Electrochemical mercury biosensors based on advanced nanomaterials

Tao Liu, Zhenyu Chu* and Wanqin Jin*

Mercury ion (Hg$^{2+}$) is a typical heavy metal ion that shows strong toxicity and readily accumulates in the human body; it is a source of severe pollution in ecological systems. The detection of trace Hg$^{2+}$ is an active research area for developing accurate and sensitive electrochemical biosensors. Recently, numerous novel nanomaterials have been successfully designed with remarkable advantages in Hg$^{2+}$ recognition due to their strong electrocatalysis, specific surface areas, conductivities, etc. This review aims to present the latest progress in advanced nanomaterials for electrochemical Hg$^{2+}$ detection.

Emphasis will be given to the main detection mechanisms and nanostructure control methodology of these nanomaterials. Meanwhile, the performance of various nanomaterials-based mercury biosensors will be compared to discuss their positive effects on the detection performance of different nanostructures. This article is expected to provide inspiration for the design of novel nanomaterials to construct high-performance electrochemical mercury biosensors.

1. Introduction

Mercury ion (Hg$^{2+}$) is an extremely toxic heavy ion that causes severe damage to the environment and human health even at low concentrations.1 Currently, it is widely present in soil, water and air due to various human industrial activities, such as gold mining, exhaust emission, wastewater discharge and combustion of fossil fuels.2,3 With the assistance of microorganisms, the inorganic mercury element can be transformed to organic mercury, which is easily absorbed by animals and plants and then spreads through the food chain into human bodies.4 The non-biodegradability and facile accumulation of mercury ions in the human body can lead to various cognitive and motor disorders as well as to death. Therefore, the maximum permitted level of Hg$^{2+}$ in drinking water is 10 nM (2 μg L$^{-1}$), as regulated by the United States Environmental Protection Agency (EPA) and the World Health Organization (WHO).

The principal issue in mercury pollution treatment is identification of the pollution source as early as possible. In the past few decades, numerous analytical methods have been continuously developed for the quantitative detection of Hg$^{2+}$, such as atomic absorption/emission spectroscopy (ABS/AES),5 atomic fluorescence spectrometry (AFS),6 inductively coupled plasma mass spectrometry (ICP-MS),7 X-ray fluorescence spectrometry8 and surface enhanced Raman scattering (SERS).9 Although these methods show good sensitivity and selectivity, most of them require great expense and long analysis periods with large, heavy instruments; this presents difficulties in on-site and immediate recognition of Hg$^{2+}$ leakage.4,10 Since the principle of biosensing technology was first proposed by Clark and Lyon in the 1960s,11 electrochemical biosensors have obtained high-speed development and wide usage in the fields of environmental protection,12 food safety,13 fermentation processes14 and medical health monitoring.15 In general, a biosensor is composed of a biological recognition unit and a signal conversion module. According to the different categories of biological recognition units, electrochemical biosensors can be categorized as enzyme biosensors,16 DNA biosensors,17 immunosensors18 and cell and tissue biosensors.19 From 2003 to 2018, there was a ten-fold increase in the number of literature reports involving the construction of novel electrochemical mercury biosensors. Especially due to the fast progress of nanoscience, increasing numbers of researchers are gaining interest in the design and synthesis of novel nanomaterials to realize comprehensive improvement of detection performance.20 A general conclusion has been confirmed that the nanostructures of the electrode material dominate the electrochemical behaviour between the biological recognition unit and the target, greatly affecting the generation, transfer and reporting of the detection signal.21 From one to three-dimensional nanostructures, control of the material morphology plays an essential role in obviously improving the sensitivity, detection limit and accuracy of biosensors. Various novel nanomaterials have emerged in the construction of advanced mercury biosensors.22,23 However, few reviews have summarized the recent progress of these nanomaterials and nanostructure control methods.
In this review, we mainly focus on the applications of new nanomaterials for electrochemical Hg$^{2+}$ recognition and analysis. The working principles and characteristics of various biosensors will be overviewed to introduce their specific requirements in electrode materials. Emphasis will be placed on discussion of the advantages and deficiencies of these nanomaterials according to the different material categories. Additionally, the preparation and control strategies of these nanomaterials will be discussed to evaluate the general possibility of their large-scale production. Finally, the overall performance of reported electrochemical Hg$^{2+}$ biosensors is compared to show the advances in nanomaterial employment. We hope to present an overview of recently developed nanomaterials and their synthesis strategies as well as the performance of these nanostructured materials in Hg$^{2+}$ detection. This review of the characteristics and synthesis methodologies of nanomaterials may inspire the design and preparation of more nanomaterials for continuous improvement of biosensing performance.

2. Detection strategies of electrochemical Hg$^{2+}$ biosensors

Electrochemical biosensors mainly function by receiving an electric signal change during reaction of a biocomponent with the target. For Hg$^{2+}$ detection, enzymes$^{24}$ and DNA$^{25}$ are often selected as the recognition biocomponents. Additionally, mercury(II) recognition can be realized using the stripping voltammetry method without any biocomponent.$^{26}$ This section will mainly discuss the above three strategies of Hg$^{2+}$ detection, referring to their working principles and recent progress.

2.1 Enzyme inhibition strategy

It is well known that heavy metal ions do great damage to the structures of proteins, causing protein denaturation. With regard to enzymes, their active centers, which may contain hydroxyl, carboxyl, thiol and selenol groups,$^{27}$ play the most critical role in the redox function to produce the electrochemical signal. Complexation reactions can easily occur between mercury ion and these groups, leading to a decrease of the enzyme activity. Accordingly, in the construction of electrochemical mercury biosensors, the enzyme inhibition strategy was adopted earlier than other methods.

