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Recent applications of carbon nanomaterials for microRNA electrochemical sensing

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Abstract: MicroRNA (miRNA) is an important tumor marker in human body, and its early detection has a great influence on the survival rate of patients. Although there are many detection methods for miRNA at present such as northern blotting, real-time quantitative polymerase chain reaction, microarrays, and others, the electrochemical biosensor has the advantages of low detection cost, small instrument size, simple operation, non-invasive detection and low consumption of reagents and solvents, so it plays an important role in the early detection of cancer. In addition, with the development of nanotechnology, nano-biosensors show great potential. The application of various nanomaterials in the development of electrochemical biosensor has greatly improved the detection sensitivity of electrochemical biosensor. Among them, carbon nanomaterials which have unique electrical, optical, physical and chemical properties have attracted increasing attention. In particular, they have a large surface area, good biocompatibility and conductivity. Therefore, carbon nanomaterials combined with electrochemical methods can be used to detect miRNA quickly, easily and sensitively. In this review, we systematically review recent applications of different carbon nanomaterials (carbon nanotubes, graphene and its derivatives, graphitic carbon nitride, carbon dots, graphene quantum dots and other carbon nanomaterials) for miRNA electrochemical detection. In addition, we demonstrate the future prospects of electrochemical biosensors modified by carbon nanomaterials for the detection of miRNAs, and some suggestions for their development in the near future.

1. Introduction

As we all know, cancer is a disease with a high fatality rate and is a huge threat to all people in the world.[1] It is reported that every minute approximately six people in the world are diagnosed with cancer.[2] Unfortunately, there are no effective treatment strategies to defeat cancer thoroughly. For example, traditional chemotherapy and radiotherapy are not selective and efficient in treatment of cancer.[3] While, the operation may also lead to a recurrence of cancer.[4] Many patients are diagnosed with cancer after the optimal period of treatment, so the cure rate of cancer is very low.[5] If there is a way to detect cancer in its early stages, it could greatly improve the cure rate for patients. Currently, ultrasound, computerized tomography and nuclear magnetic resonance tomography are used for cancer detection.[6] These diagnostic methods largely require expensive equipment and are not accurate enough to distinguish benign from malignant tumors.[7] Tumor markers are substances which exist in tumor cells and reflect the existence and growth of tumor.[8] The detection of tumor markers is important in the early diagnosis of cancer.[9] Therefore, finding effective tumor markers which can be diagnosed in the early stage of cancer, could reduce the mortality rate of patients and save millions of lives.[10]

Tumor markers are widely found in the blood, body fluids and tissues of the body.[5b, 11] They have strong analytical capabilities and can provide information about disease conditions for disease early assessment,[4b, 12] so their presence can better monitor the development of tumors.[13] Studies show that miRNA can act as a tumor marker and play an important role in early detection of cancer.[14] Because miRNA expressed in cancer cells is different from that in the normal cells, so the detection of miRNA can accurately and timely find cancer cells.[15] miRNA is a type of small non-coding RNA molecules (about 18-25 nucleotides), which is involved in regulating gene expression.[16] miRNA mainly contributes to post-transcriptional regulation through interacting with mRNA and subsequently silence gene expression.[17] It exists in all stages of biological processes, including cell growth, differentiation and development. Meanwhile it acts as a significant guiding role in gene expression. Once its level is dysregulated, it can cause many diseases, including cancer.[18] miRNA is highly stable in blood, body fluids and tissues of the body that is not easily degraded by RNAase,[19] and easy to be detected in a non-invasive way, so the successful detection of miRNA is very important for cancer patients, which can improve the cure rate of cancer with a high probability.

But the low abundance, short sequences and highly sequence homology between members of miRNA make it hard to be detected accurately.[20] Traditional methods for detecting miRNA include northern blotting, RT-PCR, microarrays, and others.[15, 21] However, each of them has its own limitations, for example, the northern blotting has the disadvantages of low detection sensitivity, cumbersome operation and the requirement of large number of RNA samples;[22] RT-PCR and microarrays have a low detection limit but higher cost.[23] In comparison with them, biosensor provides a simple and effective method for detecting miRNA. The most widely used methods are electrochemical biosensors and fluorescence biosensors.[24] Biosensors can be used to detect whether a gene's miRNA expression is abnormal, thus indicating the occurrence of a disease.[25] The fluorescence biosensors use the basic principle of fluorescence resonance energy transfer, based on fluorescence emitting or quenching to detect the target analyte.[26] Compared to fluorescent biosensors, electrochemical biosensors are constructed based on the immobilization of biological molecules on the surface of solid electrodes by specific recognition between biological molecules and target molecules. Thus, the target molecule can be analyzed qualitatively and quantitatively by the change of electrical signal.[27] Although different biosensors use different mechanisms, they all play an important role in the early diagnosis of cancer. In comparison with other detection technologies, electrochemical biosensors have many unique features such as low detection costs, miniaturized instruments, simple operation, non-invasive testing possible and low consumption of reagents and solvents, especially high sensitivity and specificity.[20a, 28] Thus, electrochemical biosensors may play an important role in future miRNA analysis. In addition, nanomaterials have been widely used to construct electrochemical biosensors due to their advantages such as high conductivity, large specific surface area and good repeatability.[29] Advances in nanomaterials may provide an encouraging new approach to improve the sensitivity and selectivity of electrochemical biosensors.[30]

As an element abundant in nature, carbon has been widely used in science and technology.[31] A variety of carbon allotropes can be synthesized by changing the hybrid combination of sp, sp^2 and sp^3, and a series of carbon-based nanostructures have emerged.[32] In recent years, researchers have gradually discovered the unique advantages of carbon nanomaterials in the field of electrochemical detection of miRNA.[33] Firstly, carbon nanomaterials can combine with electrolytes and biological probes directly, accelerating signal transduction, enhancing analytical sensitivity and reducing detection limits.[34] Secondly, the high surface area and biocompatibility of carbon nanomaterials enable them to load large quantities of specific biological probes and act as dispersible capture agents to separate and capture miRNAs.[35] Moreover, carbon nanomaterials can be used as electrode support substrate or medium to regulate electron transfer process.[36] Owing to these advantages, carbon nanomaterials can significantly improve the performance of electrochemical biosensors, making them highly compatible in miRNA electrochemical sensing.

In comparison with other nanomaterials, carbon nanomaterials are superior in hardness, optical properties, heat resistance, electrical conductivity, surface and interfacial properties for miRNAs electrochemical sensing.[37] For instance, carbon nanomaterials have unique photoelectric properties, good biocompatibility, good surface function and high reactivity.[38] In particular, carbon nanomaterials have a good ability to adsorb nucleic acids and can be used in electrochemical biosensors to detect miRNA, which has aroused widespread interests among researchers.[39] In addition, carbon nanomaterials are excellent electrode materials for electrochemical biosensors in improving electrocatalytic performance, conductivity and long-term stability in recent years.[40] Because the electrochemical biosensors modified with carbon nanomaterials usually have higher sensitivity, lower detection limit and faster electron transfer capability than traditional detection electrodes, there are more and more reports about the development and application of carbon nanomaterials in electrochemical biosensors.[38b, 41] Among these carbon nanomaterials, the most widely used are carbon nanotubes (CNTs), graphene and its derivatives, graphitic carbon nitride (g-C_3N_4), carbon dots (CDs) and graphene quantum dots (GQDs).[42]

Hence, in this article, we will focus on miRNA, an important tumor marker in human body, comprehensively review recent advances in carbon nanomaterials-based miRNA electrochemical biosensors, which is divided into six parts according to different types of carbon nanomaterials: CNTs, graphene and its derivatives, g-C_3N_4, CDs, GQDs and other carbon nanomaterials. **(Figure 1)**. Finally, possible development prospects of this field in the future are proposed.

