

Review article

Aptamer-functionalized carbon nanomaterials electrochemical sensors for detecting cancer relevant biomolecules



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ABSTRACT

As a class of low-dimensional materials, carbon nanomaterials which are mainly composed of sp^2 and sp^3 carbon atoms arranged into a seamless network have aroused considerable interest since the first exploration of fullerene by Smalley et al. in 1985. Owing to their extraordinary physical, chemical, electrical, optical, mechanical and thermal properties, carbon nanomaterials have found their wide applications in sensor, biomedicine, electrode, electrocatalysis, energy storage and conversions. Especially, with proper functionalization, carbon nanomaterials can be utilized to construct high performance electrochemical sensors for promising applications in medical diagnostics and therapies. Here, the recent progresses of electrochemical sensors based on carbon nanomaterials are reviewed. The structure related properties of carbon nanomaterials as well as the surface functionalization methods are briefly introduced. The detection mechanisms of carbon nanomaterials-based electrochemical sensors are comprehensively analyzed. Furthermore, the most recent achievements of nucleic acid aptamer functionalized carbon nanomaterials-based electrochemical biosensors for the detection of cancer relevant biomarkers including nucleic acids, protein and cells are overviewed. At the end, the future developments as well as the issues and challenges of the fabrication of carbon nanomaterials-based nucleic acid aptamer functionalized electrochemical biosensors are proposed.

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1. Introduction

Carbon nanomaterials, which are composed of sp^2 and sp^3 -bonded carbon atoms, have generated considerable interests in the past decades [1–4]. The tunable physical, chemical, mechanical and electrical properties of carbon nanomaterials and corresponding composites provide tremendous opportunities for their applications in energy storage [5–9], electrodes [10–14], water desalination [15–19], and biomedical technologies [20–24]. In particular, the superior electrical performance [25–27], redox chemistry [28,29] and biocompatibility [30–32] of carbon nanomaterials make them attractive candidates for electrochemical sensor applications. Carbon nanomaterials-based electrochemical biosensors exhibit intriguing advantages of high surface area, tunable electronic properties, component diversity, and biocompatibility, promising their potential to achieve rapid, sensitive and low-cost detection of disease relevant biomolecules [33]. Moreover, the ease to fabricate carbon nanomaterials-based patterns makes it possible to develop electrochemical sensor arrays for realizing multiple biomolecules detection simultaneously [22,34]. By rational design, the point-of-care devices based on carbon nanomaterials electrochemical biosensors can be fabricated for the detection of biomolecules, such as enzyme-based glucose [35–37] and lactate [21,38]. Also, the excellent mechanical flexibility of carbon nanomaterials make it possible to construct flexible and wearable biosensor to enable continuously monitoring the electrochemical signals emanating from the human body [39,40]. In this regard, the design and development of carbon nanomaterials-based electrochemical biosensor for high sensitivity, specific and *in situ* detection of vital biomolecules is critical for realizing early disease diagnostic.

The working principle of carbon nanomaterials-based electrochemical biosensors is the change of redox potential or current upon the binding of analytes [35]. The carbon nanomaterials-based electrochemical biosensor can directly adsorb and conjugate biomolecules such as nucleic acids [41–45] or protein [46] on the

surface of carbon nanomaterials through electrostatic or π - π interactions to realize detection. Alternatively, by incorporating biomolecular recognition elements through covalent or non-covalent methods [47], carbon nanomaterials also can be used to construct high sensitivity electrochemical biosensors for the diagnosis of specific diseases. The achievement of high performance and selectivity electrochemical biosensors requires the design of optimized nanostructures, the synthesis of new multifunctional nanomaterials as well as the select of highly stable biorecognition molecules. Up to now, the fast growing number of new nanomaterial structures [48,49] and specific biorecognition elements has significantly promoted the development of carbon nanomaterials-based electrochemical biosensor. The typical biorecognition elements [50] including antibodies, enzymes, lectins, cells, peptides, nucleic acids, molecularly imprinted polymers and nucleic acid aptamers have been extensively investigated as electrochemical biosensors. Among them, nucleic acid aptamers, which are isolated from oligonucleotides libraries by an *in vitro* selection process, exhibiting similar properties to antibodies in the selectivity and binding affinity. In comparison to antibody, nucleic acid aptamer with tunable properties is more stable and can be synthesized by chemical method. A variety of nucleic acid aptamer-based electrochemical biosensors [51–54] have been designed to specific sensing of a range of biomolecules ranging from small molecules to protein and cells. Nucleic acid aptamer-based electrochemical biosensors exhibit outstanding advantages in terms of high sensitivity, superior selectivity, fast response, low cost and the potential for miniaturization.

In this review, the recent progresses of nucleic acid aptamer functionalized carbon nanomaterials-based electrochemical biosensors are overviewed (Fig. 1). Firstly, the carbon nanomaterials composites and corresponding assemblies with various nanostructures being employed for electrochemical sensors are discussed. The structure related sensor properties are also introduced in detail. Then, the surface functionalization methods including the covalent and noncovalent approaches toward the

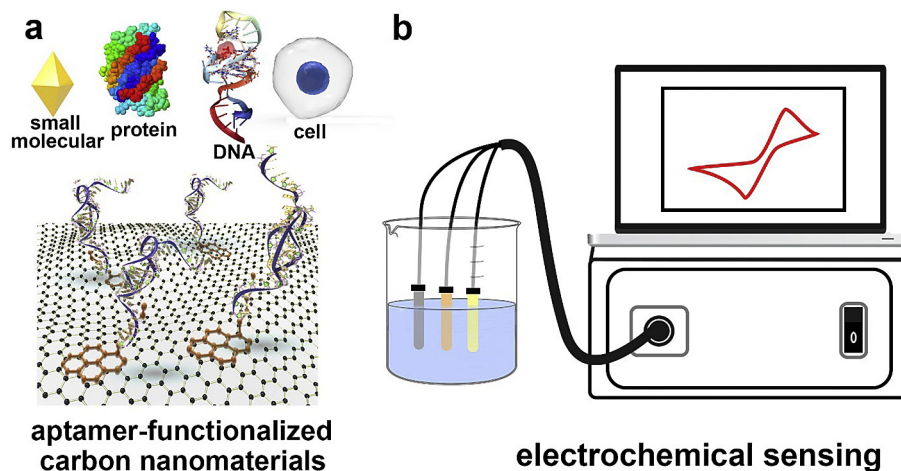


Fig. 1. (a) Schematic models of nucleic acid aptamer-functionalized carbon nanomaterials biosensor; (b) Schematic images of a typical electrochemical biosensor. (A colour version of this figure can be viewed online.)

construction of nucleic acid aptamer functionalized carbon nanomaterials electrochemical biosensors are briefly summarized together with the examples reported in the literature. As a further step, the working principles as well as the current state-of-art developments of nucleic acid aptamer functionalized carbon nanomaterials-based electrochemical biosensor for the specific diagnosis of cancer relevant biomolecules are comprehensively reviewed. Finally, the emerging challenges and opportunities regarding the design of high performance nucleic acid aptamer functionalized carbon nanomaterials-based biosensors are proposed. Overall, this review is devoted to present the recent advancements and future challenges of nucleic acid aptamer functionalized carbon nanomaterials for the diagnosis of cancer relevant biomolecules.

2. Carbon nanomaterials and assemblies

As we have illustrated, carbon nanomaterials exhibit superior electrical, magnetic, mechanical, thermal stability, electrochemical activity, ease to modify, chemical diversity, and biocompatibility, ensuring their wide applications in high performance electrochemical biosensors. The commonly used carbon nanomaterials include fullerene, carbon dots (CDs), carbon nanohorns (CNHs), carbon nanotubes (CNTs) and graphene (G) as well as their corresponding derivatives (Fig. 2). Upon controlling synthesis conditions, carbon nanomaterials especially CNTs and G can be assembled into films, foams, arrays and sponges, further increases the electrical conductivity and available surface area for biosensor applications [33]. This part will introduce the properties of carbon nanomaterials as well as their nucleic acid aptamer conjugates for electrochemical biosensors.

