cancer treatment [210]. Lee and his team loaded the chemotherapy drug curcumin into graphene quantum dots in order to improve its hydrophilicity [214]. Ge and coworkers focused on the potential of singlet oxygen generation by graphene quantum dots, aiming to create an effective sensitizer for photodynamic therapy [215]. They successfully created graphene quantum dots smaller than 6 nm. They added norpempidine and studied the generation of ($^1\text{O}_2$) under irradiation. They also used a scavenger for singlet oxygen to prove that no other reactive oxygen species were generated. Furthermore, no significant diffusion of GQDs was observed at the injection site. In vivo studies in mice using this method resulted in regression of the tumors after a few days.

It is also possible to use magnetic nanoparticles to generate heat under an alternating magnetic field in order to kill cancer cells. Yao and his team used a composite of graphene quantum dots and magnetic silica nanoparticles to study this effect [216]. Near-infrared radiation can be used to amplify this effect. A different group used folic acid to functionalize graphene quantum dots and then loaded them with IR780. They used a NIR laser to target tumors in mice, which had been given the drug. After a few minutes, the temperature of the target was increased, which had a significant effect on the cancer cells. The tumors almost disappeared after two weeks [217].

In their thorough review, Ailuno and her colleagues indicated the potential of quantum dots and boron carbide nanoparticles in cancer treatment. Boron carbide (B₄C) was investigated as a potential material for boron neutron capture therapy (BNCT) agents due to its high neutron absorptivity. Several studies have explored different approaches to functionalize B₄C nanoparticles (NPs) for targeted BNCT applications. These studies collectively demonstrate the promising features of modified B₄C NPs and boron-doped quantum dots in targeted BNCT applications, providing promising avenues for cancer therapy [218].

4.4. GQDs in Theragnostic Systems

GQDs can be utilized for bioimaging and sensing for early disease diagnosis as well as in drug delivery systems targeting particularly cancer cells. Since GQDs can be quite easily functionalized, many studies have investigated their potential use in theragnostic systems [219].

The functionalization of GQDs can be maintained during the synthesis process or the molecular binding step, and it generally decreases their toxicity, thus improving their biocompatibility and increasing their internalization and endocytosis [219]. Polymers (e.g., PEG), lipid-like materials, and peptides can be used to increase biocompatibility and internalization [220]. In other studies, therapeutic monoclonal antibodies have been attached to GQDs for advanced immunotherapy through transmembrane receptors [221] and drugs such as DOX for efficient chemotherapy [222]. Their fluorescent properties make them desirable for bioimaging and simultaneous targeting.

4.5. Cytotoxicity of GQDs

Graphene quantum dots (GQDs) have gained significant attention for biological applications due to their unique properties in relation to other carbon nanomaterials. However, understanding the toxicity of graphene quantum dots is crucial, since they can differ from other carbon nanomaterials that have been studied, and thus, new research is required. The research suggests that there are many factors that influence the toxicity of carbon nanodots. A key factor is their size, and, in this case, it seems that their incredibly small diameter is an advantage over other carbon nanomaterials. The research on cell viability by Wang and coworkers proved that toxicity is very low when the nanodot size is under 10 nm [223]. As for any substance, the concentration is extremely important when assessing the toxicity of nanomaterials. In this area, there have been some conflicting results, indicating that more research is needed. Simulations provided some theoretical results based on size and concentration [224]. The simulations showed that graphene quantum dots with smaller sizes had the ability to penetrate the POPC membrane [225]. The presence of GQDs affected the thickness of the POPC lipid membrane by their permeation. Larger graphene quantum dots were only absorbed by the membrane. Something else to consider when assessing toxicity are the functional groups at the surface of graphene quantum dots. The reports again vary. Studies on hydroxylated graphene quantum dots, by one group, showed low cell viability for A549 and H1299 cells [226]. At the same time, a different group did not observe any significant toxic effects on various cancer cells (KB, MDA-MB231, A549) or normal cell lines (MDCK). They followed these results with *in vivo* studies that focused on any long-term effects, and again, did not find any significant damage [227].

Unfortunately, clear information concerning the influence of various functional groups on GQD toxicity is still lacking in the available literature.

5. Conclusions and Future Remarks

In the past twenty years, significant research efforts have been dedicated to studying CBNs, which have emerged as a highly utilized class of nanomaterials. With their exceptional mechanical, optical, electrochemical, and electrical features, CBNs have found extensive applications in various fields. Particularly, their multifunctional surficial characteristics, size, and shape have made them a subject of great interest in biomedical engineering. It is noteworthy that CBNs exhibit inorganic semiconducting characteristics, as well as organic π - π stacking properties, enabling their effective interaction with biomolecules and a light response. This unique combination makes CBNs highly promising for future biomedical applications. Nonetheless, the potential toxicity of CBNs in biological systems has raised concerns. To confront this, diverse chemical functionalization techniques have been effectively employed for bio-applications like drug delivery, tissue engineering, biomolecule detection, and cancer treatment.

The applications of CNTs are numerous, and for this reason, they are considered the prevalent type of CBNs in biomedicine, particularly in sensing, drug delivery, cancer treatment, and tissue engineering. Compared to the other available CBNs, CNTs are considered a very good choice. Although the general applications of graphene and graphene oxide are significant, the biomedical ones are limited, and this could be attributed to the concerns about their toxicity. Scientific effort has been reported in their functionalization to increase their biocompatibility, and it is possible that in the near future, more biomedical and pharmaceutical applications will be available. CQDs are very promising materials, particularly due to their fluorescent potential, and for this reason, the expectations for these materials are extremely high, focusing mainly on theragnostic approaches. CQDs, as a part of hybrid materials, will allow for molecular imaging and the controlled release of a bound drug in clinical routine soon enough.

The present review highlights notable advancements in the utilization of CBNs for biomedical purposes and sheds light on recent discoveries regarding their key features and superior applications in the field. However, further systematic studies are necessary to comprehensively assess the toxicity and pharmaco-kinetics of CBNs.

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