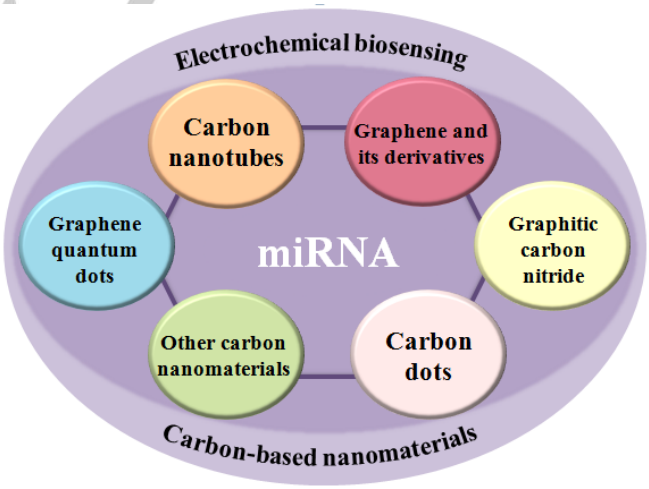

Figure 1. Carbon nanomaterials for miRNA electrochemical biosensing.

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2. Carbon nanomaterials for microRNA electrochemical biosensing

Due to the excellent properties of carbon nanomaterials, such as good electrical conductivity, large specific surface area, biocompatibility and non-toxicity and etc.,[38c, 43] many types of carbon nanomaterials such as CNTs, graphene and its derivatives, g-C_3N_4, CDs and GQDs are widely used in miRNA electrochemical detection. In this section, we will review some achievements of miRNA electrochemical sensing based on carbon nanomaterials, mainly CNTs, graphene and its derivatives, g-C_3N_4, CDs and GQDs and others, in the past few years.

2.1. Carbon nanotubes for microRNA electrochemical biosensing

As a very excellent electrode material for electrochemical biosensors, CNTs have attracted much attention in recent years because of their advantages such as high surface area and suitable volume ratio, excellent electronic properties and the presence of defects like edge planes.[44] Due to these excellent characteristics, CNTs have been widely used in the detection of tumor markers.[45] What's more, CNTs can be divided into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs).[46] SWCNTs are carbon atoms arranged on a graphene sheet,[47] in which the graphene sheet is formed into a cylindrical structure.[48] While, MWCNTs are multiple. Whether SWCNTs or MWCNTs, the perfect combination of them with electrochemical biosensors is remarkable. Here are some specific examples as follows. Li et al.[49] developed a simple, label-free and sensitive electrochemical biosensor using MWCNTs. This biosensor can detect miRNA-24 by monitoring the oxidation signal of guanine, a synthetic DNA probe complementary to miRNA-24 which was immobilized on the surface of a MWCNTs-modified glassy carbon electrode (GCE) by covalent cross-linking. The probes were hybridized with different concentrations of miRNA-24. The formation of hybrids on the electrode surface was evaluated by differential pulse voltammetry (DPV). Due to the hybridization between the probe and miRNA-24, a change in the guanine oxidation signal was observed, thereby miRNA-24 could be detected. The results showed that under the optimal conditions, the proposed miRNA-24 biosensor had good sensitivity (4.963 μA cm^{-2} $decade^{-1}$), low detection limit (1 pM), good selectivity and reproducibility. The biosensor also had acceptable recoveries for miRNA-24 detection in complex miRNA samples. The same simple and unlabeled electrochemical biosensors using MWCNTs have been developed by different researchers. For example, Tran's group proposed to use miRNA-141 as a biomarker to detect prostate cancer.[50] They used an unlabeled and reagent-free test which included an interpenetrated network of MWCNTs and an electroactive polymer with embedded quinone groups in the backbone to covalent attach the DNA probe. When miRNA-141 targets were added, the signal response was observed and causing a current increase, but it was not sensitive to other tumor markers. The detection limit of this sensor was 8 fM, which had a very good application prospect in prostate cancer.

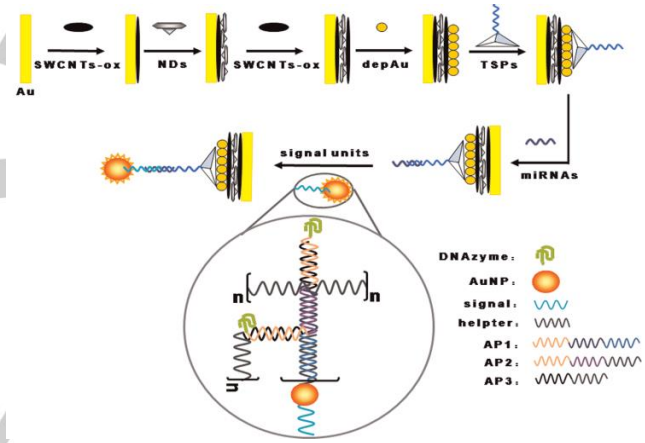

Figure 2. Schematic illustrations of the electrochemical biosensor. Note: n represents the number of cycles. Reprinted with permission from ref. 51. Copyright 2015 Elsevier.

Moreover, CNTs can form composites with other carbon nanomaterials to improve the detection of miRNA. For example, Liu et al. assembled the electrode layer by layer with oxidized SWCNTs and nano-diamond to make an electrochemical sensor. A tetrahedron-structured probe with a free-standing probe at the top can be used as a receptor for direct hybridization with the target miRNA **(Figure 2)**.[51] The probe was attached to the deposited Au nanoparticles (AuNPs) by a strong Au-S bond, which can detect electrical signals. The electrochemical signal was recorded by DPV. The electrochemical signal increased linearly with the concentration of miRNA-21 and demonstrated good repeatability and stability, high sensitivity with the linear detection range of 10 fM to 1.0 nM and detection limit of 1.95 fM (S/N=3). Similarly, Tran et al. proposed a strategy, using a secondary antibody which conjugated to horseradish peroxidase (HRP) to set up an electrochemical ELISA-like amplification system, based on screen-printed gold electrodes modified with reduced graphene oxide (rGO) and CNTs.[52] Hydroquinone was oxidized to benzoquinone under HRP/H_2O_2 catalysis. Then, benzoquinone was electrically reduced to hydroquinone at the

electrode. The catalytic reduction current was related to the amount of HRP fixed on the surface, and the amount of HRP itself was related to miRNA. In this way, miRNA levels in cancer cells were detected, and the detection limit was up to 8 fM. Because of the excellent electrochemical properties of carbon nanomaterials, many researchers used different kinds of nanomaterials to modify electrode to achieve the ideal detection limit. Therefore, the detection sensitivity of other carbon nanomaterials modified CNT electrodes will be improved, but the experimental operation will be relatively difficult.