2.1. Fullerene

C_{60} fullerene is a cage structured carbon nanomaterial composed of packed carbon atoms [55]. As one of carbon allotropes, fullerene possesses notable physical, chemical, and electrical properties, making it ideal candidates for constructing nano-assemblies for various applications [56–58]. The spherical structure

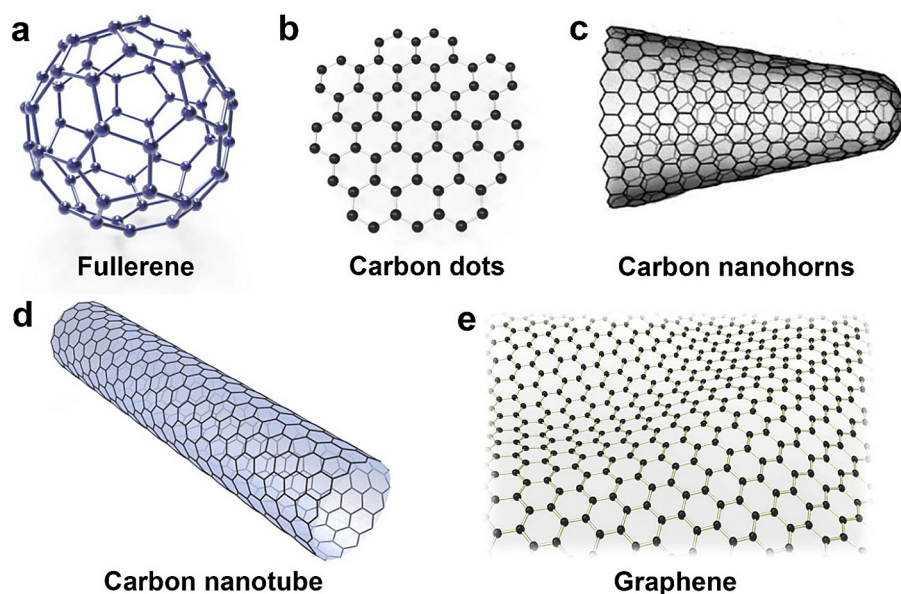


Fig. 2. Schematic models of various carbon nanomaterials: (a) Fullerene; (b) Carbon dots; (c) Carbon nanohorns (adapted from Ref. [77]); (d) Carbon nanotube; (e) Graphene. (A colour version of this figure can be viewed online.)

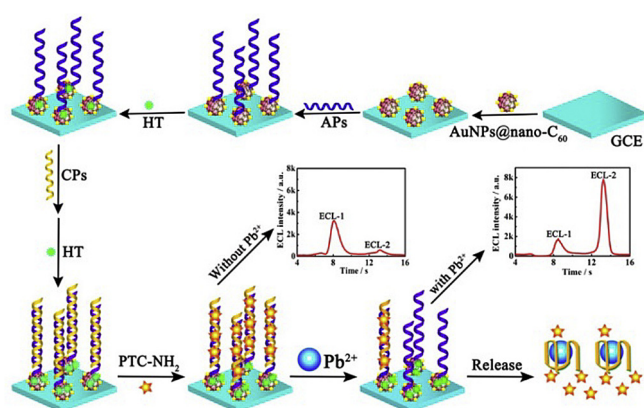


Fig. 3. Schematic illustration of the preparation process of the electrochemical ions biosensor based on nucleic acid aptamer modified fullerene/AuNPs. CPs denote the capture probes (Pb^{2+} specific nucleic acid aptamer). HT represents hexanethiol, which is used to eliminate the nonspecific binding effect. Reproduced from Ref. [63]. (A colour version of this figure can be viewed online.)

accelerates electrons transfer and charge shift [59]. Fullerene also can be functionalized by covalent or noncovalent interaction to form a multifunctional material with tunable physical and chemical properties, promising their applications in electrochemical biosensors [60]. The surface of fullerene can be grafted with redox active enzymes to form an electrochemical biosensor for high sensitivity and selectivity glucose sensing [61], or be directly used for DNA detection by surface adsorption effect [62]. To enhance detection sensitivity, Lei, Y. -M. et al. fabricated a hybrid electrochemiluminescence (ECL) system based on nucleic acid aptamer modified fullerene/gold nanoparticles (nano- C_{60} /AuNPs) composites for specific and label-free lead ions (Pb^{2+}) detection [63] (Fig. 3). The nano- C_{60} /AuNPs coated on the glass carbon electrode (GCE) not only provide a high surface area and active interface for the immobilization of assistant probes (APs) through Au–S bonds but also enhance the ECL of the $O_2/S_2O_8^{2-}$ system. The sensing mechanism is based on the change of ECL signal between the amino-terminated perylene derivative (PTC-NH₂) and $O_2/S_2O_8^{2-}$

resonance energy transfer system upon the presence of Pb^{2+} . The resulting electrochemical biosensor shows a linear range from 1.0 nM to 10 μM and a detection limit of 0.35 pM, which is due to the rational designed resonance energy transfer system combined with highly conductive fullerene and specific nucleic acid aptamer. This ratiometric method also eliminates the false positive or negative errors to make the detection results more accurate, providing a guideline for personalized medicine.

2.2. Carbon dots

Carbon dots (CDs), as a type of 0D carbon nanomaterials, have attracted enormous interest due to their outstanding optical and electrochemical performances arising from the quantum confinement and edge effects [64,65]. CDs can be classified into carbon quantum dots (CQDs) [66,67] and graphene quantum dots (GQDs) [68,69] according to different structure. CQDs are quasi-spherical amorphous or nanocrystalline nanoparticles with diameters smaller than 10 nm [70]. GQDs are similar to the 2D plane of G except that the lateral dimensions of GQDs are smaller than 20 nm [71]. In this regard, the properties of GQDs are similar to that of G and QDs. Owing to their intriguing characteristics such as high surface area, ease to modify, and excellent biocompatibility, the electrochemical performances of CQDs and CQDs-based electrochemical biosensors have been extensively investigated during the past few years [72–74]. A work by Zhao, J. et al. designed a simple and general voltammetric electrochemical biosensor platform based on single strand DNA or nucleic acid aptamer attached on the GQDs for the specific detection of complementary DNA or protein [75] (Fig. 4a, Table 1). The presence of target DNA or protein induces the formation of probe DNA-target conjugates and these conjugates were detached from the surface of GQDs, thus resulting in an electrochemical signal change and specific sensing. The detection limits for the target single-strand DNA and protein are all 100 nM.

The superior sensing performance is attributed to the large surface area for surface functionalization of probe molecules, abundant oxygen-containing groups around the edges of GQDs facilitate the electrostatic interactions with probe molecules, as well as the intrinsic excellent electrical conductivity of GQDs which benefits electrons transport. However, the relatively narrow linear range from 200 nM to 500 nM need to be overcome through engineering hybrid GQDs-based structure with multiple electrochemical signal.

2.3. Carbon nanohorns

Carbon nanohorns (CNHs) are conical carbon nanostructures which are composed of sp^2 and sp^3 carbon sheets [76–79]. The diameter of CNHs is about 2–5 nm and the length is about 40–50 nm. CNHs exhibit distinct advantages such as: (1) low toxicity, which is due to the absence of toxic catalyst during the synthesis [48]; (2) mass production at room temperature, which is significantly higher than CNTs and G [80]; (3) The unique electrical properties arise from the conical structure [81]; (4) The high porosity and adsorption capabilities enable them to serve as nanocarriers or substrates for biomedical applications [82]. Similar to CNTs and G, CNHs can be partially oxidized to include oxygen-containing groups on their surface through heat or chemical treatment. The introduction of nanopores also can increase the surface area and pore volume of CNHs, ensuring their capabilities to be functionalized with biomolecules. These prominent properties provide opportunities for them to construct effective biosensors and electrodes for electrochemical purposes [83–85]. The as-prepared CNHs can be directly used as an electrochemical biosensor for the detection of dopamine, uric acid, and ascorbic acid in biological samples [86]. Alternatively, Liu, Z. et al. developed a label-free ECL biosensor for highly sensitive detection of adenosine triphosphate (ATP) based on nucleic acid aptamer modified single walled CNHs (SWCNHs) [87] (Fig. 4b).

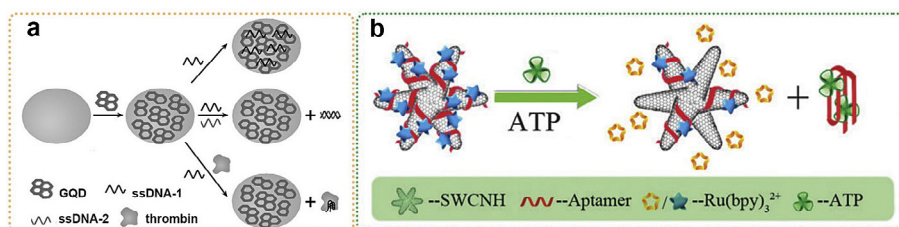


Fig. 4. (a) The preparation process of DNA modified GQD electrochemical biosensor platform for the detection of target DNA and protein. (b) Schematic illustration of the sensing mechanism of nucleic acid aptamer/CNH ECL biosensor for the detection of ATP. Adapted from Refs. [75,87]. (A colour version of this figure can be viewed online.)

Table 1

Typical nucleic acid aptamer functionalized carbon nanomaterials-based biosensors for the detection of cancer relevant biomarkers.