The working mechanism of an electrochemical enzyme inhibition-based biosensor is shown in Fig. 1a. Normally, the substrate is catalyzed by the active center of the corresponding enzyme to generate electron transfer to the supporting electrode. In the presence of a certain inhibitor, the active center will be rapidly damaged and induce weak catalysis, causing an obvious decrease in the response signal. Hence, the concentration of the inhibitor can be determined indirectly through changes in the signal intensity. In addition, common inhibitors include heavy metal ions (e.g. Hg$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Cr$^{4+}$ and Cu$^{2+}$), drugs, insecticides, and nerve agents.$^{28}$

To our best knowledge, in 1962, Guilbault et al.$^{1}$ first developed an electrochemical biosensor based on the enzyme inhibition principle.$^{29}$ In their work, the enzyme cholinesterase was employed to detect nerve agents which act as enzyme inhibitors. Since then, this type of biosensor has attracted increasing attention. In 1981, Liu and his co-workers provided an enzyme inhibition strategy for the detection of Hg$^{2+}$ with the assistance of glucose oxidase for the first time.$^{30}$ The detection limit of this biosensor was 500 μM. In addition to the most commonly applied glucose oxidase, urease,$^{31}$ invertase$^{32}$ and hydrogen peroxide$^{33}$ are applied in the Hg$^{2+}$ assay. Among these enzyme inhibition-based biosensors, the average detection limit of Hg$^{2+}$ is one to several μM, and the lowest limit can reach 18 pM.

However, due to the irreversible damage to enzymes caused by mercury(n), this type of biosensor can only be used one time. In addition, as mentioned before, there are many substances that can cause inhibition effects. Hence, enzyme inhibition-based Hg$^{2+}$ biosensors often show poor specificity, restricting their further applications in real water samples.

2.2 DNA probe principle

Due to the weak selectivity of the enzyme inhibition method, a superior detection strategy is desirable. In 2004, Akira Ono and Humika Togashi first reported a sensing system which could specifically assay mercury(n).$^{34}$ Selective binding between mercury(n) and thymine–thymine (T–T) base pairs occurred in DNA duplexes. In the thymine structure, as shown in Fig. 1b, the imino proton of N3 was directly replaced by mercury(II), bridging two thymine bases in order to generate the T–Hg$^{2+}$–T mismatch. This structure showed great selectivity and was even more stable than the Watson–Crick type base pairs in natural DNA via coordination bonds. Based on the stable and selective mismatch structure, numerous biosensors have been designed for trace detection of mercury(n).

Indeed, most of these biosensors contain thymine-rich single-stranded DNA, which shows excellent flexibility and mercury(n) recognition ability. In the presence of Hg$^{2+}$, the response signal changed through the redox reaction intensity of the signal indicators by the electrode due to the transformation between the flexible single-stranded DNA and rigid double-stranded DNA. In this way, the concentration of mercury(n) could be measured quantitatively and accurately.
Actually, there are four different sensing strategies based on T–Hg$^{2+}$–T complexes. Electrochemical redox indicators are widely utilized to report signal changes during the last three detection processes.

**Simple approach strategy.** This is the simplest strategy for the detection of Hg$^{2+}$ through T–Hg$^{2+}$–T without any electrochemical redox indicators. Zhang et al. developed a graphene-based Hg$^{2+}$ biosensor, where [Ru(NH$_3$)$_6$]$^{3+}$ was applied to indicate the hybridization of two oligonucleotides. In this work, dopamine was adopted to reduce the graphene oxide and modify the surface of rGO to provide more amino groups for immobilization of the DNA probe. In the presence of mercury(II), the capture probe and target probe hybridized via T–Hg$^{2+}$–T coordination chemistry, contributing to an enhancement in the peak current of [Ru(NH$_3$)$_6$]$^{3+}$. As a result, the limit of detection (LOD) of this biosensor could reach 5 nM with a linear range from 8 to 100 nM. Although this type of biosensor can be easily designed and prepared, it suffers from high background noise and low accuracy. In order to achieve a stronger response signal, some electrochemical indicators have been applied. Normally, these indicators are required to possess excellent redox ability and conductivity in order to evidently enhance the electric signal and rapidly transfer electrons. As confirmed, the distance between the electrode and the indicator is another key characteristic that greatly affects the redox degree and the signal strength. In this case, another type of DNA response method was developed to focus on this issue.

**Conformational switch-based signal-on biosensors.** With regard to signal-on biosensors, the electrochemical response signal is enhanced in the presence of Hg$^{2+}$ through the conformational switch of the DNA probe, pushing the indicator close to the electrode. In order to obtain a lower detection limit through greatly improving the strength of the response signal, Zhang et al. constructed a sensitive biosensor through a coupling target-induced conformational switch of DNA hairpins. The hairpin probe was immobilized on the electrode through a terminal thiol with ferrocene (Fc) labeled in the middle of the sequence. In the presence of mercury(II), the T–Hg$^{2+}$–T coordination caused a conformational change at the end of the probe to open the hairpins. With the transformation from rigid hairpins to flexible single-stranded DNA, the ferrocene indicator moved close to the electrode for generation of the signal. This biosensor showed good sensitivity and repeatability, with a linear range from 5 nM to 1 μM and a detection limit of 2.5 nM. In addition to ferrocene, methylene blue (MB) is a popular electrochemical indicator in the construction of Hg$^{2+}$ biosensors. H. Fan’s group recently reported a novel biosensor based on a hairpin hindrance method with methylene blue as an indicator. In their research, hairpin capture DNA and linear signal DNA labelled with methylene blue (MB) were immobilized on Au nanoparticles. When mercury(II) appeared, with the help of the specific helper probe DNA, the formation of a T–Hg$^{2+}$–T mismatch opened the hairpin structure of the capture DNA, bringing the MB molecular close to the electrode. In this way, a strong differential pulse voltammetry (DPV) peak appeared via the redox reaction of methylene blue. This biosensor had a wide linear range from 0.35 pM to 3.5 nM, with a LOD of 0.21 pM.