Because CNTs have a large surface area and good electrical conductivity, they can load multiple molecules to achieve multiple sensing and improve detection efficiency.[53] To achieve this goal, Deng et al. designed two DNA probe fragments to hybrid with miRNA-21 and miRNAs model analytes.[54] DNA probe 1 (P1) was assembled on an AuNPs modified electrode, and miRNA-21 was fixed to P1. Then, a large amount of thionin (Thi) was loaded on shortened multi-walled carbon nanotubes (S-SWCNTs) by covalently bound probe 2 (P2). S-SWCNTs were synthesized as signal nanoprobes and the conductivity of the high-load Thi and AuNPs hybridized with miRNA-21 on S-MWCNTs thus increased, resulting in an increased current response at the electrode. The increased electrochemical current enabled it to quantitatively detect miRNA-21. Chen et al. developed a new electrochemical biometric method for the detection of miRNA-21 by combining a one-step biometric reaction based on vertically aligned nanotubes with T7 exonuclease assisted target recovery on a biosensor **(Figure 3)**.[55] When target miRNA-21 was hybridized with DNA, it can also trigger T7 exogenous auxiliary target recovery to realize signal amplification, thus further effectively identifying miRNA-21. It showed a linear range from 0.01 pM to 100 pM and a detection limit of 3.5 fM. Due to the good biocompatibility of CNTs, it is a good method to combine enzyme molecules with CNTs to improve the sensitivity. Compared to the electrode modified by CNTs alone, the electrochemical sensor constructed by combining enzyme molecules with CNTs has a lower detection limit.

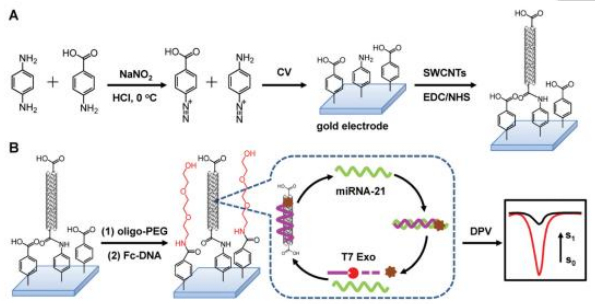

Figure 3. Schematic illustration of (A) the preparation process of the vertically aligned SWCNTs-modified electrode and (B) the miRNA-21 biosensor along with its electrochemical signal transduction principle. Reprinted with permission from ref. 55. Copyright 2019 The Royal Society of Chemistry.

Nanocomposites based on CNTs and other conductive materials are also developed for the construction of electrochemical biosensors. AuNPs are widely used as excellent electrode surface materials for biosensing including miRNA detection due to their high surface energy, catalytic efficiency, biocompatibility and conductivity.[56] Therefore, a great number of papers have reported to use CNTs to combine with AuNPs for optimal miRNA detection.[57] For instance, Abbas Sabahi et al. designed an electrochemical sensor to detect miRNA based on thiolated receptor probe-functionalized dendritic gold nanostructures which grafted SWCNTs on the surface of the fluorinedoped tin oxide electrode via self-assembly monolayer process.[58] The sensor combined the advantages of electrochemical methods and nanomaterials. Moreover, the sensor utilized cadmium ions (Cd^{2+}) as a signal unit and signal amplification to mark the miRNA-21. The detected oxidation Cd^{2+} signal was linear with the miRNA-21 concentration. It showed a low experimental detection limit of 0.01 fM. It also can be used to detect miRNA-21 in human serum samples and to diagnose prostate cancer in clinic. In comparison with the electrochemical sensor based on sole CNTs modified electrode, the biosensor on the basis of the composite nanometer electrode material had better detection sensitivity which was largely attributed to the excellent conductive properties of the AuNPs material, though the experimental operation was relatively tedious.[59] Therefore, combining CNTs and other conductive materials to construct hybrid electrodes is a trend of electrochemical sensors.

Taken together, as one of the carbon nanomaterials, CNTs have the advantages of super large surface area, extremely low background signal, label free detection and high sensitivity. In the field of miRNA electrochemical sensing, the excellent conductivity of CNTs can significantly realize signal conversion and amplification. In particular, the specific surface area of CNTs endows them with ability to absorb nucleic acid probes effectively, thus improving the sensitivity and the detection throughput of electrochemical sensors. Compared to other nanomaterials, CNTs can effectively immobilize nucleic acid probe molecules and protect these biomolecules from being digested or degraded by enzymes in the biological environment. Moreover, the special rigid structure endows them with improved detection stability during electrochemical detection process.

2.2 Graphene for microRNA electrochemical biosensing

Graphene is a single-layered structure composed of carbon atom layers combined into a honeycomb lattice.[24, 60] Since the successful preparation of graphene in 2004,[61] graphene and its derivatives show broad application prospects in the field of biochemistry due to their large specific surface area, excellent electrical conductivity, and good biocompatibility, including the field of electrochemical biosensors.[62] Moreover, significant progresses have been made in the successful detection of miRNA, which provide an excellent approach for the early detection of tumors.[5b, 63] Tugba Kilic et al. reported an electrochemical biosensor using graphene-modified disposable pencil graphite electrode (GME) for electrochemical detection of miRNA-21 in cell lysates.[64] In comparison with the unmodified pencil graphite electrode (PGE), the detection limit of this biosensor was lower, which was 2.09 mg/mL. Moreover, this biosensor worked well in breast cancer cell lysates. Based on the good detection performance of graphene, its derivatives also have significant achievements in the detection of miRNA. As an example, Abdollah Salimi et al. proposed an amino graphene (AGr)-based sensing platform for the detection of miRNA-155, and analyzed its complementary target miRNA-155 hybridization using DPV.[65] The as-prepared biosensors were able to detect miRNA-155 at nanogram concentration levels.

Apart from graphene, graphene oxide (GO) is the most important graphene derivative with multiple oxygen-containing functional groups which has also been utilized for miRNA detection.[66] Its large surface area makes it an excellent support

material to attach biomolecules.[67] For instance, Ece Eksin et al. used disposable pencil graphite modified GO as electrode to make an electrochemical sensor for miRNA detection by measuring guanine oxidation signals.[68] The amino linked DNA probes were immobilized with GO-modified PGE as the solid phase, and thus complementary target RNA sequences (miRNA-34a) were identified. The results showed that the sensor had good specificity. Similarly, Sun et al. developed an electrochemical biosensor for quantitative detection of miRNA-21 based on GO. DNA labeled with Ag nanoparticles (AgNPs) was decorated the surface of the electrode.[69] When DNA probes hybridized with miRNA-21, AgNPs-labeled DNA probes were released back into the solution. The concentration of miRNA can be calculated by detecting the silver dissolution current of AgNPs.

With the unique advantages of graphene and its derivatives, new electrochemical biosensors have developed for improved sensitivity and lower detection limits.[70] The electrochemical biosensors using electrochemical impedance spectroscopy (EIS) technology to record impedance changes for miRNA detection have made a new breakthrough in the improvement of detection limit and sensitivity.[43b, 71] For example, GO modified graphite electrodes to detect the impedance of miRNA-34a was described in a study by Deniz Isin's group.[72] After chemical activation with covalent agent (CA) on the surface of PGE, GO modification was performed on the chemically activated PGE surface. After hybridization between the miRNA-34a target and its complementary DNA probe, the hybrid was immobilized on the surface of CA-GO-PGEs. By optimizing the effect of GO concentration, DNA probe concentration and miRNA-34a concentration on biosensor response, the selectivity of miRNA-34a biosensor was tested under optimal conditions. Similarly, Gulsah Congur et al. designed an electrochemical biosensor for the specific identification of miRNA-34a by modifying PGE with GO.[73] The DNA probe complementary to miRNA-34a was combined with the miRNA-34a target and fixed on the surface of GO-PGE. The EIS technique was used to record the change in charge transfer resistance value after each modification and fixation step. It can provide the impedance change before and after the biometric recognition process on the electrode surface to estimate the detection limit of miRNA-34a and selectivity to other miRNA sequences. Compared to other detection methods, the method of recording the resistance value using EIS technology is more accurate for miRNA detection.