Materials	Mechanism	Target	Detection limit	Linear range	Refs.
DNA modified GQD	voltammetric	DNA		200–500 nM	75
G/Fe ₃ O ₄ /AuNPs	ECL	HeLa cells	8 cells mL ⁻¹	20–10000 cells mL ⁻¹	115
rGO-Chit/aptamer	voltammetric	HER2	0.21 ng mL ⁻¹	2–75 ng mL ⁻¹	123
GNM/HER2 aptamer	FET	HER2	0.6 fM	1–200 ng mL ⁻¹	132
aptamer/PPy-NDFLG	FET	VEGF	100 fM		133
DNA-decorated G	FET	DNA	1 pM		164
3D GFs on Au substrate	voltammetric	miR-155	5.2 pM		166
HO-GNRs/G	voltammetric	CEA	1.5 pg mL ⁻¹	5 pg mL ⁻¹ –50 ng mL ⁻¹	172
Aptamer/rGO	impedimetric	CEA	0.1 fg mL ⁻¹	0.1 fg mL ⁻¹ –5.0 pg mL ⁻¹	173
GQDs-IL-NF	voltammetric	CEA	0.34 fg mL ⁻¹	0.5 fg mL ⁻¹ –0.5 ng mL ⁻¹	174
rGO/aptamer/GCE	voltammetric	CEA	80 ag mL ⁻¹	80 ag mL ⁻¹ –950 fg mL ⁻¹	175
aptamer-PTCA/CCG	impedimetric	HeLa cells	794 cells mL ⁻¹	1 × 10 ³ –1 × 10 ⁶ cells mL ⁻¹	184
MWCNTs@PDA@AuNPs	voltammetric	CCRF-CEM cells	50 cells mL ⁻¹	1 × 10 ² –1 × 10 ⁶ cells mL ⁻¹	185
rGO–DEN/GCE	voltammetric	CCRF-CEM cell	10 cells mL ⁻¹	1 × 10 ² –5 × 10 ⁴ cells mL ⁻¹	186

Note: VEGF indicates the antivascular endothelial growth factor.

Specifically, the SWCNH modified on the GCE surface serves as both scaffold for the interaction of ATP specific nucleic acid aptamer *via* π -stacking and quencher of Ru (bpy) $_3^{2+}$ conjugated on the surface of nucleic acid aptamer through electrostatic interaction. The presence of ATP target induces the conformation change of nucleic acid aptamer and further weakens the SWCNH–Ru (bpy) $_3^{2+}$ interaction, resulting in the ECL signal-on of the Ru (bpy) $_3^{2+}$ as well as specific and quantitative determination of ATP. The sensor exhibits a detection limit of 1 nM, wide linear range (5 nM–0.5 mM) and surprisingly high selectivity. These prominent properties arise from the excellent electrical conductivity of CNHs, large surface area and plenty of active sites for surface functionalization, as well as the superior selectivity of specific nucleic acid aptamer. Nevertheless, the aggregation of CNHs during the synthesis procedures which needs a further dispersion step limits their widespread application.

2.4. Carbon nanotube and carbon nanotube assemblies

Carbon nanotubes (CNTs), a type of 1D tubular nanomaterials similar to rolled G nanosheet, exhibit excellent electrical, mechanical and conductivity, has received considerable attention since its first isolation in 1991 [88,89]. In comparison to the 0D fullerene and CDs, CNTs with high aspect ratio exhibit superior electrical conductivity as electrochemical biosensor since CNTs can be connected with each to form an interconnected conductive network. CNT can be classified into multi-walled CNTs (MWCNTs) and single-walled CNTs according to the wall numbers [90–92]. The surface of CNTs can be modified with chemical functional groups or biomolecules to tune the dispersibility, conductivity, mechanical strength, surface area, and optical absorption capabilities to afford electrochemical sensors with enhanced performance [93–95]. In this regard, CNTs have been widely used as substrate for the construction of electrochemical biosensor. Additionally, the combination of CNTs with other nanomaterials can construct a multifunctional electrochemical platform with optimized performance [96–98]. As an example, a solid-contact potentiometric sensor for specific thrombin detecting was achieved by covalently grafting the thrombin nucleic acid aptamer onto SWNT surface [99]. The affinity interaction of aptamer with thrombin results in a distinct potentiometric signal that can be directly recorded within 15 s. The linear range is 0.1 μ M–1 μ M and the detection limit reaches 80 nM. To further improve the sensor performance, Liu, X. R. et al. reported an ultrasensitive voltammetric electrochemical sensor based on thrombin-binding nucleic acid aptamer attached on the MWCNTs surface for specific thrombin sensing [100] (Fig. 5a). The MWCNTs with high exposed surface area not only serve as the immobilized carrier for loading of capture DNA (Cpt-DNA) molecules with large amount but also amplify the electrochemical signal. Upon the presence of target thrombin, the specific aptamer contains ferrocene (Fe-Tgt-aptamer) hybridizes with thrombin and dissociates from the Cpt-DNA, resulting in significantly decreased peak current intensity. Consequently, a wide linear range of 1.0 pM–0.5 nM and low detection limit of 0.5 pM were obtained. In a separate report, Pilehvar, S. et al. immobilized hydroxylated polychlorinated biphenyls (OH-PCB) specific nucleic acid aptamer on the modified MWNT-COOH electrode to detect OH-PCB in the blood serum as well as distinguish OH-PCB between other similar structured species such as butyl paraben, matairesinol and bisphenol A *etc* [101] (Fig. 5b). The excellent sensor performance is due to the presence of high selectivity nucleic acid aptamer and highly conductive CNTs which enable high sensitivity. It is worth mentioning that a primary advantage of CNT is its ability to assemble into free-standing films [102], sponges [103] and arrays [104] with high mechanical strength and impressive electrical conductivity, promising their

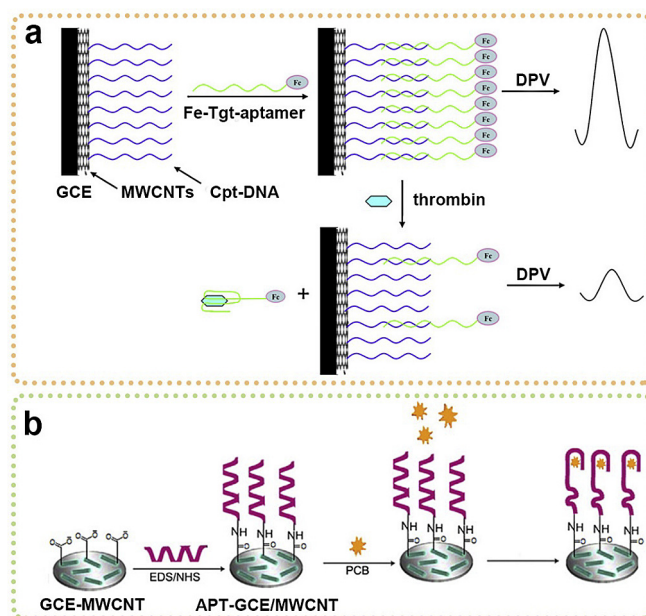


Fig. 5. The preparation process of (a) the CNT-enhanced electrochemical nucleic acid aptamer sensor for the detection of thrombin and (b) nucleic acid aptamer conjugated MWCNTs-based sensing platform for detecting PCB in blood serum. DPV indicates differential pulse voltammetry. Reproduced from Refs. [100,101]. (A colour version of this figure can be viewed online.)

opportunities for microscale analysis and decentralized assay. Additionally, the CNT assemblies also facilitate their abilities to act as free-standing electrodes for portable and wearable electrochemical biosensors. Especially, the CNT assemblies with high surface area can provide enough available active sites for analytes detection, and thus improve the detection sensitivity and reduce the limit of detection. In addition, it has been reported that the doping of CNTs with hetero-atoms to include defects in its surface could further increase the selectivity and sensitivity of CNT-based electrochemical biosensors [105]. However, as for FET biosensor applications, CNTs face the problems of poor chirality control in the growth process. In addition, the preparation and organization of high density and aligned CNT arrays to satisfy integrated multiplex FET bioelectronics is also a critical problem to be overcome.

2.5. Graphene and graphene architectures

Graphene (G), as one of the most promising carbon nanomaterials which is composed of a monolayer of sp^2 -bonded carbon atoms packed into a honeycomb lattice, has attracted enormous interest since its first discovery in 2004 by A. Geim et al. [106]. Generally, the family of G materials includes single and multilayer G, graphene oxide (GO), reduced graphene oxide (rGO), and GQDs [33]. Each type of G materials exhibits different and tunable physical, chemical, defect density, electrical and mechanical properties, promising its applications in a variety of electrochemical sensors with tunable electrochemical properties. In comparison to CNTs, G exhibits relatively high surface area, abundant surface functional groups, easy to be dispersed, and can be patterned to form enormous sensor arrays. The large surface area of G nanomaterials enables its ability to adsorb and conjugate with biomolecules, which provides a potential platform for multifunctional sensor applications [49]. Particularly, the presence of epoxides, alcohols, carboxylic, ethers, carboxylate and hydroxides defects on the basal plane of GO and rGO promise their capability to conjugate with

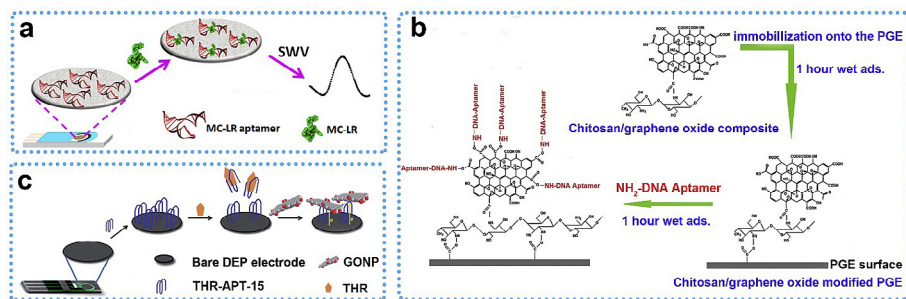


Fig. 6. Fabrication of (a) nucleic acid aptamer-functionalized G modified screen printed carbon electrodes for the detection of MC-LR. (b) Schematic drawing of nucleic acid aptamer functionalized chitosan/GO-based impedimetric sensor for lysozyme detection. (c) Schematic illustration of the detection of THR with THR-APT-15 modified electrode through electrochemical reduction of GO nanoplatelets. Reproduced from Ref. [109–111]. (A colour version of this figure can be viewed online.)