**Conformational switch-based signal-off biosensors.** In contrast, in some designs, the conformational switch of DNA causes the indicator to move away from the electrode, resulting in a weak response signal; this is called a signal-off biosensor. Xiong et al. constructed a dual-signaling electrochemical ratiometric biosensor to realize the specific and reusable detection of Hg$^{2+}$. The methylene blue (MB)-labeled T-rich hairpin DNA probe was immobilized onto a gold electrode, showing a high peak intensity at a potential of −0.3 V. After the addition of Hg$^{2+}$, another T-rich DNA probe which was labeled with ferrocene (Fc) could hybridize the MB-labeled probe through T–Hg$^{2+}$–T coordination. The methylene blue moved away from the electrode as the ferrocene came close to the electrode, leading to a decrease in the peak at the potential of −0.3 V as well as peak enhancement at 0.3 V. This biosensor possessed a linear range between 0.5 and 5000 nM with a low detection limit of 0.08 nM. In another study, Zhang et al. developed a signal-off electrochemical biosensor based on the remarkable difference in the affinity of graphene, as shown in Fig. 2a. In this detection platform, a ferrocene-tagged ssDNA probe was immobilized on the surface of graphene with a strong redox response signal. While detecting Hg$^{2+}$, the Fc-labeled probe was hybridized with the target probe via a T–Hg$^{2+}$–T base pair, in which case the rigid duplex DNA complex formed while the ferrocene indicator moved away from the graphene-modified electrode with low signal intensity. This biosensor exhibited a linear range from 25 pM to 10 μM with a detection limit of 5 pM.

**Signal amplification-based biosensors.** However, in some cases, the signal change produced by the indicator is not enough to indicate trace Hg$^{2+}$, especially with strong background noise.

![Fig 2](image-url)
In order to construct a highly sensitive Hg$^{2+}$ biosensor without the risk of indicator invalidation, signal amplification strategies have been designed and rapidly developed in recent years. Fu et al. constructed a homogeneous electrochemical biosensor which was based on an exonuclease III (Exo III)-assisted recycling amplification process. Two probes which contained ploy T sequences were designed, one of which was labeled with methylene blue (MB) at the terminal as a report probe. The Exo III specifically recognized the double-stranded structure and cut specific sites to release MB-labeled fragments. With no target in the detection system, the Exo III digested the original double-stranded structure. The long unmodified fragments were repulsed from the electrode because of their same charge. When Hg$^{2+}$ was present, the two probes formed a stable T–Hg$^{2+}$–T structure, which was recognized by Exo III for the short MB-labeled fragments. These MB-labeled fragments could diffuse to the electrode surface to enhance the electrochemical signal, while the released Hg$^{2+}$ took part in the next cycle to generate more MB-labeled fragments. Under the optimal conditions, this biosensor provided a low detection limit of 0.38 nM with a linear range from 1 to 500 nM.

Hong et al. designed another route via hybridization chain reaction (HCR) amplification, as shown in Fig. 2b. In this route, DNA probe A was first immobilized on the surface of the electrode, which could be recognized by nicking endonuclease (NEase). Then, another probe B was hybridized with probe A via the T–Hg$^{2+}$–T base pair, leading to selective digestion of the duplex structure by NEase as well as the release of probe B and Hg$^{2+}$ for the next cycle. Additionally, a double helix structure was formed via HCR; this was triggered by the initiators and two hairpin-shaped signal probes labeled with methylene blue, resulting in a significant signal increase. The biosensor based on this strategy exhibited high selectivity, with a linear range from 10 pM to 50 nM and a low detection limit of 1.6 pM.

### 2.3 Other strategies

Additionally, anodic stripping voltammetry (ASV), square wave voltammetry (SWV) and even the combination of these two voltammetric techniques (SWASV) have been widely used in the detection of Hg$^{2+}$ because of their ability to achieve high sensitivity and selectivity as well as niche applications.

Anodic stripping voltammetry (ASV) is a well-known sensitive electrochemical method which involves two steps for Hg$^{2+}$ detection, as shown in Fig. 3. A pre-concentration process is first required in order to attract mercury ions (Hg$^{2+}$) onto the electrode during a certain accumulation time. In this case, Hg$^{2+}$ is reduced to elemental mercury in the presence of a cathodic potential. Afterwards, scanning of the potential from negative to positive proceeds in order to oxidize the elemental mercury back to Hg$^{2+}$. In this process, a current peak can be obtained at the characteristic potential of Hg$^{2+}$. The peak intensity is associated with the Hg$^{2+}$ concentration. ASV can be utilized to assay inorganic Hg$^{2+}$ in aqueous solutions, even in the presence of dissolved oxygen.

ASV can be also applied in collaboration with the square wave voltammetry (SWV) method as square wave anodic stripping voltammetry (SWASV) for improving the ability of high frequency operation. The combination of these two techniques provides more adjustable parameters for optimization during the detection process, which is beneficial for improving the detection limit and accuracy.