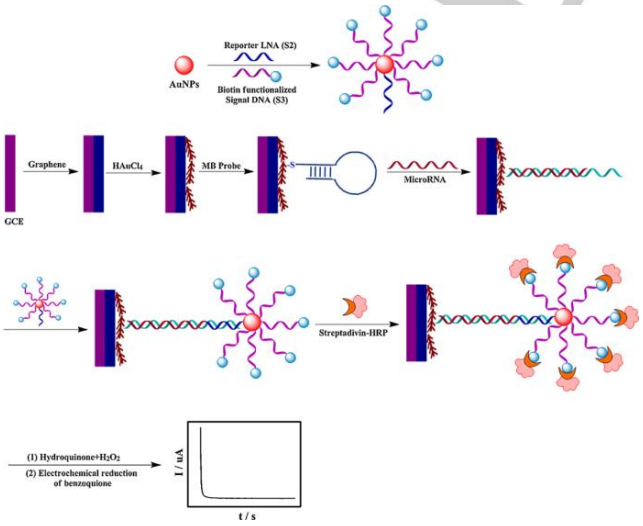

Figure 4. Chronoamperometry determination of miRNA hybridization through three steps of amplification. Reprinted with permission from ref. 77. Copyright 2012 Elsevier.

In addition to graphene and its derivatives, their nano-composites also have been devoted to miRNA detection.[7, 74] The composite materials of AuNPs and graphene are widely used as electrode materials for electrochemical biosensors due to their excellent electron transfer ability and large specific surface area.[75] Based on the nanocomposites between AuNPs and graphene, lower detection limit can be achieved, which are preferred by cancer patients.[76] For instance, Yin et al. modified the glassy carbon electrode with AuNPs and graphene nanosheets to construct an electrochemical biosensor for the detection of miRNA-21 without miRNA-21 markers **(Figure 4)**.[77] Sulfydryl functionalized locked nucleic acid integrated with hairpin molecule beacon probe was acted as miRNA-21 capture probe. The biosensor exhibited good selectivity and high sensitivity, and the detection limit was 0.06 pM. Moreover, miRNA-21 expression could be analyzed in human liver cancer bel-7402 cells and normal liver L02 cells based on this sensor. Furthermore, studies have shown that miRNA-199a-5p is an important tumor marker in the early diagnosis of Triple Negative Breast Cancer.[78] Ammar Ebrahimi et al. designed an electrochemical biosensor to detect miRNA-199a-5p in serum using a modified GCE consisting of GO and gold nanorods which was immobilized with a thiolated probe.[79] This electrochemical biosensor had an ideal sensitivity and accuracy in the evaluation of miRNA-199-5p, and can be used to determine the relatively low concentration of miRNA-199-5p in serum samples. Similarly, Bao et al. successfully developed a sensitive and effective electrochemical miRNA sensing platform by integrating AuNPs/polypyrrole reduced graphene oxide (Au/ppy-rGO), catalytic hairpin assembly (CHA), and hybrid chain reaction multi-signal amplification strategy.[80] The Au/ppy-rGO electrode position onto the bare GCE greatly improved conductivity through effectively immobilizing the probe and smoothly detecting electrochemical signals. The sensor was able to detect miRNA-16 at concentrations ranging from 10 fM to 5 nM, with a minimum detection limit of 1.57 fM (S/N=3). Moreover, Tian et al. developed an electrochemical biosensor for the detection of miRNA-155 with high sensitivity and specificity which used a tetrahedral DNA nanostructure-based biomolecular probe modified on 3D nitrogen-doped rGO/AuNPs (3D N-doped rGO/AuNPs) electrode surface.[81] After added the target molecule miRNA-155, gold and silver nanorods/thionine/complementary DNA (AuAgNR/Thi/F) were hybridized with the target molecule for signal amplification and detection of miRNA-155. The detection limit was 10^{-12} M, and the specificity in serum was very high. In addition, Wang et al. prepared a self-powered miRNA electrochemical biosensor using sulfur-selenium co-doped graphene/AuNPs as electrode modification materials.[82] The obtained biosensor showed a detection limit of 1.5×10^{-16} M with a linear range from 0.5 to 10000 fM, which had a great potential in clinical application. As above-mentioned, the graphene electrode combined with AuNPs has better chemical conductivity, so its sensitivity is better for miRNA detection.

To further enhance detection performance, the electrochemical biosensor was fabricated with gold nanometer and GO composite modified electrode. And the method of target recovery assisted by double chain specific nuclease was applied to detect miRNA.[83] For example, Han et al. proposed a cascade amplification system based on duplex-specific nuclease (DSN)

assisted target recovery and electrocatalytic water pyrolysis and AuNP/GO composite modified electrode for detection of miRNA.[84] In the system, RNA heteroduplexes hybridized with DNA probes on the surface of AuNPs, which can be hydrolyzed by DSN. Then miRNA was released into solution and can be recycled to multiple DNA probes. This process utilized AuNPs/GO nanocomposites to provide a large surface area, a special affinity for water molecules, and to facilitate mass diffusion of the water fission reaction. The biosensor achieved a limit detection of 1.5 fM in 80 minutes, with a linear detection range of about 4 orders of magnitude which had a great clinical application potential. Futhermore, Xiao et al. designed an electrochemical biosensor with carboxylate-rGO (COOH-rGO) and AuNPs modified electrodes for miRNA-21 detection **(Figure 5)**.[85] And then, capture DNA (cDNA) was self-assembled on the surface of gold electrode (GE) and hybridized with the target miRNA-21 to form cDNA/miRNA-21 hybrids. Then the DSN digested cDNA and released miRNA-21 for circulation, resulting in a significant reduction in cDNA sequence length. Moreover, the sensor had an excellent applicability to detect the target miRNA-21 in human serum samples. On the basis of the combination of graphene and AuNPs, DSN was added. Although the experimental steps were relatively complex, the presence of enzyme molecules made the electrochemical sensor more accurate in the detection of miRNA.

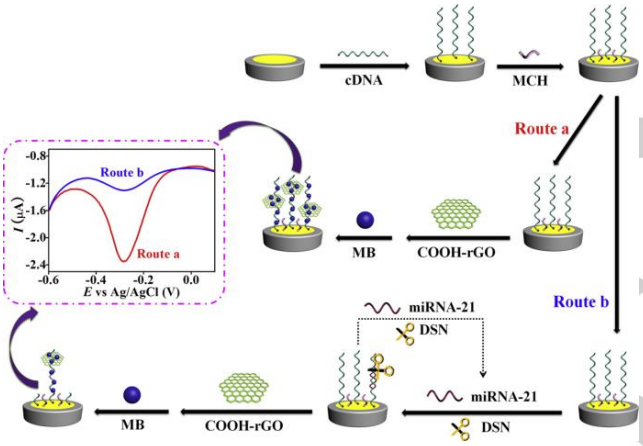

Figure 5. Fabrication process of electrochemical biosensor and its application for miRNA-21. Reprinted with permission from ref. 85. Copyright 2019 Elsevier.