multifunctional molecules through π - π stacking, hydrogen-bond, electrostatic interactions, and covalent bonding [107]. The conjugated molecules self-assembled on G are also emerging tools for tailoring the band structure of G, which is of vital importance for the achievement of field-effect transistor (FET) biosensors with high sensitivity [108]. The monolayer G and assembled G films can be easily patterned into aligned patterns to form sensor arrays and realize multifunctional sensing. For these reasons, G and G derivatives are promising candidates for fabricating various electrochemical biosensors. Eissa, S. et al. designed a label-free voltammetric biosensor based on nucleic acid aptamer modified G assembled on the screen printed carbon electrodes for sensitive detection of microcystin-LR in fish samples [109] (Fig. 6a). In this biosensor, G serves as the active sites for nucleic acid aptamer immobilization and accelerates the electron transfer rates. Distinct from the sensing mechanism of protein induced conformational change of aptamer and the release of aptamer from G surface, the detection mechanism of microcystin-LR relies on the part conformation change of aptamer without release from G surface. Despite of excellent electrical conductivity, pure G suffers from the limitations in surface area. As an alternative, GO with various surface oxygenated functionalization groups endows a relatively high surface area. Erdem, A. et al. fabricated an electrochemical impedance spectroscopy biosensor based on a mixture of chitosan/GO composites modified with antilysozyme DNA nucleic acid aptamer for lysozyme detection [110] (Fig. 6b). This designed sensor is insensitive to interference species and reaches a limit of detection of 28.5 nM for lysozyme. The elimination of oxygenated surface functional groups on GO also can be used to construct electrochemical biosensors. For instance, Loo, A. H. et al. utilized the signal generated by electrochemical reduction of GO to realize sensing [111] (Fig. 6c). Specifically, the thrombin (THR) specific nucleic acid aptamer (THR-APT-15) was immobilized on the electrode surface. The presence of target THR results in the conformational change of THR-APT-15 and further leads to the partial removal of THR-APT-15 from the electrode surface. Then, the contact of GO with the exposed electrode surface leads to the reduction of GO and generates a great electrochemical signal. This enlarged surface area and superior electrochemical activity of the designed sensor system results in a wide detection concentration range of 3 pM–0.3 μ M. The electrochemical activity can be further improved through doping of GO and GO derivatives with heteroatoms such as nitrogen and sulfur [112,113]. However, the intrinsic electrical insulation performance of GO limits its application as electrode in electrochemical biosensor. The elimination of partial oxygenated groups of GO through chemical, thermal or electrochemical reduction methods to obtain rGO with relatively high electrical conductivity and large surface area is an efficient

way to construct electrochemical biosensors with high sensitivity. It is worth mentioning that the excellent mechanical strength of monolayer G facilitates their applications in flexible and wearable biosensor devices. However, it is particularly difficult to obtain a free-standing large-area G with sufficient mechanical strength, significantly inhibits its ability to contact with tissues or skins intimately. Since the CNT films show superior mechanical strength, the combination of CNT and G films may be a promising solution towards the fabrication of free-standing large-area sensor elements with superior mechanical flexibility.

2.6. Carbon nanomaterials-based hybrid assemblies

Carbon nanomaterials-based electrochemical biosensors exhibit prominent properties in sensitivity due to the increased electron transfer rate. The sensitivity can be further enhanced through surface functionalization of carbon nanomaterials with nanostructures with synergistic electrochemical performances. The carbon nanomaterials can act as a substrate for 0D nanoparticles, 1D nanorods and nanotubes, 2D nanosheets or nanodots, polymers, and enzymes to improve the electrochemical performance in regards conductivity or anchoring sites [36]. The nanostructures can be deposited onto the surface of carbon nanomaterials or directly grown on the surface through covalent or noncovalent interactions, which is depending on the surface functional groups of carbon nanomaterials. The typical used methods for the construction of hybrid nanoassemblies include hydrothermal, solvothermal, sol-gel, electrochemical deposition, and chemical/physical vapor deposition [35]. As for electrochemical biosensor applications, the developments of hybrid assemblies can improve the sensor sensitivity, enlarge the available reaction sites, enhance the stability and reproducibility, as well as reduce the limit of detection. There have been enormous researches on the improvement of biosensor performance by rational design of carbon nanomaterials-based hybrid assemblies. As an example, Guo, Y. et al. included Orange dye in the rGO electrochemical nucleic acid aptamer biosensor to prevent the agglomeration of G nanocomposites as well as provide rGO with electroactive properties [114] (Fig. 7a). This optimized structure with high surface area and electrochemically active as well as high specificity results in highly sensitive and selective detection of thrombin and lysozyme. Despite of the enhanced electrochemical activity, the fabrication of hybrid nanoarchitectures also can be utilized to construct biosensors with multifunctional performances in a single device. Gu, W. et al. developed a high sensitivity and reproducibility ECL biosensor based on nucleic acid aptamer (AS1411) modified multifunctional G/iron oxide/gold nanoparticles (G/Fe₃O₄/AuNPs) nanoarchitecture for sensing of human cervical carcinoma (Hela)

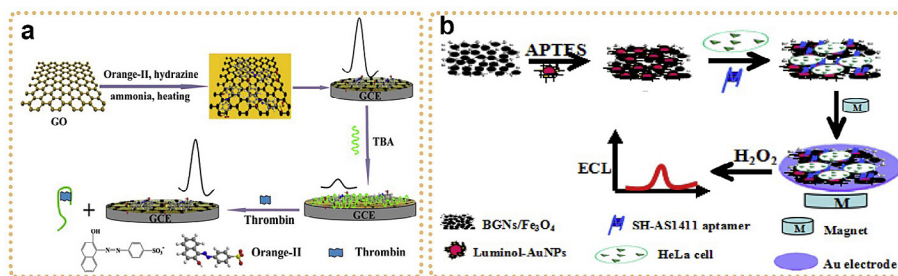


Fig. 7. The preparation process of (a) Orange II functionalized G nanosheets-based nucleic acid aptamer sensor for specific detection of thrombin; (b) nucleic acid aptamer functionalized G/Fe₃O₄ hybrids and luminol capped AuNPs-based ECL sensor for the detection of HeLa cells. Reproduced from Refs. [114,115]. (A colour version of this figure can be viewed online.)

cells [115] (Fig. 7b, Table 1). In this hybrid system, G serves as the conductive substrate for efficient electron transfer, and AuNPs serve as the ECL marker with high emission intensity. Meanwhile, the presence of magnetic Fe₃O₄ nanoparticles facilitates the construction of magnetically-controlled and portable solid-state biosensing platform. The specific sensing of HeLa cells relies on the decrease of ECL intensity due to the hindered electron transfer in the presence of cell membrane. As a result, the fabricated biosensor exhibits a linear range of $1\text{--}20 \times 10^4$ cells mL⁻¹, high reproducibility, and superior stability. In addition to the improved surface area, the topography structure of the hybrid assemblies also can be utilized to isolate analytes in the complex biological samples. Yoon, H. J. et al. utilized the morphological features of noncovalently functionalized GO on the patterned Au surface to improve the sensitivity toward the isolation and separation of circulating tumor cells (CTCs) from the blood of cancer patients [116]. The topography feature of the GO/Au nanostructures combined with the specific capture ability results in a high capture efficiency of 73% under a particularly low cell concentration ($3\text{--}5$ cells mL⁻¹).

Another interesting example is the creation of hybrid assemblies by combining different types of carbon nanomaterials via π stacking, such as the integration of G with OH-functionalized CNTs results in a hybrid ink with improved electrical conductivity and mechanical stability [35]. The incorporation of G into the CNT electrode also can relieve the problems of fouling which is always observed in CNT-based electrochemical biosensor. Recently, Yang, Y. J. et al. designed an electrochemical biosensor composed of cetyltrimethyl ammonium bromide (CTAB) and GO/MWNT hybrid composites for high sensitivity simultaneously detection of small molecules such as dopamine, uric acid and ascorbic acid [117]. The enhanced electrochemical performance is attributed to the highly porous 3D structure of CTAB-GO/MWNT composite foams with improved active sites. The 2D G can also combine with other 2D transition metal dichalcogenides like MoS₂, WS₂ and WSe₂ to obtain hybrid architectures with variable performance for biosensor applications [118–120]. In addition, as we have mentioned, carbon nanomaterials can also be assembled into various macroscopic free-standing nanostructures such as films, arrays, yarns, and foams, enabling their applications as free-standing electrochemical electrodes of biosensor. As an example, Choi, B. G. et al. constructed an organophosphate biosensor based on free-standing flexible electrochemical rGO/naftion hybrid films [121]. The superior electrical conductivity and high surface area of rGO films lead to a relatively low detection limit (1.37×10^{-7} M) and fast response time (<3 s).