The selectivity and sensitivity of biosensors mostly depends on their detection strategies. Enzyme inhibition-based Hg$^{2+}$ biosensors show worse sensitivity and selectivity than other strategies. In the detection process, Hg$^{2+}$ will irreversibly damage the enzyme, leading to a decrease of the conductivity and resulting in poor detection performance. In addition, many other substances can cause inhibition effects, restricting the selectivity of this type of biosensor. ASV-based Hg$^{2+}$ sensors exhibit the best sensitivity among the three types because the pre-concentration process greatly attracts Hg$^{2+}$ to the surface of the electrode during the detection procedure. However, ASV sensors show unsatisfactory selectivity because other substances will be also oxidized at a high detection potential. DNA biosensors for Hg$^{2+}$ detection exhibit both high sensitivity and selectivity. The excellent detection performance is due to the specificity of T–Hg$^{2+}$–T coordination, which has special recognition of Hg$^{2+}$ rather than other metal ions or organic molecules and can form even at low concentrations. Therefore, the DNA strategy for mercury detection dominates the current research on biosensor construction.

In addition, the surface area, conductivity, electron transfer capability and morphology of nanomaterials have been confirmed to have great influences on the sensitivity of biosensors. Good conductivity and electron transfer capability are beneficial to transfer and amplify the response signal. Additionally, a large surface area with certain regular structures can provide more active sites for the immobilization of bioactive substances. Almost all nanomaterials reviewed in the next section demonstrate the above advantages.

### 3. Advanced nanomaterials for electrochemical Hg$^{2+}$ biosensors

Mercury in water often co-exists with many other substances, causing difficulty in accurate recognition. Therefore, accuracy, sensitivity and selectivity of a prepared biosensor are essential for its practical application. The electrode material always determines the detection performance of the electrochemical mercury biosensor. Relying on the electric signal transfer, the material is always required to have high electrocatalysis and conductivity in order to increase the signal production and accelerate its transportation. Nanomaterials, which have received

![Fig. 3 Schematic of the anodic stripping voltammetry process.](image)
rapid development in recent years, have been widely confirmed to demonstrate advanced electrochemical performance due to their high specific areas, electrocatalytic activity and low transfer resistance.\textsuperscript{36,47} In this case, the design of novel nanomaterials has gradually become an active research topic in mercury biosensor construction.\textsuperscript{12} These nanomaterials can often spontaneously crosslink with bioactive substances via coordination bonds. Mounting evidence has revealed that the material nanostructures can greatly affect the detection performance of Hg\textsuperscript{2+} biosensors. Herein, as shown in Fig. 4, recently reported nanomaterials will be briefly discussed, from their nanostructure control methods to their promotion effects on Hg\textsuperscript{2+} detection.

Most nanomaterials discussed in this paper can be also used to detect other metal ions if the detection strategy is based on an enzymatic or DNA biosensor. In this case, the nanomaterial mainly serves as a bridge between the bioactive substances and the electrode. Therefore, the nanomaterials should accelerate electron transfer and enhance the response signal. Moreover, the nanomaterials are also responsible for providing binding sites to immobilize DNA and enzymes via covalent bonds.

However, in contrast with other metal ions, mercury ions can easily accumulate and are non-degradable in the human body, leading to various cognitive and motor disorders as well as to death. According to the Chinese national standard, the maximum permitted level of Hg\textsuperscript{2+} in drinking water is much lower than those of other heavy metal ions (Hg\textsuperscript{2+}: 1 ng mL\textsuperscript{−1}, Cd\textsuperscript{2+}: 5 ng mL\textsuperscript{−1}, Cr\textsuperscript{6+}: 50 ng mL\textsuperscript{−1}, Pb\textsuperscript{2+}: 10 ng mL\textsuperscript{−1}, Ag\textsuperscript{+}: 50 ng mL\textsuperscript{−1}, etc.). In this case, nanomaterials adopted for Hg\textsuperscript{2+} biosensors are required to show better accuracy, chemical stability and low detection limits in signal amplification and electron transport to detect Hg\textsuperscript{2+} at much lower concentrations. Generally, in order to achieve lower detection limits, a nanomaterial for Hg\textsuperscript{2+} detection is expected to possess a high surface area to provide more binding sites for DNA and enzyme immobilization.

Hence, the prepared biosensor can accept more response signals from the reaction between mercury ion and the DNA or enzymes.

### 3.1 Noble metal materials

Materials containing noble metals, such as Au, Pt and Ag, are well known as some of the earliest applied electrocatalytic materials. Their high catalysis and conductivity is often satisfactory to strengthen the response signals from electrochemical biosensing reactions. Although these materials have been well studied, control of their nanostructures to realize new morphologies is receiving increasing research interest to pursue performance enhancement. Normally, these nanostructures have greatly increased specific areas to provide abundant active sites, benefiting the immobilization of more biomolecules (such as DNA, RNA and enzymes) via coordination bonds.

Among the various noble metal materials, gold nanoparticles (AuNPs) are the most commonly applied to construct mercury biosensors. Zhang et al. prepared a gold nanoparticles–reduced graphene oxide (AuNPs–rGO) film for electrochemical Hg\textsuperscript{2+} detection.\textsuperscript{48} In their work, the film was synthesized via one-step reduction of a mixture of graphene oxide and HAuCl\textsubscript{4} by a NaBH\textsubscript{4} solution, which acted as an indicator to generate the electrochemical signal with excellent electrocatalytic activity and conductivity. In the presence of Hg\textsuperscript{2+}, the AuNPs–rGO modified ssDNA moved away from the electrode, leading to a sudden decrease of the response signal. This biosensor showed high selectivity to Hg\textsuperscript{2+} with a wide linear range of 0 to 2000 nM and a low detection limit of 0.04 nM. Additionally, the biosensor could be used to assay Hg\textsuperscript{2+} in real water samples with some satisfactory results.