Using the nanocomposites of graphene and metal oxide to modify the electrodes of the electrochemical sensor is also a good detection strategy. Tungsten oxide (WO_3) is an important transition metal oxide with the advantages of low cost, non-toxic, abundant source and simple manufacturing process.[86] Graphene and WO_3 composites have attracted extensive attention due to their high surface area, good electrical conductivity and chemical stability.[87] As an example, Shuai's group designed an ultra-sensitive electrochemical sensor using WO_3-graphene composite and AuNPs combined with catalytic hairpin assembly target recovery and enzyme signal amplification to detect miRNA **(Figure 6)**.[88] The two hairpin DNA probes (H1 and H2) coexisted stably in the solution. When the target miRNA was added, the target miRNA would hybridize with target H1. Subsequently, H2 would hybridize with H1 in the solution, releasing miRNA for the next round of H1 hybridization. During this process, each target miRNA can be hybridized with a large number of signal probes for highly sensitive miRNA detection, the detection limit of which can be as low as 0.05 fM (S/N=3). Moreover, magnesium oxide (MgO) nanostructures which have good physical and chemical properties have attracted significant attention in biosensing.[89] For instance, Shuai et al. developed an ultra-sensitive sandwich-type miRNA electrochemical biosensor based on MgO nanoflower and GO-AuNPs hybrids coupling with electrochemical-chemical-chemical (ECC) detection system.[90] When miRNA was present, DNA probe and miRNA would form a sandwich complex for ECC reaction in the system to amplify detection signal and improve detection sensitivity. This strategy had good dynamic characteristics in the range of 0.1-100 fM with the detection limit of 50 aM. In comparison with the combination of graphene and AuNPs with DSN, the combination of oxidized substances makes the experimental procedures clearer and easier. In addition, sandwich-type strategy used in biosensor fabrication usually can increase its specificity and sensitivity greatly,[91] and the detection limit of miRNA was lower.

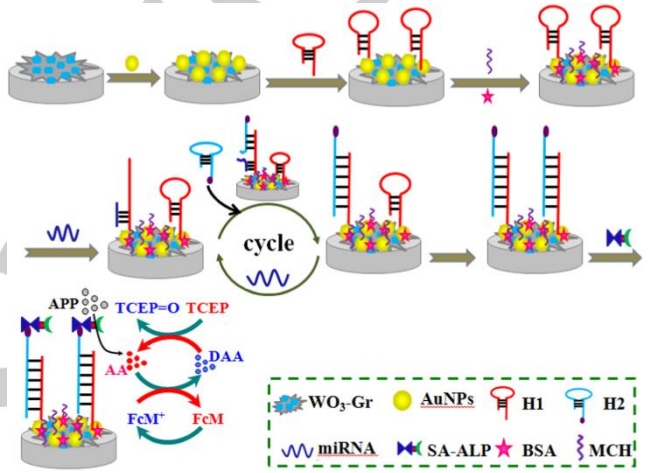

Figure 6. Schematic illustration of sensors based on CHA target miRNA recycling and signal amplification of ALP coupled with ECC redox cycling for miRNA detection. Reprinted with permission from ref. 88. Copyright 2016 Elsevier.

Apart from excellent electrical conductivity, ductility and covalent modification capabilities, graphene and its derivatives have a larger surface area and aromatic structure, thus miRNA can be stacked on the surface of them through π-π stacking interactions and/or Vander Waals forces. Benefiting from these advantages, graphene and its derivatives can be ideal electrode materials for miRNA electrochemical sensing. In comparison with other carbon nanomaterials, graphene and its derivatives have superior advantages such as cheaper fabrication methods, being free of metallic impurities which may cause domination of electrochemical signals.

2.3 Graphitic carbon nitride for microRNA electrochemical biosensing

g-C_3N_4 is a kind of carbon-based nanomaterial with controllable morphology and active surface area.[92] In specific, g-C_3N_4 is a metal-free polymer semiconductor with a medium band gap of 2.65 eV that forms a two-dimensional (2D) layered structure through sp^2 hybridization between carbon and nitrogen atoms, resulting in a graphene-like coupled electron system.[93] Benefiting from its unique structure, g-C_3N_4 has the advantages of high thermal stability, good physicochemical stability, good visible light activity, simple preparation, non-toxic and so on, in

which is preferred for electrochemical sensing and other related fields.[94]

For miRNA sensing, g-C_3N_4 is widely used in the construction of electrochemiluminescence (ECL) biosensors and photoelectrichemical (PEC) biosensors due to its excellent photoelectric characteristics.[95] In addition, g-C_3N_4 is a new ECL emitter with high quantum yield, it's high load capacity and good catalytic activity have attracted wide attention.[96] To further improve the performance of luminescent groups and expand their application scope, researchers usually combined g-C_3N_4 with metals and metal oxides.[97] For example, Shao et al. reported a novel Faraday cage-type ECL biosensor based on potential analytical strategy.[94c] In this biosensor, hairpin DNA1 (HP1) and hairpin DNA2 (HP2) were immobilized on the Fe_3O_4@Au nanocomposites as the capture units. Besides, the g-C_3N_4@AuNPs nanocomposites tagged with signal DNA1 (sDNA1) and the ruthenium-based metal organic framework nanosheets tagged with signal DNA2 (sDNA2) were used as signal units. Based on this, miRNA-141 and miRNA-21 double targets can be detected within the linear range of 1 fM-10 pM with the detection limit of 0.3 fM. Meanwhile, the proposed miRNA detection method is highly selective and sensitive, and can be used for actual analysis in human serum. Apart from metal organic framework nanosheets, AuNPs also has been used to combine with g-C_3N_4 for enhanced electrical activity and effective surface area, thus improving the detection performance of miRNA.[98] For exmaple, Feng's group used g-C_3N_4 nanosheets and AuNPs synthesizing a kind of nanocomposite to fabricate a dual-wavelength ratiometric ECL biosensor, which can accurately quantify the concentration of miRNA-21 over a wide range of 1.0 fM to 1.0 nM **(Figure 7)**.[99] In this strategy, a hairpin structure molecular beacon and enzyme-assisted cycle amplification were used to improve the sensitivity and for resonance energy transfer (RET) between AuNPs-g-C_3N_4 nanohybrid and $Ru(bpy)_3^{2+}$. Only after hybridization with target miRNA, the hairpin structure would open up, accompany with the close of probe DNA-$Ru(bpy)_3^{2+}$ and AuNPs-g-C_3N_4 nanohybrid, resulting in an energy transfer from AuNPs-g-C_3N_4 nanohybrid to $Ru(bpy)_3^{2+}$. Both the decline of ECL intensity from AuNPs-g-C_3N_4 nanohybrid at 460 nm and the increase of ECL intensity from $Ru(bpy)_3^{2+}$ at 620 nm could reflect the amount of target miRNA. Compared to single wavelength ECL detection, the method of dual-wavelength is more reliable.

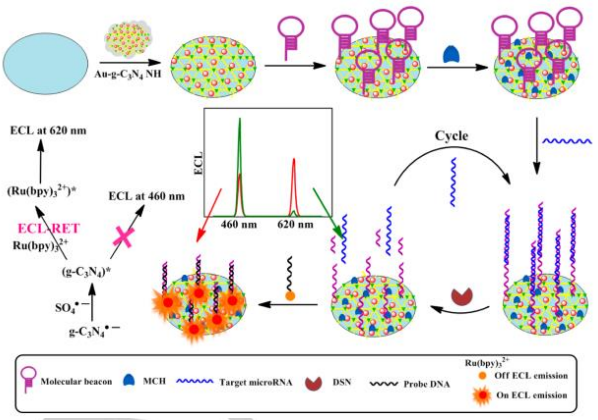

Figure 7. Schematic illustration of the dual-wavelength ratiometric ECL-RET biosensor configuration strategy. Reprinted with permission from ref. 99. Copyright 2016 American Chemical Society.