3. Surface functionalization with nucleic acid aptamer

To achieve the specific molecular recognition or detection as

well as endow the carbon nanomaterials with multifunctional structure and physicochemical properties for applications in biosensors and biomedical, surface functionalization is required in most cases. Typically, the functionalization of carbon nanomaterials includes covalent and noncovalent methods according to different inter-molecular interactions. The covalent functionalization methods can significantly deteriorate the sp² structure of the carbon honeycomb lattice and induce the formation of defects on the carbon nanomaterials, thus undermining the electronic properties. In contrast, noncovalent functionalization approach cannot interrupt the intrinsic structure, mechanical and electronic properties of carbon nanomaterials while it also introduces new functionalization groups on the material surface. These new functionalization groups enable the carbon nanomaterials with superior biocompatibility, improved dispersibility, and sensing properties. In this subsection, we will give an overview of surface functionalization methods with regard to carbon nanomaterials for biosensor applications.

3.1. Covalent method

The covalent functionalization of nucleic acid aptamer on the carbon nanomaterials often occurs by the formation of amide bonds. In a characteristic example, Zelada-Guillén, G. A. et al. designed a potentiometric biosensor for selectively and real-time detecting *Salmonella Typhi* [122] (Fig. 8a). The nucleic acid aptamer with an amine group was covalently combined with the carboxylated SWNTs through the formation of amide bonds. The presence of target bacteria promotes the conformational change of nucleic acid aptamer and separates the phosphate groups from the SWNT surface, thus inducing the charge change to the SWNT and further change of recorded potential. A limit of detection of 1 CFU and dynamic range of $0.2\text{--}10^3$ CFU mL⁻¹ were obtained by this highly sensitive and selective biosensor. Tabasi, A. et al. also demonstrated an electrochemical nucleic acid aptamer-based voltammetric biosensor for human epidermal growth factor receptor 2 protein (HER2) detection [123] (Fig. 8b, Table 1). In this sensing platform, nucleic acid aptamer was covalently linked to the amine groups functionalized rGO surface through the formation of amide bond. Liu, Z. B. et al. demonstrated that the covalent functionalization of nucleic acid aptamer on the GO surface can address the problems of possible nonspecific probe displacement and the false positive signal present in the physisorbed GO biosensor [124] (Fig. 8c). They showed that the covalent sensor is more resistant to nonspecific probes than noncovalent one. Although the covalent functionalization method shows superior electrochemical performance in the analysis of specific molecules, the disruption of the π -electronic networks and reduction of electrical conductivity is an unavoidable problem.

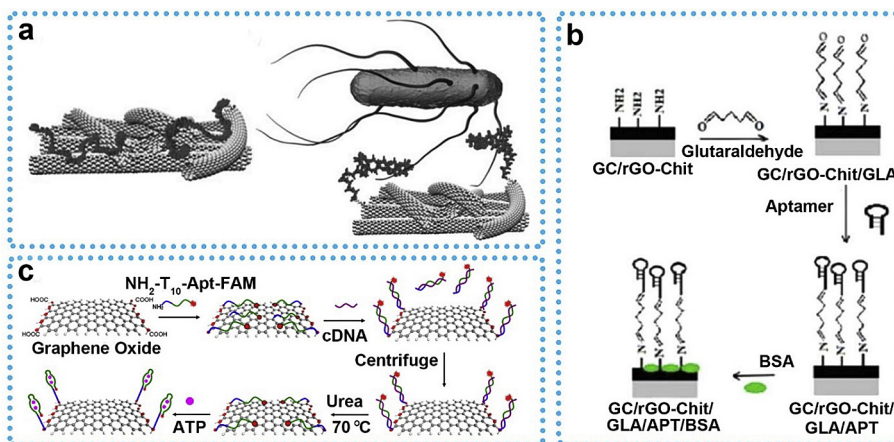


Fig. 8. (a) Schematic models of CNT-based potentiometric nucleic acid aptamer sensor for the detection of *Salmonella Typhi* in real-time. Fabrication of nucleic acid aptamer functionalized (b) rGO and (c) GO-based biosensor with covalent method. GC/rGO-Chit indicates the rGO-chitosan composites modified on the GC electrode. GLA represents glutaraldehyde, and BSA is Bovine Serum Albumin. Reproduced from Ref. [122–124]. (A colour version of this figure can be viewed online.)

3.2. Noncovalent method

The driving forces for noncovalent functionalization of carbon nanomaterials with organic molecules or polymers involve the π - π interaction, van der Waals force, electron donor-acceptor complexes, ionic interaction, and hydrogen bonding [108]. The π - π interactions are often observed between carbon nanomaterials and molecules with extended π systems, and the interaction of aliphatic chains with the G basal plane allows the molecules to lie on the surface. The van der Waals force occurs between carbon nanomaterials with highly or partially hydrophobic organic molecules, surfactants and polymers for the effective dispersion or stabilization of carbon nanomaterials in aqueous or organic media. The ionic interaction and hydrogen bonding are usually involved within the carbon nanomaterials with enormous oxygenated groups. The hydrogen bonding and hydrophobic interaction always direct the formation of 2D supramolecular systems on the G/GO surface.

Single-stranded DNA molecules with aromatic bases can be easily immobilized on the surface of carbon nanomaterials through the π - π interactions with the hexagonal carbon lattice. The as-formed carbon nanomaterials/DNA composites can be directly used as biosensors for the detection of complementary DNA strands. For instance, Lu, C.-H. et al. constructed a DNA sensor based on the fluorophore-labeled single-strand DNA immobilized on GO [125]. The strong π -interaction of DNA and GO induces the quench of fluorescence of dye. After the presence of complementary DNA strand, the quenched

fluorescence is recovered due to the strong interaction between the complementary DNA strands results in the dissociation of DNA strands from GO. This sensor also can distinguish the complementary DNA strands with single-based mismatched DNA strands. With the same principle, Yang R. H. et al. designed a molecular beacon-SWNT complex for the detection of complementary DNA [126]. In addition to the optical signal, the electrochemical behavior of G also changes when the probe DNA hybridized with target DNA. In a characteristic example, Bonanni, A. et al. developed a G/hairpin-DNA sensing platform for the sensitive and fast detection of single nucleotide polymorphism based on the electrochemical impedance spectroscopy [127] (Fig. 9a). As a result, a detection limit of 50 nM was obtained. To further decrease the detection limit, they also utilized the intrinsic redox properties of GO as electroactive labels for single nucleotide polymorphism analysis. The estimated detection limit was 500 pM and the differentiation between the wild-type and mutant DNA reaches 10 nM. When the immobilized DNA molecules are replaced with nucleic acid aptamer with specific recognition ability, the constructed biosensor can be developed for detecting RNA, DNA, protein, bacteria and cells correlated with specific diseases based on the variation of electrochemical signal. So, H.-M. et al. constructed a nucleic acid aptamer-functionalized SWNT FET for detecting *Escherichia coli* [128]. The biotinylated nucleic acid aptamer was immobilized on the SWNT sidewalls through the noncovalent interaction with streptavidin functionalized SWNT. Wu, L. et al. developed a reliable and

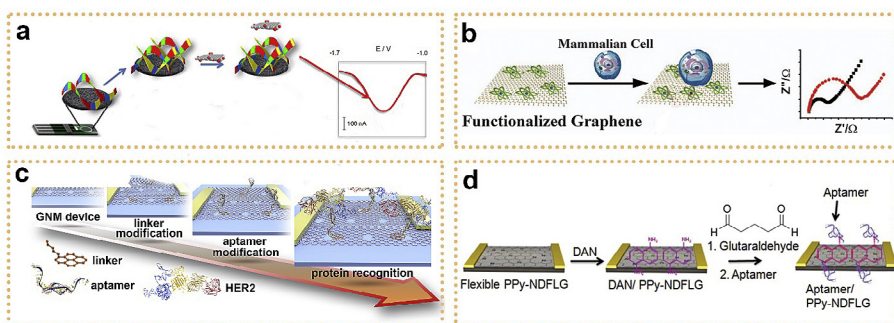


Fig. 9. The preparation process of (a) GO nanoplatelets-based sensor through the π - π interaction between GO and nucleic acid aptamer. (b) Schematic illustration of the fabrication of functionalized G-based sensor through noncovalent interaction. (c) Schematic drawing of the fabrication of nucleic acid aptamer modified graphene nanomesh (GNM) FET biosensor through PBSE linker. (d) Schematic representation of the surface modification of polypyrrole-converted nitrogen doped few-layer G (PPy-NDFLG) with nucleic acid aptamer through DAN linker. Reproduced from Refs. [127,129,132,133]. (A colour version of this figure can be viewed online.)

sensitive electrochemical impedance monitoring assays by grafting nucleic acid aptamer on the G surface through π -stacking for cancer relevant cell sensing and discriminating [129] (Fig. 9b).