Due to their excellent optical and electrical properties, AuNPs have been also applied in photoelectrochemical biosensors to show both high sensitivity and stability during Hg\textsuperscript{2+} detection. Wang et al. developed a label-free electrochemiluminescence (ECL) biosensor in order to assay Hg\textsuperscript{2+} with reliable results.\textsuperscript{49} Au nanoparticles (AuNPs) which were prepared by the citrate reduction of HAuCl\textsubscript{4} with an average diameter of 13 nm were utilized to realize the “turn-on” detection mode through selective assembly on the ITO surface. Without any Hg\textsuperscript{2+} in the detection system, the ssDNA preferred to adsorb on the AuNPs in the solution. However, when mercury ions were introduced, the ssDNA immediately formed double-stranded structures via thymine–Hg\textsuperscript{2+}–thymine coordination to escape from the surface of the AuNPs. Then, the free AuNPs could be deposited on the ITO surface to show better conductivity and catalytic activity, producing a significantly enhanced ECL signal. This biosensor was able to quantitatively detect Hg\textsuperscript{2+} with a low detection limit of 2 pM and a wide linear range from 8 pM to 2 nM in addition to good specificity and selectivity. Additionally, in order to achieve the ultrasensitive detection of trace Hg\textsuperscript{2+}, Zhang et al. developed an amplified signal strategy to strengthen the performance of AuNPs.\textsuperscript{50} They electrodeposited graphene and AuNPs respectively on a bare electrode to obtain improved conductivity after the immobilization of thiol-modified ssDNA. The AuNPs were dispersed uniformly on the graphene surface, with sizes of around 100 nm. The as-prepared mercury biosensor exhibited...
an extremely low detection limit of 0.001 aM, with an ultrawide linear range between 1 aM and 100 nM. Meanwhile, it also showed great specificity and sensitivity for the assay of mercury ions, which is promising for application in online pollution analysis in real lake water.

In addition to nanoparticles, numerous other Au nanostructures have been created to construct various Hg$^{2+}$ biosensors. Zeng et al. controlled the shapes of gold nanomaterials to form nanopores through a dealloying method$^{51}$ in which Ag was selectively dissolved from an Ag/Au alloy in a nitric acid solution. As shown in Fig. 5, this controllable three-dimensional structure of nanoporous Au (NPG) with a high specific area showed outstanding catalytic activity and electron transfer efficiency during the electrochemical process. Aneesh et al. synthesized gold atomic cluster–chitosan nanocomposite (AuAc–chit) nanostructures via an electrodeposition approach in acidic aqueous solution by cycling the potential within the range of $-1.30$ to $+1.20$ V 16 to 20 times with a scan rate of 50 mV s$^{-1}$. The as-prepared AuAc–chit possessed good water solubility, good stability and outstanding biocompatibility and showed outstanding Hg$^{2+}$ detection performance.

Similar to gold, silver (Ag) is a widely applied noble metal in electrochemical mercury biosensors. However, compared with Au, it shows much more active properties with weak stability. Therefore, it is often employed to form different core–shell nanostructures to repair this defect.

Among these core–shell materials, Ag@Au nanoparticles are the most commonly used. Ezhil et al. reported a DNA electrochemical Hg$^{2+}$ biosensor with a very low working potential using an Ag@Au core–shell nanoparticles-modified electrode.$^{53}$ AgNPs were first synthesized to form an aqueous suspension, in which HAuCl$_4$·3H$_2$O solution was added to fully cover the particle surface. With crosslinking of PEI, 20 nm core–shell crystals were successfully obtained and showed much higher electrocatalytic performance than AuNPs or AgNPs alone. Due to the synergetic amplifying effects of the Ag@Au nanoparticles on the high specificity of the thymine–Hg$^{2+}$–thymine mismatch, this biosensor showed a low detection limit of 6 pM with a linear range from 10 pM to 160 pM.

Interestingly, the surface charge of Ag@Au NPs can be controlled to absorb on the substrate via electrostatic forces. Li et al. constructed positively charged Ag@Au core–shell nanoparticles ($^{+}$Ag@Au CSNPs).$^{54}$ In their experiment, AgNPs were first prepared as the core with NaBH$_4$ as a reducing agent. After that, HAuCl$_4$, NH$_2$OH·HCl and cetyl-trimethyl ammonium bromide (CTAB) were added to the dispersion of AgNPs to form the core–shell structures through continuous stirring. The average size of these nanoparticles was about 32 nm, while the core size was 22 nm. In the process of Hg$^{2+}$ detection, the ($^{+}$)Ag@Au CSNPs could adsorb on the negatively charged dsDNA polymers to enhance the response signal. In addition, the positively charged Ag@Au CSNPs showed better physical and chemical properties than the individual AuNPs or AgNPs due to the enhanced localized electric field from the core–shell structure, leading to dual signal amplification. This biosensor exhibited a low detection limit of 3.6 pM as well as a wide linear range from 30 pM to 1.5 nM with high selectivity for Hg$^{2+}$.