In addition to ECL method, PEC detection method based on g-C_3N_4 has also attracted extensive attention because the excitation light source and electrical readout signal are part of different energy forms, endowing it with a low background signal and high sensitivity.[93b, 100] For instance, Wang's group reported a novel PEC biosensor for miRNA detection based on a ternary MoS_2/g-C_3N_4/black TiO_2 heterojunction as the photoactive material, and the AuNPs carrying Histostar antibodies (Histostar@AuNPs) for signal amplification.[101] In this PEC biosensor, MoS_2/g-C_3N_4/black TiO_2 was first deposited on an indium tin oxide electrode surface, then AuNPs and probe DNA were assembled on the modified electrode. The hybridization formed by probe DNA and miRNA-396a can be recognized by the S9.6 antibody. Then, the captured antibody can further conjugate with the secondary IgG antibodies of Histostar@AuNPs, which lead to the immobilization of the HRP. In the presence of HRP, H_2O_2 accelerated the oxidation of 4-chloro-1-naphthol, producing the insoluble product benzo-4-chloro-hexadienone on the electrode surface and resulting in a significant decrease of photocurrent. This strategy could detect miRNA-396a at concentrations from 0.5 fM to 5000 fM, with a detection limit of 0.13 fM. Similarly, Yin et al. proposed a more simple detection method by using the antibody to detect miRNA.[102] In this work, a signal-on PEC biosensor was fabricated for miRNA detection using the prepared g-C_3N_4-AuNPs nanocomposites as photoactive material, anti-DNA: RNA antibody as miRNA recognition unit and alkaline phosphatase (ALP) as catalytic signal amplification unit. After the probe DNA was hybridized with the target miRNA, the DNA-RNA hybrid can be recognized by RNA antibodies, which can be further captured on the electrode surface. The antibodies specifically interacted with the alkaline phosphatase labeled IgG (ALP-IgG) to capture ALP-IgG and catalyze the hydrolysis of ascorbic acid-2-phosphate (AAP) to produce ascorbic acid as an electron donor. Thus, strong photocurrent can be obtained, and the expression level of miRNA can be detected according to the change of photocurrent.

To sum up, g-C_3N_4 nanomaterials exhibit the characteristics of high stability, good biocompatibility and non-toxicity. In particular, the synergistic effect of g-C_3N_4 combined with other nanomaterials, such as AuNPs or MoS_2, can further improve light absorption efficiency, electron transfer and signal response. Therefore, both of ECL sensors and PEC sensors constructed based on g-C_3N_4 show great potential for the sensitive detection of miRNA.[93c, 103] In addition, due to the 2D structure and high specific surface area of g-C_3N_4 nanomaterials, they are more easily integrated with other functional materials than CDs and GQDs in ECL or PEC sensing.[104]

2.4 Carbon dots for microRNA electrochemical biosensing

CDs, as one of the emerging carbon nanomaterials in recent years, have its unique properties.[105] Their advantages such as large specific surface area, abundant edge position, intrinsic catalytic activity, high affinity and specificity to the target make it popular in the field of electrochemical biosensor.[106] In particular, because of their excellent optical properties, CDs have made great achievements in the detection of nucleic acids, proteins, glucose and other aspects.[107] Moreover, for the detection of miRNA, CDs are a kind of excellent electrode material, generally existing as composites which are combined with other materials to improve sensitivity.[108] Owing to the excellent photoelectric characteristics of CDs, they have been more and more widely

studied in the ECL biosensor.[109] For example, Zhong's group prepared a novel autocatalytic ECL luminescent group by loading amino-modified CDs onto the AuNPs-modified TiO_2NPs (CDs-Au-PEI@TiO_2) surface and applied it to construct an ECL biosensor for the sensitive detection of miRNA-21.[110] With the concentration of miRNA-21 increased from 0.1 fM to 10 pM, the response of ECL gradually decreased, and the detection limit was 0.03 fM. In the system, the initial ECL strength of the prepared CDs-Au-PEI@TiO_2 nanocomposite was strong, which could accelerate the development of TiO_2NPs to the electrochemical reduction of co-reactive H_2O_2, overcoming the lack of strong oxidization of persulfate in the traditional CDs-based ECL biosensors and improving the detection sensitivity.

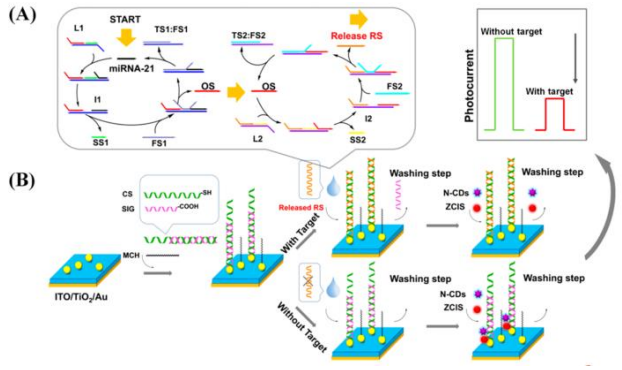

Figure 8. (A) Schematic illustration of dual cascade TSDA initiated by miRNA-21; (B) Construction process of the designed PEC biosensor for miRNA-21 detection. Reprinted with permission from ref. 115. Copyright 2018 American Chemical Society.

The photochemical properties of CDs enable the photochemical sensor to successfully detect miRNA.[111] On this basis, PEC bioanalysis, as a new analytical technique, has been proved to be a good biological detection method.[112] Currently, many PEC detection methods have been developed for biomolecular detection.[113, 114] For instance, a PEC biosensor for miRNA-21 detection based on cosensitization of biocompatible $CuInS_2$/ZnS QDs (ZCIS QDs) and N-doped CDs (N-CDs) coupled with dual cascade toehold-mediated strand displacement amplification (dual cascade TSDA) was developed by Chu et al. **(Figure 8)**.[115] In this sensor, TiO_2/Au hybrid structure was utilized to immobilize double stranded DNA, which could capture glutathione stabilized ZCIS QDs and N-CDs. The original TiO_2/Au/ZCIS/N-CDs structure could form a cascade band gap arrangement, which improved PEC performance vividly. Target miRNA initiated enzyme-free dual cascade TSDA, and consumed both the substrate and the fuel, eventually released reporter strands to suppress photocurrent signals. The detection limit of miRNA-21 can be as low as 0.31 pM.

In this part, two types of miRNA biosensor (ECL biosensor and PEC biosensor) based on CDs are reviewed. Using TiO_2/Au/ZCIS/N-CDs co-sensitive structure and enzyme-free dual cascade TSDA, a PEC biosensor for miRNA-21 detection was realized. Although this detection method had the advantages of environmental protection, simple post-processing and lower cost, its detection sensitivity was lower than ECL biosensor, which used the method combining CDs composite nanomaterials with DSN auxiliary loop amplification. In general, CDs show high affinity and specificity in miRNA electrochemical sensing. Moreover, CDs also have the advantages of large surface area, non-toxicity, and good stability, making them suitable for potential clinical miRNA electrochemical sensing. Compared to other nanomaterials, CDs have good photoelectric properties and abundant active sites. Though their applications in the field of miRNA electrochemical sensing are relatively fewer, they show great development potentials.