On the other hand, nucleic acid aptamer can be modified on the surface of carbon nanomaterials through the formation of covalent bonds with the organic molecules conjugated on its surface through π - π interactions. The usually used organic linker molecules with aromatic groups include pyrene, pyridine, benzene, and quinoline derivatives [108]. For example, Zhang, J. Z. et al. reported the functionalization of rGO with a series of phenyl or pyrene terminated polyethylene glycols (PEG) through π - π interactions to form a nanocomposite with enhanced mechanical performance [130]. Apart from the enhanced mechanical strength, the electrical conductivity change of rGO is negligible due to the conjugated phenyl group *via* π stacking. Representative examples are the works of Kim, D. -J. [131] and Yang Y. B. et al. [132] who showed the fabrication of electrical G FET sensor by linking amino groups functionalized nucleic acid aptamer with 1-pyrenebutanoic acid succinimidyl ester (PBSE) conjugated G through the formation of covalent amide bonds (Fig. 9c, Table 1). Kwon, O. S. et al. also fabricated a similar FET-type sensor by immobilizing RNA nucleic acid aptamer on the nitrogen-doped G (PPy-NDFLG) functionalized with glutaraldehyde-conjugated 1,5-diaminonaphthalene (DAN) *via* Schiff-base reaction [133] (Fig. 9d, Table 1). In these processes, the aromatic groups of organic linkers interact with the G basal plane through π -stacking, while the ester or carboxylate groups simultaneously covalent react with amino terminated nucleic acid aptamer to form an amide bond.

4. Electrochemical analysis of cancer biomarkers

4.1. Working principle of electrochemical biosensors

An electrochemical biosensor is composed of a biorecognition element integrated with an electrochemical signal transducer such

as electrode and FET. Generally, the working mechanism of the electrochemical biosensor is based on the change of current or voltage when the analyte is recognized by the biorecognition molecules. According to the signal transport behavior, electrochemical biosensors can be classified into voltammetric/amperometric, impedimetric, conductometric, potentiometric, and FET biosensor [134] (Fig. 10). Among all of the biosensors, voltammetric sensor is the most widely used approach and is flexible in signal output. Specifically, the voltammetric biosensor is operated by applying a potential to the working electrode and recording the current arises from the electrochemical oxidation/reduction of the analytes [135] (Fig. 10a). The applied voltage is ramped at a constant rate and the recorded current value is proportional to the concentration of analytes. Similar to voltammetric sensor, amperometric sensor also records the current when the working electrode is exposed to analyte molecules except that the potential is set at a desired value. Besides the biorecognition element, the amperometric biosensor also provides additional selectivity since the applied oxidation/reduction potential is the typical characteristic of analyte molecules. The usually used electrochemical techniques of voltammetric/amperometric biosensors can be organized into seven main types, including linear sweep voltammetry, alternating current voltammetry, cyclic voltammetry, chronoamperometry, chronocoulometry, chronopotentiometry, and pulse methods *etc.* [136–140] Impedimetric biosensor relies on the measurement of electrochemical impedance spectroscopy of the entire circuit in the analyte system [141] (Fig. 10b). In the presence of analytes, the migration rate of the redox species to the electrode surface is limited by the analyte molecules attached on the electrode surface at high frequency, resulting in a frequency-dependent phase lags between the alternating current voltage and current variation to realize selective sensing. The conductometric and capacitive biosensors measure the changes in the electrical conductivity and capacitance of the sensor as the component of the analyte solution changes during a chemical reaction [142] (Fig. 10c). Potentiometric

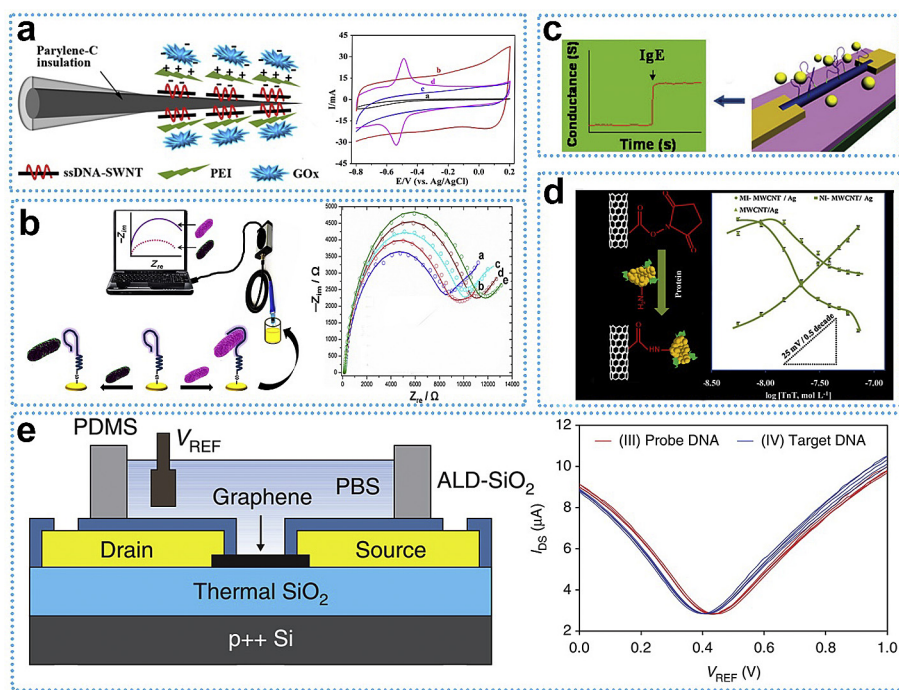


Fig. 10. The working principle of (a) single-stranded DNA functionalized SWNTs-based voltammetric sensor; (b) AuNP-modified screen-printed carbon electrode-based impedimetric sensor; (c) nucleic acid aptamer functionalized single polyaniline nanowire-based conductometric sensor; (d) MWNTs-based potentiometric biosensor; (e) G-based FET biosensor. Reproduced from Refs. [135,141–144]. (A colour version of this figure can be viewed online.)

biosensors include an electrochemical cell equipped with two reference electrodes and an ion-selective membrane [143] (Fig. 10d). The biological elements such as enzyme catalyst is used in the potentiometric sensor to produce the charged ions that can be react with the ion-selective membrane and then generate measurable potential. FET-based biosensor is composed of a semiconductor channel, two source/drain electrodes, and a solution gate electrode [144] (Fig. 10e). When the semiconductor channel incorporated with the biorecognition element is contact with the analyte environment, the source-drain current (carrier density) change is measured and the current is proportional to the analyte concentration. The FET-based biosensor exhibits the advantages of high sensitivity because that a FET sensor is composed of a sensor and an amplifier.

4.2. Specific biosensing

The qualification and quantification of trace amount of cancer biomarkers is of vital importance for the early diagnosis, prognosis, and predictive of cancer because of its high rates of recurrence and potential lethality [145–151]. Currently, the cancer relevant biomarkers can be classified to small organics, peptides, nucleic acids, protein and cells. Carbon nanomaterials-based electrochemical biosensors possess many advantages such as fast, low-cost, label-free, high sensitivity, simplicity, and real-time, providing a potential horizon in the field of diagnosis [152–155]. In this section, we will focus on the exploration of nucleic acid aptamer functionalized carbon nanomaterials-based electrochemical biosensor for the analysis of cancer relevant biomolecules.

4.2.1. Nucleic acids sensing

The development of real-time and sensitive sensing method for the quantification of trace amount of DNA is critical since DNA molecules can provide adequate information in the field of molecular biology, disease diagnostic and therapy, as well as

environmental monitoring [156,157]. So far, electrochemical biosensors and FET biosensors have been extensively investigated and explored for the detection of DNA [158–162]. Du, D. X. et al. fabricated a voltammetric electrochemical DNA biosensor by modifying the peptide nucleic acid (PNA) on the G electrode through a linker. Methylene blue (MB) was used as the electrochemical indicator [163] (Fig. 11a). The differential pulse voltammetry was recorded during the hybridization of PNA and DNA. The presence of G increases the electrode surface area, accelerates the electron transfer and enhances the electrochemical response. The neutral PNA molecules also eliminate the electrostatic repulsion between the PNA and DNA hybridized strands, resulting in a high affinity and selectivity for DNA. Consequently, this DNA sensor shows a wide dynamic range from 0.1 μM to 1.0 μM and a limit of detection of 0.5 μM . Compared with electrochemical sensors, FET sensors do not need electrochemical tags. Chen, T, -Y. et al. immobilize the probe DNA on the chemical vapor deposition (CVD) grown G to serve as a FET sensor for the detection of complementary DNA [164] (Fig. 11b, Table 1). With its high transconductance and large surface area, this designed FET biosensor shows a limit of detection of down to 1 μM . To further improve the sensing performance, Cai, B. J. et al. employed PNA modified rGO as the FET sensor platform for detecting DNA [165] (Fig. 11c). The use of PNA reduces the detection limit down to 100 fM, which is 1 order of magnitude lower than that of DNA conjugated probe. This sensor also can discriminate the complementary DNA from one-base mismatched DNA. The G and GO films also can be patterned into arrays to fabricate multifunctional FET biosensors for multiple DNA molecules sensing. In addition to the DNA molecules, the detection of microRNA can be used for classification, staging, and progression of cancer diagnostic and prognostic [166]. For instance, Kong, D. Q. et al. developed an *in situ* growth method to obtain 3D G films on Au substrates to serve as a voltammetric electrochemical platform for circulating miR-155 sensing [166] (Fig. 11d, Table 1). Typically, the G film with high electrical