Among all noble metal materials, platinum shows superior chemical stability and satisfactory electrocatalysis. Its nanostructures are often built as nanoparticles or nanotubes for mercury detection. Our group constructed a novel electrochemical DNA sensing platform on the basis of platinum nanotube arrays (PtNAs) for trace Hg$^{2+}$ recognition, as shown in Fig. 6a.$^{55}$ In order to achieve this special architecture, a ZnO nanotube film was first formed as a template. This could be realized by a hydrothermal route on carbon fibers. Based on the previously obtained ZnO nanotubes, a layer of Pt film was then synthesized to surround each nanotube through a second hydrothermal reaction. It is interesting that during the Pt crystallization, the environmental pH value continuously decreased to dissolve the ZnO template. Finally, the ZnO nanotubes were totally erased, leaving only the hollow nanotubic structure of the Pt arrays with lengths and diameters of ca. 2 µm and 100 nm, respectively, as shown in Fig. 6b.

Liu et al. reported urchin-like platinum nanoparticles to fabricate a novel lab-on-a-chip biosensor for the quantitative detection of multiple heavy metal ions, including Hg$^{2+}$, Cu$^{2+}$ and Pb$^{2+}$. These nanoparticles were prepared through the reduction...
Table 1 Advanced nanomaterials-based Hg$^{2+}$ biosensors in the literature