2.5 Graphene quantum dots for microRNA electrochemical biosensing

In recent years, GQDs have attracted tremendous attention for their potentials in the field of biology, photoelectricity and others.[35a, 116] As a new kind of carbon nanomaterial, GQDs not only have the good properties of CDs, but also own some excellent properties of graphene,[42, 117] such as large surface area, unique electron mobility and chemical stability.[118] Due to their unique electron mobility, they have made some achievements in the field of electrochemistry.[119] For example, Hu et al. demonstrated an electrochemical biosensor assisted by HRP catalyzed reaction to establish a specific and sensitive method for the quantitative detection of miRNA **(Figure 9)**.[120] The electrochemical biosensor was constructed based on double-stranded DNA structure, in which the activated GQDs were assembled on NH_2-DNA. Then GQDs were used as a platform for non-covalent assembly and immobilization of HRP. The biosensor modified by HRP can effectively catalyze the oxidation reaction of 3, 3, 5, 5-tetramethylbenzidine mediated by H_2O_2, which changed the color of the solution from colorless to blue and increased the electrochemical current signal. Due to GQDs and enzyme catalysis, the biosensor can sensitively detect miRNA-155 between 1 fM to 100 pM, with detection limit of 0.14 fM. Making use of the combination of GQDs and enzyme to detect miRNA, this method had the advantages of simple, easy operation and effective improvement of detection sensitivity.

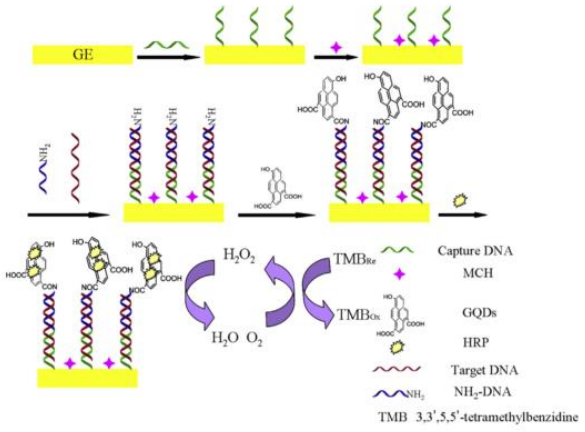

Figure 9. Principle of the enzyme catalytic amplification of miRNA-155 detection with GQDs-based electrochemical biosensor. Reprinted with permission from ref. 120. Copyright 2016 Elsevier.

In addition, GQDs also have made progress in photochemical sensors because of their excellent optical properties and electrical conductivity.[56b, 71a] On the basis of electrochemical detection, many papers have put forward ECL detection and achieved good results.[121] In this respect, Zhang et al. constructed a photochemical biosensor which used GQDs combined with boron (named as BGQDs) to detect oncogene miRNA-20a.[122] BGQDs-labeled hairpin DNA can be self-assembled on the surface of AuNPs modified platinum electrodes. After hybridization with the target molecule miRNA-20a, the spatial structure of hairpin DNA changed, leading to changes in ECL signal. The ECL biosensor with BGQDs as the

electrode material was within the linear range of 0.1-1 × 10^4 pM. The quantitative limit was 0.1 pM. And the detection of miRNA-20a had a good analytical performance and good specificity. Some studies have incorporated the method of exonuclease assisted circulation amplification into photochemical sensors based GQDs. For instance, Zhang et al. prepared a 3D DNA-mediated silver-enhanced biosensor based on T7 exonuclease assisted circulation amplification and GQDs for miRNA analysis.[123] In this biosensor, aminated-3, 4, 9, 10-perylenetetracarboxylic acid (PTCA-NH_2) was introduced to load GQDs through π-π stacking (the product was named as GQDs/PTCA-NH_2) to obtain the solid-state GQDs. Then, Fe_3O_4-Au core-shell nanocomposites (Au@Fe_3O_4) was adopted as probe anchor to form a novel ECL signal tag of GQDs/PTCA-NH_2/Au@Fe_3O_4, which made the ECL signal marker modified on the electrode surface. Afterwards, hairpin probe modified on the electrode was opened by helper DNA. Finally, the 3D DNA network of T7 exonuclease and AgNPs were used to enhance the ECL signal. After two signal amplifications, the ECL signal of GQDs was significantly enhanced, and the as-prepared biosensor had a high sensitivity, with a detection limit of 0.83 fM. Both of the above-mentioned two methods are excellent in miRNA detection, but the former method is simpler to operate, only combining GQDs and boron to assemble on the surface of platinum electrode modified with AuNPs, and can also conduct quantitative detection. Moreover, compared to the pure electrochemical detection method, its linear range is more extensive.

Similar to CDs, GQDs exhibit low toxicity, excellent solubility, satisfactory surface grating and good biocompatibility, which enable them to be efficiently applied in miRNA electrochemical sensing. In comparison with other nanomaterials, GQDs have the advantages of graphene and CDs. In addition, GQDs have large surface area and abundant edge sites, which make them as good electron donors and electron acceptors.

2.6 Other carbon nanomaterials for microRNA electrochemical biosensing

In addition to the above-mentioned several carbon nanomaterials, there are other carbon-based materials have been developed for miRNA electrochemical sensing, such as fullerenes, carbon black (CB), carbon nanaofibers (CNFs), carbon sphere (CS), carbon nanohorns (CNHs), carbon nanoonions (CNOs) and so on. Fullerenes have excellent electrical conductivity, charge transfer, physical behavior, and effective charge separation.[124] Fullerene-based sensors have been extensively studied in the detection of various biomolecules including miRNA. For example, Zhou's group developed a double-amplification strategy to detect low levels of miRNA-141 by combining click-chemistry mediated enzyme-assisted target recovery with amino and sulfhydryl multifunctional fullerene nanoparticles (FC60).[125] The FC60 was modified on an Au electrode for signal amplification. Meanwhile, two broken nucleic acid chains contained G-quadruplex were connected through click chemistry. And the obtained DNA hairpin loop which was complementary with the target sequence can hybrid with the target sequence forming DNA-RNA duplexes. And the resulted DNA-RNA duplexes could be cleaved by a DSN, accompany with the release of the free miRNA, thus triggering another cycle and realizing exponential amplification of the signal. In another work, Liu et al. developed an ECL biosensor based on functionalized fullerenes.[126] In this work, pyrrolidyl fullerenes derivative was recruited as an unadulterated and congruent nano-hub to converge three zinc porphyrins on its monopole. Such peculiar assembly was convinced via micro-imaging and spectrophotometry. A multiplied ECL emission bursted out from the porphyrin trinity in a synergistic manner by taking full advantage of fullerenyl proficiency in catalytic singlet O_2 generation and excited-state preservation. Without any prebio-conjugation, this orderly ECL-active individual turned to anchor in the toroid of a peripherally modified gamma-cyclodextrin in a good shape match. This in situ terminal labeling strategy during the identification of induced allosteric events enabled signal enhancement for the detection of miRNA markers.