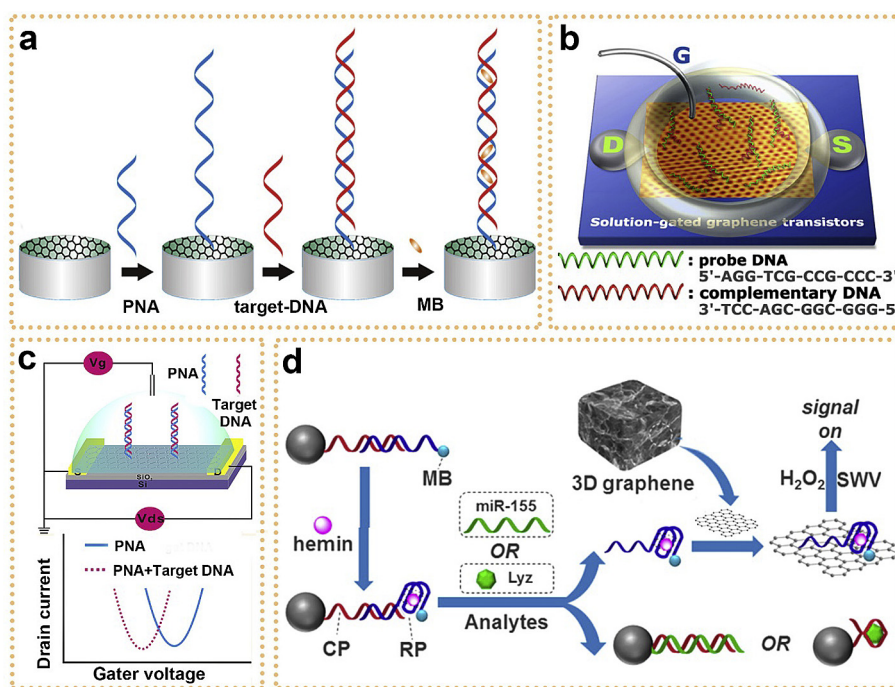


Fig. 11. (a) The fabrication procedure of electrochemical rGO modified GCEs. (b) Schematic model of solution-gated G FET. (c) Schematic drawing of the DNA sensing based on rGO FET biosensor. (d) The fabrication and working principle of 3D G films-based biosensor. CP represents the capture probe, and RP denotes the reporter probe. Reproduced from Ref. [163–166]. (A colour version of this figure can be viewed online.)

conductivity and large surface area acts as the electrochemical platform, and magnetic nanoparticles as carriers for probe DNA. Upon the introduction of target miRNA, the strand displacement and specific binding occurs, resulting in the high sensitivity signal-on detection of miRNA. After the optimization of conditions, this biosensor can be used in human serum samples and shows a low detection limit of 5.2 pM. This designed biosensor also can be utilized to detect protein such as lysozyme (Lyz) with high sensitivity and specificity, illustrating that the designed biosensor provides a powerful platform for a wide range of applications associated with clinical diagnostic and disease treatment. As a further step, the 3D G films with superior mechanical flexibility and strength can be applied as promising candidates to construct portable, flexible and wearable biosensor for monitoring analytes in real time.

4.2.2. Protein detection

Currently, a number of nucleic acid aptamer specific cancer relevant proteins have been developed such as, carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR) in glioblastoma, HER2 and MUC1 in breast carcinomas, CD30 and CD4 in lymphoma cells, epithelial cell adhesion molecule (EpCAM), prostate membrane-specific antigen (PMSA), α -Fetoprotein (AFP) in hepatocellular carcinoma, and Mucin 1 glycoprotein [167–169]. The recent developments of carbon nanomaterials have promoted their combinations with protein biomarkers specific nucleic acid aptamer for fast and selective sensing [170,171]. As a representative example, CEA is the mostly used cancer biomarker model to investigate the electrochemical performance of carbon nanomaterials-based biosensor. For example, Wen, W. et al. reported a triplex signal amplification amperometric electrochemical strategy based on hairpin-shaped nucleic acid aptamer (HO) functionalized gold nanorods (HO-GNRs) and G for highly sensitive sensing of CEA [172] (Fig. 12a, Table 1). Specifically, the GNR signal enhancer acts as a carrier for loading of horseradish peroxidase (HRP) and biotin modified HO. The presence of target CEA induces the binding reaction between CEA and HO, and then exposes the biotin. Subsequently, the HRP-GNRs-HO conjugates were captured on the streptavidin modified G electrodes owing to the strong binding interactions between biotin and streptavidin. The accumulation of HRP on the surface of G electrode promotes the catalytic oxidation of *o*-phenylenediamine (oPD) to form

2,3-diaminophenazine (DAP) and generates an electrochemical signal, resulting in the specific sensing of CEA. The fabricated biosensor exhibits a wide dynamic range of 5 $\mu\text{g mL}^{-1}$ to 50 ng mL^{-1} and relatively low detection limit of 1.5 $\mu\text{g mL}^{-1}$ due to the high surface area and superior electrical conductivity of G as well as the signal enhancement effect of GNRs which improves the sensitivity. Owing to the high stability of nucleic acid aptamer, this biosensor maintains excellent sensing properties when it was used for detection of serum samples from patients. This triplex signal amplification strategy also can be used to detect various cancer biomarkers by simply replace HO with different functional DNA nanostructures. As a further step, Wang, W. X. et al. [173] (Fig. 12b, Table 1) and Huang, J. -Y. et al. [174] (Fig. 12c, Table 1) further decrease the detection limit of CEA down to femto-gram level by exponential signal amplification strategy. Specifically, the former developed an impedance biosensor based on G as the sensing platform coupled with nucleic acid aptamer-switched bidirectional DNA polymerization strategy which combines the target recycling and exponential signal amplification ability. The latter designed a voltammetric electrochemical biosensor based on Pb^{2+} dependent DNAzyme-assisted signal amplification and GQDs-ionic liquid nafen (GQDs-IL-NF) composites as substrate for DNA immobilization. Specifically, the hairpin DNA with CEA specific aptamer and DNAzyme recognizes CEA and exposes the DNAzyme chains. Then, the DNAzyme hybridizes with MB-substrate chain and introduces a specific nicking site for Pb^{2+} . After the introduction of Pb^{2+} , the substrate chain is detached from the double-stranded DNA and cleaved into two fragments. At the same time, the released CEA-aptamer complex performs the signal amplification reaction and yields large amount of MB-substrates. Subsequently, the interaction of MB-substrates with GQDs produces a distinct and measurable electrochemical signal, resulting the specific CEA detection. Ge, L. et al. constructed an affinity-based differential pulse voltammetry sensor based on nucleic acid aptamer modified GO as recognition element and transducer for CEA detection [175] (Fig. 12d, Table 1). Methylene blue was used as the electrochemical probe and T7 exonuclease-assisted target recycling amplification was utilized to improve the detection sensitivity. This ultrasensitive biosensor also realizes the detection of CEA in a homogeneous solution without the immobilization of probe on electrode and acquires a limit of detection of 80 ag mL^{-1} . This homogeneous detection system holds great promise to analyze clinical biomolecules for disease

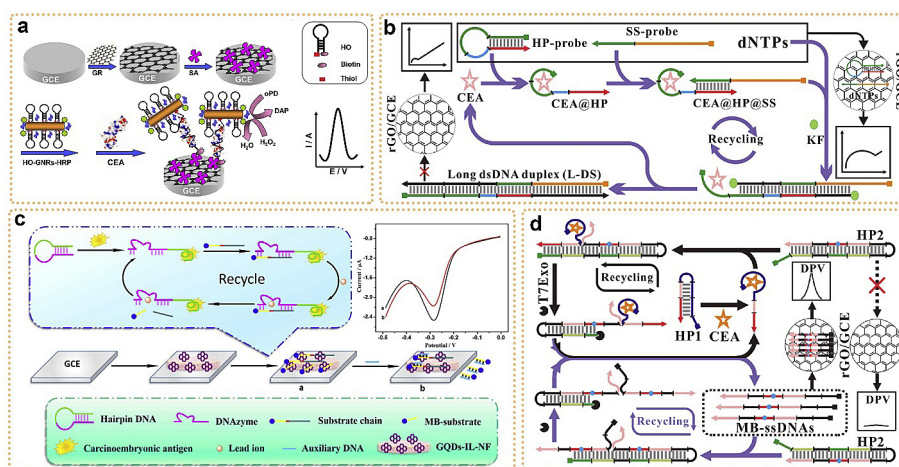


Fig. 12. (a) The fabrication procedures and sensing strategy of nucleic acid aptamer functionalized GNRs and G electrochemical sensing platform. (b) Schematic of G-assisted bidirectional DNA polymerizations impedance signal amplification strategy for the detection of CEA. (c) The preparation process and working principle of electrochemical CEA biosensor based on DNAzyme-assisted signal amplification and GQDs-IL-NF composites. (d) The working principle of affinity-based differential pulse voltammetry sensor based on nucleic acid aptamer modified rGO. Reproduced from Ref. [172–175]. (A colour version of this figure can be viewed online.)

diagnostic and biomedicine. Despite of CEA, other protein biomarkers such as MUC1, angiogenin, EpCAM, EGFR and HER2 have also been highly sensitivity detected by nucleic acid aptamer functionalized carbon nanomaterials electrochemical biosensors [132,176,177].