<table>
<thead>
<tr>
<th>Material</th>
<th>Nanostructure</th>
<th>Preparation method</th>
<th>Limit of detection (nM)</th>
<th>Linear range (nM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Noble metal materials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Au</td>
<td>Nanoparticle</td>
<td>Chemical synthesis method</td>
<td>$2 \times 10^{-3}$</td>
<td>$8 \times 10^{-3}$–2</td>
<td>49</td>
</tr>
<tr>
<td>Au</td>
<td>Nanoparticle</td>
<td>Electrochemical deposition method</td>
<td>0.01</td>
<td>0.1–2.5 $\times 10^3$</td>
<td>80</td>
</tr>
<tr>
<td>Au</td>
<td>Nanoparticle</td>
<td>Electrochemical deposition method</td>
<td>0.05</td>
<td>0.1–30</td>
<td>81</td>
</tr>
<tr>
<td>Au</td>
<td>Nanoparticle</td>
<td>Electrochemical deposition method</td>
<td>$6.84 \times 10^{-4}$</td>
<td>$10^{-3}$–1000</td>
<td>82</td>
</tr>
<tr>
<td>Au</td>
<td>Nanoparticle</td>
<td>Self-assembly</td>
<td>$5.1 \times 10^{-3}$</td>
<td>0.05–10</td>
<td>83</td>
</tr>
<tr>
<td>Au</td>
<td>Nanoparticle</td>
<td>Ion sputtering method</td>
<td>$8 \times 10^{-3}$</td>
<td>$10^{-3}$–100</td>
<td>84</td>
</tr>
<tr>
<td>Au atomic cluster</td>
<td>Nanocluster</td>
<td>Electrochemical deposition method</td>
<td>$8 \times 10^{-5}$</td>
<td>$10^{-5}$–1000</td>
<td>52</td>
</tr>
<tr>
<td>Au</td>
<td>Nanoflower</td>
<td>Chemical synthesis method</td>
<td>$6.2 \times 10^{-7}$</td>
<td>$10^{-7}$–1</td>
<td>85</td>
</tr>
<tr>
<td>Au/graphene</td>
<td>Nanoparticle</td>
<td>Electrochemical deposition method</td>
<td>$1 \times 10^{-12}$</td>
<td>$10^{-9}$–100</td>
<td>50</td>
</tr>
<tr>
<td>AuNPs–rGO</td>
<td>Nanosheet</td>
<td>Chemical synthesis method</td>
<td>0.04</td>
<td>0–2000</td>
<td>48</td>
</tr>
<tr>
<td>AuNPs/rGO</td>
<td>Nanofilm</td>
<td>Electrochemical reduction and deposition method</td>
<td>$7.5 \times 10^{-3}$</td>
<td>0.05–5</td>
<td>86</td>
</tr>
<tr>
<td><strong>Silver</strong></td>
<td>Nanoparticle</td>
<td>Chemical synthesis method</td>
<td>0.028</td>
<td>0.1–10</td>
<td>87</td>
</tr>
<tr>
<td>Ag@Au</td>
<td>Nanoparticle</td>
<td>Chemical synthesis method</td>
<td>$6 \times 10^{-3}$</td>
<td>0.01–0.16</td>
<td>53</td>
</tr>
<tr>
<td>(±) Ag@Au</td>
<td>Nanoparticle</td>
<td>Chemical synthesis method</td>
<td>$3.6 \times 10^{-3}$</td>
<td>0.03–1.5</td>
<td>54</td>
</tr>
<tr>
<td>Au@Ag</td>
<td>Nanoparticle</td>
<td>Chemical synthesis method</td>
<td>0.03</td>
<td>0.01–100</td>
<td>88</td>
</tr>
<tr>
<td>Au@Ag</td>
<td>Nanorod</td>
<td>Chemical synthesis method</td>
<td>$2.5 \times 10^{-3}$</td>
<td>0.01–10</td>
<td>89</td>
</tr>
<tr>
<td>Fe$_3$O$_4$@Au</td>
<td>Nanoparticle</td>
<td>Chemical synthesis method</td>
<td>1.7</td>
<td>10–100</td>
<td>90</td>
</tr>
<tr>
<td><strong>Platinum</strong></td>
<td>Nanotube array</td>
<td>Hydrothermal method</td>
<td>0.03</td>
<td>0.1–100</td>
<td>55</td>
</tr>
<tr>
<td><strong>Platinum</strong></td>
<td>Urchin-like nanoparticle</td>
<td>Chemical synthesis method</td>
<td>1.8</td>
<td>2–200</td>
<td>56</td>
</tr>
<tr>
<td><strong>Carbon materials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graphene</td>
<td>Nanosheet</td>
<td>Chemical synthesis method</td>
<td>$2.1 \times 10^{-4}$</td>
<td>$3.5 \times 10^{-3}$–3.5</td>
<td>91</td>
</tr>
<tr>
<td>Graphene</td>
<td>Nanosheet</td>
<td>Chemical synthesis method</td>
<td>$5 \times 10^{-3}$</td>
<td>$2.5 \times 10^{-3}$–10$^3$</td>
<td>40</td>
</tr>
<tr>
<td>Graphene</td>
<td>Nanosheet</td>
<td>Chemical vapor deposition</td>
<td>0.04</td>
<td>0.1–100</td>
<td>66</td>
</tr>
<tr>
<td>3D graphene/Au</td>
<td>Nanoporous</td>
<td>Hydrothermal method</td>
<td>$5 \times 10^{-8}$</td>
<td>$10^{-7}$–1000</td>
<td>65</td>
</tr>
<tr>
<td>GO</td>
<td>Nanosheet</td>
<td>Self-assembly</td>
<td>$3.33 \times 10^{-9}$</td>
<td>$10^{-5}$–$10^{-3}$</td>
<td>92</td>
</tr>
<tr>
<td>GO</td>
<td>Nanosheet</td>
<td>Electrochemical deposition method</td>
<td>0.01</td>
<td>0.01–10</td>
<td>93</td>
</tr>
<tr>
<td>Reduced GO</td>
<td>Nanosheet</td>
<td>Chemical synthesis method</td>
<td>5</td>
<td>8–100</td>
<td>36</td>
</tr>
<tr>
<td>Reduced GO</td>
<td>Nanosheet</td>
<td>Self-assembly</td>
<td>$5.4 \times 10^{-3}$</td>
<td>0.01–100</td>
<td>94</td>
</tr>
<tr>
<td>MWCNTs–AuNPs</td>
<td>Nanotube</td>
<td>Self-assembly</td>
<td>0.03</td>
<td>0.1–20</td>
<td>59</td>
</tr>
<tr>
<td>SWCNT</td>
<td>Oriented nanotube</td>
<td>Template-assisted method</td>
<td>$3 \times 10^{-6}$</td>
<td>$10^{-3}$–1000</td>
<td>60</td>
</tr>
<tr>
<td>SWCNT</td>
<td>Nanotube</td>
<td>Self-assembly</td>
<td>0.01</td>
<td>0.05–$10^4$</td>
<td>61</td>
</tr>
<tr>
<td>SWCNT</td>
<td>Nanotube</td>
<td>Self-assembly</td>
<td>0.022</td>
<td>0.05–5</td>
<td>62</td>
</tr>
<tr>
<td><strong>Hybrid materials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N(en)$_3$Ag$_4$I$_4$</td>
<td>Oriented nanorod</td>
<td>Hydrothermal method</td>
<td>$5 \times 10^{-3}$</td>
<td>0.015–500</td>
<td>70</td>
</tr>
<tr>
<td>GO–AgNPs–ABEI</td>
<td>Nanofilm</td>
<td>Self-assembly</td>
<td>$3.1 \times 10^{-3}$</td>
<td>0.01–$10^6$</td>
<td>72</td>
</tr>
<tr>
<td>GO–ZnO–Cds</td>
<td>Nanofilm</td>
<td>Hydrothermal method</td>
<td>$1.5 \times 10^{-3}$</td>
<td>$5 \times 10^{-3}$–0.5</td>
<td>74</td>
</tr>
<tr>
<td>AuNPs–BSA–rGO</td>
<td>Nanofilm</td>
<td>Self-assembly</td>
<td>0.03</td>
<td>0.1–130</td>
<td>71</td>
</tr>
<tr>
<td>Chitosan@3D-rGO</td>
<td>Nanohybrid</td>
<td>Self-assembly</td>
<td>0.016</td>
<td>0.1–10</td>
<td>95</td>
</tr>
<tr>
<td>Zn$_4$[PO$_4$_2]@MWCNT</td>
<td>Nanobelt</td>
<td>Self-assembly</td>
<td>0.071</td>
<td>0.1–50</td>
<td>96</td>
</tr>
<tr>
<td>CD–AuNPs</td>
<td>Nanoparticle</td>
<td>Chemical synthesis method</td>
<td>0.1</td>
<td>$10^{-4}$–4</td>
<td>97</td>
</tr>
<tr>
<td>Pt/ppy</td>
<td>Nanoparticle</td>
<td>Chemical synthesis method</td>
<td>0.27</td>
<td>5–500</td>
<td>98</td>
</tr>
<tr>
<td>β-CY–PdNPs</td>
<td>Nanoparticle</td>
<td>Chemical synthesis method</td>
<td>$7.5 \times 10^{-6}$</td>
<td>$1.5 \times 10^{-4}$–3</td>
<td>73</td>
</tr>
<tr>
<td>Fe$_{1-x}$ZnO–Ag</td>
<td>Nanohybrid</td>
<td>Self-assembly</td>
<td>0.1</td>
<td>0.5–1000</td>
<td>75</td>
</tr>
<tr>
<td>Prussian blue–Au</td>
<td>Irregular composite</td>
<td>Electrochemical deposition method</td>
<td>0.03</td>
<td>0.1–10</td>
<td>99</td>
</tr>
<tr>
<td>Au@Ag/MoS$_2$</td>
<td>Nanohybrid</td>
<td>Self-assembly</td>
<td>$5 \times 10^{-3}$</td>
<td>0.01–100</td>
<td>100</td>
</tr>
<tr>
<td>TiO$_2$/Cds</td>
<td>Nanohybrid</td>
<td>Self-assembly</td>
<td>$3.3 \times 10^{-6}$</td>
<td>$10^{-5}$–2000</td>
<td>101</td>
</tr>
<tr>
<td><strong>Quantum dots</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZnS</td>
<td>Quantum dot</td>
<td>Chemical reaction method</td>
<td>$2 \times 10^{-3}$</td>
<td>0.01–1000</td>
<td>78</td>
</tr>
<tr>
<td>CdTeSe@CdS</td>
<td>Core–shell quantum dot</td>
<td>Chemical reaction method</td>
<td>$3.5 \times 10^{-3}$</td>
<td>0.01–1000</td>
<td>79</td>
</tr>
<tr>
<td>CdS</td>
<td>Quantum dot</td>
<td>Chemical reaction method</td>
<td>0.6</td>
<td>3 to 100</td>
<td>102</td>
</tr>
<tr>
<td>CdTe/CdS</td>
<td>Quantum dot</td>
<td>Chemical reaction method</td>
<td>$2 \times 10^{-9}$</td>
<td>$2 \times 10^{-8}$–2000</td>
<td>103</td>
</tr>
</tbody>
</table>