CB is a low-cost conductive nanomaterial consisting of amorphous and quasi-graphitized nanoparticles with particle sizes of 3.0-100 nm.[127] CB has excellent electrical conductivity, numerous defect sites and fast electron transfer dynamics.[128] Moreover, CB can dispersible in solvents and easy to be functionalized.[129] Based on these advantages, CB is emerging to be a valuable nanomaterial in the construction of miRNA sensors. For example, Yammouri et al. prepared an economical, simple and sensitive electrochemical biosensor for miRNA-21 detection based on the nanocomplexes based on CB and AuNPs.[130] The biosensor used methylene blue (MB) to label mercapto capture probe (miRNA-21 complementary sequence). After hybridization with the target miRNA-21, the direction of the labeled capture probe changed, leading to a decrease in MB oxidation reaction. The response of MB before and after hybridization was monitored by DPV to detect the concentration of miRNA. The results showed that the detection limit of this strategy reached 1 fM and had a wide linear range between 2.9 fM-0.7 fM. In addition, good reproducibility, stability and excellent selectivity were achieved, along with satisfactory results when miRNA-21 was analysed in serum.

CNFs are a kind of nano-sized carbon material with fiber structure whose outer wall has many edge sites without any hollow core.[131] In addition, they have excellent mechanical, thermodynamic and electrical properties, so that it is a promising nanomaterial and show the potential for wide applications.[132] For instance, Erdem's group developed a traceless biosensor for miRNA-34a detection by using CNF enriched disposable screen printed electrodes (CNF-SPEs).[133] In this work, an amino linked DNA probe that complementary to miRNA-34a target was immobilized onto the surface of CNF-SPEs. After measuring the guanine oxidation signal by DPV, sequence selective hybridization detection was performed between the DNA probe and the miRNA-34a target, or other miRNA sequences. miRNA-15a or miRNA-660 were selected as non-complementary sequences. Finally, the detection limit was 10.98 μg/mL with the linear concentration range of target miRNA-34a from 25 to 100 μg/mL by using CNF-SPEs. The further improvement of CNF sensor has made it possible for the development of miRNA sensor platforms to achieve faster and more economical detection.

CS is a kind of ideal electrochemical biosensor electrode material due to their high surface area, ideal electrical conductivity, negligible aggregation and the ability to fill space efficiently.[134] In comparison with other carbon nanomaterials, CS has the advantages of high packing density and high capacity.[135] In particular, combining CS with other nanomaterials is an effective way to realize the sensitive detection of miRNA. For instance, Chen et al. designed a sandwich-type electrochemical biosensing platform based on

CS-MoS_2 and target recycling amplification of CHA for ultra-sensitive determination of miRNA.[91] The capture probe was fixed on the AuNPs/CS-MoS_2 modified GCE through the Au-S bond. When the target miRNA-21 showed up, the CHA occurred and then a mass of avidin-HRP enzymes were brought onto the electrode as a signal indicator via specific binding of avidin-biotin. Finally, the miRNA-21 can be detected within the linear range of 1×10^{-16} to 1×10^{-10} M with the detection limit of 1.6×10^{-17} M.

3 Summary and outlook

Tumor markers are a class of substances that can reflect the existence and growth of tumors, which are produced by tumor cell gene expression, or by the body's response to tumor cells. The detection of tumor markers has important practical value in the early screening of tumors, auxiliary diagnosis, observation of curative effects, disease monitoring, prognosis judgment and molecular targeted therapy. In particular, as an important tumor marker in the body, miRNA can regulate genes in the human body, whose detection is of great importance for the early diagnosis of tumor. In recent years, because of their excellent physical and chemical properties, different carbon nanomaterials such as CNTs, graphene and its derivatives, g-C_3N_4, CDs, GQDs and others have been widely used in the construction of electrochemical sensors for miRNA detection.

This review article mainly focuses on the use of electrochemical biosensors for miRNAs detection, highlighting the excellent detection capabilities of electrochemical biosensors modified with different carbon nanomaterials. Up to now, CNTs and graphene have been most widely used in the miRNA electrochemical sensing. CNTs have rigid strength, good elasticity, fatigue resistance and isotropy, which greatly improve the performance of electrochemical sensors. However, the modification process is too complicated. And there are many defects after modification, such as uneven diameter growth and poor length control, which need to be improved. Graphene and its derivatives show great potential for the construction of miRNA biosensors owing to their unique conductive properties and nucleic acid adsorption capacity. In particular, GO contains many carboxyl and hydroxyl groups on its surface, endowing it with excellent water solubility, which is suitable for simple covalent functionalization and biomolecular adsorption. Therefore, based on the excellent properties of graphene and its derivatives, more functional graphene-based nanocomposites should be explored in the future. Similar to graphene, g-C_3N_4 is a kind of 2D carbon nanomaterial, which has excellent thermal stability and physicochemical stability. Although it has strong binding ability with other functional materials, its functional properties are not as good as graphene. CDs and GQDs are newly emerging carbon nanomaterials. In comparison with other carbon nanomaterials, they have excellent photoelectric properties and are widely used in ECL and PEC sensing platforms, which exhibit high detection sensitivity for miRNA detection. However, more convenient strategies need to be explored to avoid cumbersome detection steps. Other carbon nanomaterials such as fullerenes, CB, and CNFs also have excellent electron transfer ability and biocompatibility, but their applications in miRNA electrochemical sensing are few and need to be further explored.

In general, the electrochemical biosensors modified by carbon nanomaterials have higher sensitivity, better selectivity, wider detection range and lower cost. In particular, the nontoxicity of carbon nanomaterials makes electrochemical biosensors have a wider range of applications especially biomedical applications. Although electrochemical biosensors incorporating carbon nanomaterials or their nanocomposites have many advantages, a huge room challenges are still remained to overcome for further applications. For example, there are a large part of technologies are still at their theoretical stage, which should be combined with clinical practice for further applications. In addition, seeking out carbon nanocomposites with better properties or better surface modification methods is in urgent need for improving detection performance to meet the requirements of high biocompatibility, long-term stability and high repeatability. For intance, AuNPs with excellent conductivity are good candidates to combine with carbon nanomaterials to increase detection sensitivity. In addition, developing electrochemical biosensors with higher stability and repeatability is another challenge.

The application of carbon nanomaterials and its nanocomposites for miRNA detection has yet to be fully explored. Further research effort should be devoted to investigating their potential for clinical application. For instance, the size, shape and morphology of carbon nanomaterials should be controlled and adjusted appropriately to obtain higher biocompatibility. Signal amplification strategies, such as hybrid chain reaction, CHA, rolling cycle amplification and DSN assisted target recovery can be considered to combine with carbon nanomaterials to achieve high sensitivity and improved detection performance. Nevertheless, multi-mode detection method such as ECL or PEC detection based on carbon nanomaterials have achieved breakthroughs, which indicate the feasibility of using carbon nanomaterials and its nanocomposites for innovative applications.

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Keywords: electrochemical sensing, miRNA, carbon nanomaterials

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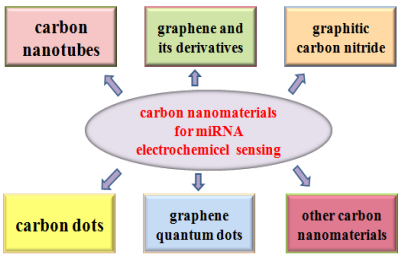

miRNA is an important tumor marker in human body, which is of great significance to human health. In this paper, the applications of different carbon nanomaterials (carbon nanotubes, graphene and its derivatives, graphitic carbon nitride, carbon dots, graphene quantum dots and other carbon nanomaterials) in miRNA electrochemical sensing are reviewed systematically, and some future development prospects are also proposed.