4.2.3. Cell sensing

In comparison to biomarkers such as nucleic acids and proteins, tumor cells possess large surface area and more binding sites [169]. A variety of nucleic acid aptamers target different cell types as well as crucial molecules on the cells surface have been exploited, which ensures the possibility to directly detect cancer cells in the practical blood samples [178–182]. Nucleic acid aptamer modified carbon nanomaterials-based electrochemical biosensors have been extensively designed to detect cancer cells [183]. Feng, L. Y. et al. simply employs nucleic acid aptamer AS1411 anchored on G to construct an impedance electrochemical biosensor for the target recognize nucleolin overexpressed on the cancer cell surface to realize specific cell detection and isolation [184] (Fig. 13a, Table 1). This sensor reaches a limit of detection of $794 \text{ cells mL}^{-1}$ and can be reused and regenerated with subsequent DNA hybridization technique. This simple design also can realize the detection of protein, small molecules, and nucleic acids by using different DNA nanostructures. However, this proposed strategy suffers from an intrinsic limitations in sensitivity since each or several nucleic acid aptamers can only capture one target cells. To solve this problem, Liu, H. Y. et al. designed a signal amplification supersandwich assay with nucleic acid aptamer-DNA concatamer quantum dots (QDs) as the recognition probe and MWCNTs/polydopamine/gold nanoparticles (MWCNTs@PDA@AuNPs) composites as the signal probe for highly sensitive detection of CCRF-CEM cells [185] (Fig. 13b, Table 1). As a result, a wide linear range of $10^2 \sim 10^6 \text{ cells mL}^{-1}$ and limit of detection of 50 cells mL^{-1} were obtained. The sensor also can discriminate cancer cells from normal cells due to the inclusion of human acute lymphoblastic leukemia (CCRF-CEM) cell specific nucleic acid aptamer. These superior performance arises from the following reasons: (1) The MWCNTs@PDA@AuNPs with high

electrical conductivity and large surface area promise the immobilization of concanavalin A (ConA) with high stability and bioactivity for specificity and sensitivity cell detection; (2) The signal amplification of nucleic acid aptamer-DNA concatamer QDs further improves the sensitivity dramatically. In addition to the specific detection of cancer cells, the efficient capture and enrichment of trace amount of cancer cells from serum or blood samples is of vital importance for the improvement of detection sensitivity and the realization of early cancer diagnostic. To improve the cell capture efficiency, Chen, X. J. et al. demonstrated a multivalent recognition and nucleic acid aptamer signal amplification strategy for sensing of CCRF-CEM cells and evaluating of N-glycan expression [186] (Fig. 13c, Table 1). This biosensor was composed of mannose binding protein Con A conjugated rGO modified with poly (amidoamine) dendrimers (rGO-DEN), as well as nucleic acid aptamer and horseradish peroxidase-modified AuNPs (HRP-nucleic acid aptamer-AuNPs) nanoprobe. The rGO-DEN accelerates the interface electron transfer rate as well as provides a multivalent recognition interface for efficient conjugation of Con A to improve the carbohydrate-protein interaction and enhance the cell capture efficiency. The AuNPs conjugated nucleic acid aptamer and HRP act as ultrasensitive electrochemical probe to improve the electrochemical catalysis signal of hydroquinone. Consequently, with its excellent conductivity, multivalent recognition ability, and biocompatibility, this designed ultrasensitive voltammetric electrochemical biosensor shows an ultralow limit of detection of 10 cells mL^{-1} . Based on the aforementioned results, the engineering of nucleic acid aptamer functionalized carbon nanomaterials has great potential in future clinical diagnosis and therapy.

5. Issues and challenges

During the past few decades, the nucleic acid aptamer functionalized carbon nanomaterials-based electrochemical biosensors in bioanalytical field have produced numerous advances due to their high sensitivity, low-cost and easy operation. However, it is prudent to give a comprehensive discussion of the issues and challenges that nucleic acid aptamer functionalized carbon nanomaterials-based biosensor faced before their practical clinical applications. In terms of materials preparation, the mechanical cleavage and CVD grown G exhibits excellent electrical conductivity, but the intrinsic hydrophobicity, low throughput and small size limit its application in electrochemical biosensors. The selection of suitable surface functionalization method and agents for engineering G nanoarchitectures with hydrophilicity and high surface area is an effective solution. However, the increase of yield and quality of single layer G is still to be resolved. GO with various surface oxygenated functionalization groups endows a relatively high surface area, while the electrical insulator performance inhibits its sensitivity as electrochemical biosensor. The elimination of partial oxygenated groups of GO or the combination of GO with high electrical conductivity CNT can make carbon nanomaterials with ideal electrochemical performance. For biosensor applications, the fabrication of large-area nanomaterials with high quality as well as the development of reliable surface modification technologies is critical for obtaining high performance biosensor with high reproducibility. Another challenge is the tailoring of bandgap of G through noncovalent approach is less successful than covalent method.

As for the practical electrochemical biosensor applications, the long-term toxicity analysis of the carbon nanomaterials or corresponding derivatives is vital importance for the *in vivo* recording the electrochemical signals. Additionally, the long-term stability of nucleic acid aptamer especially the RNA nucleic acid aptamer in the real biological and complex samples should be considered. The

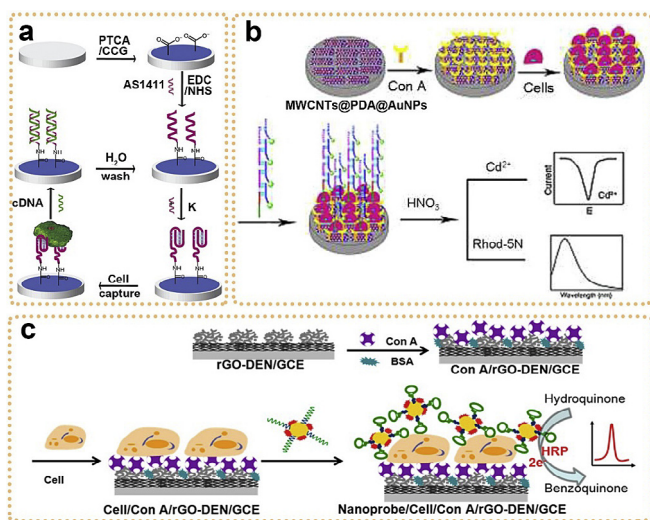


Fig. 13. (a) Schematic drawing of the AS1411 modified G electrochemical biosensor for the detection of cancer cell. PTCA/CCG represents the perylene tetracarboxylic acid modified G, and cDNA denotes complementary DNA. (b) The fabrication of MWNT@PDA@AuNPs-based supersandwich biosensor for highly sensitive detection of CCRF-CEM cells. (c) The fabrication procedures and working principle of rGO-DEN-based multivalent recognition and nucleic acid aptamer signal amplification biosensor for CCRF-CEM cell sensing. Reproduced from Ref. [184–186]. (A colour version of this figure can be viewed online.)

declared high sensitivity and selectivity are all performed at precisely controlled laboratory conditions. The presence of large amount of nontarget molecules, ions and particles would influence the specific recognition and undermine detection sensitivity. The relatively inadequate nucleic acid aptamer is an important issue and limits its real applications. Further developments of cell-SELEX techniques may offer more promising results and opportunities. The designing of functional nanocomposites to enable multifunctional systems for highly efficient, sensitive and selective detecting of multiple biomolecules simultaneously will significantly improve the detection accuracy and promote the development of precision medicine. The combination of precise diagnostic and fast therapy can provide new opportunities for current clinical medical. One advantage of carbon nanomaterials is that it can serve as both the sensing component and electrode with miniature size, promising its application in implanted and portable health device. However, the lack of suitable light-weight and flexible power source limits its development. In order to overcome these difficulties, further efforts are needed in the exploration of new multifunctional nanomaterials and sensor technologies to satisfy the requirements. Nevertheless, we believe that the nucleic acid aptamer functionalized carbon nanomaterials-based electrochemical biosensors would be an effective solution to current clinical diagnostic after resolving challenges they faced.

6. Conclusions and outlook

This review has focused on the recent progress in the development of nucleic acid aptamer functionalized carbon nanomaterials-based electrochemical biosensors from the perspective of carbon nanomaterials and biosensor applications. The carbon nanomaterials including fullerene, CDs, CNHs, CNT and G used for the construction of electrochemical biosensor were comprehensively reviewed and discussed. This review will provide a guideline for researchers to develop nucleic acid aptamer modified electrochemical biosensor in the respect of material synthesis and selection, as well as sensor fabrication. While the challenges in the optimization of materials and sensor fabrication techniques to enable high sensitivity, selectivity and reproducibility electrochemical biosensor are still need to be addressed. The future prospects of nucleic acid aptamer modified carbon nanomaterials-based electrochemical biosensor rely on the development of point-of-care and portable electrochemical devices with small size to satisfy the demands for mobile clinic diagnostic devices in medical fields. Additionally, the developments of digital diagnosis platform require the integration of carbon nanomaterials-based electrochemical biosensor with wireless transmitter, data processing system and memory circuits to satisfy the personal healthcare. Also, the requirement for real-time detection of disease provides new opportunities for developing flexible and wearable nucleic acid aptamer modified carbon nanomaterials-based sensors.

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