As shown in Table 1, noble metal materials-based electrochemical Hg$^{2+}$ biosensors exhibit excellent detection performance at low concentrations. The Au/Graphene modified biosensor possessed the lowest detection limit among nanomaterials-based biosensors because Au and graphene show excellent conductivity and catalytic activity to assist the amplified signal strategy for Hg$^{2+}$ detection. Otherwise, nanoporous gold-based biosensors have the widest detection ranges among all noble metal material-based biosensors; this is attributed to the...
to their large pore volumes, which efficiently enhance the catalytic area.

3.2 Carbon materials

Carbon materials, which normally include graphene, graphene oxide, carbon nanotubes and fullerene, have been widely applied in electrochemical biosensors, mainly due to their superior conductivity and specific surface areas. Although they possess similar elemental compositions, these materials can demonstrate various electrochemical properties attributed to their different nanostructures.

Carbon nanotubes (CNTs) are one of the earliest applied carbon materials to construct \( \text{Hg}^{2+} \) biosensors. Early in the last decade, for the first time, multi-walled carbon nanotubes (MWCNTs) were applied by Yi to construct a mercury ion biosensor based on the ASV strategy.\(^{57}\) Later, in 2008, Gao et al. used single-walled carbon nanotubes (SWCNTs) for the detection of \( \text{Hg}^{2+} \) based on the DNA probe principle.\(^{58}\)

In 2012, Lu et al. constructed an electrochemical \( \text{Hg}^{2+} \) biosensor based on MWCNTs and AuNPs.\(^{59}\) In their experiment, MWCNTs were first dropped on the electrode to form a thin film. Using the electrochemical deposition method with a constant potential of \(-0.2 \ \text{V} \) \( \text{AuNPs} \) were obtained on the surface of the MWCNTs. After that, the specific DNA probe could crosslink with \( \text{AuNPs} \) via \( \text{Au–S} \) bonds. The MWCNTs and \( \text{AuNPs} \) composite acted as an efficient sensing platform because of its combination of unique electrical properties and good biocompatibility. This biosensor showed a low detection limit of 0.03 nM with a linear range from 0.1 to 20 nM. Recently, our group developed a highly sensitive and reusable electrochemical mercury biosensor that exhibited an ultralow \( \text{Hg}^{2+} \) detection limit of 3 fM and a wide linear range from 10 fM to 1 \( \mu \text{M} \) in real lake water.\(^{60}\) In this work, two different self-assembled monolayers (SAMs) were applied to pretreat the electrode. Both the soft SAMs template and the rigid template had terminals of –SH groups and –NH\(_2\) groups, respectively. The –SH group combined with the Au electrode and the –NH\(_2\) group interacted with the SWCNTs via peptide bonds in order to control the fabrication of the SWCNTs. The rigid SAMs-modified electrode could form tunable vertical SWCNTs with particular orientations and homogeneities, while the growth of SWCNTs on the soft SAMs modified electrode was disordered. The tunable vertical SWCNTs exhibited a large specific area, good electrical conductivity and excellent binding strength, resulting in outstanding electrochemical performance for \( \text{Hg}^{2+} \) monitoring.

The above two studies mainly dedicated effort to developing SWCNTs-based amperometric mercury biosensors. Wang et al. used this material to construct a field effect transistor (FET)-based biosensor, as shown in Fig. 7a.\(^{61}\) In this design, SWCNT/FET was constructed via a spray method with high humidity conditions on an \( \text{SiO}_2/\text{Si} \) substrate to form covalent crosslinks with Hg-DPR via peptide bonds. The SWCNTs, which possessed one-dimensional structures, provided outstanding electronic, mechanical and optical properties. The FET coupling with the SWCNTs showed great potential in low concentration detection and real time assays. In this case, the biosensor could decrease the detection limit to 10 pM, with two different linear ranges of 50 pM to 100 nM and 100 nM to 10 \( \mu \text{M} \).

In addition, the outstanding optical properties of SWCNTs improve their application in electrochemiluminescence biosensors. Recently, an ECL approach for the detection of \( \text{Hg}^{2+} \) was developed by Huang’s group.\(^{62}\) SWCNTs were utilized in their research in order to greatly enhance the electrochemical conductivity and catalyse the reaction of tri-\( \text{n} \)-propylamine (TPrA), which acted as an ECL co-reactant. Under normal conditions, capture ssDNA immobilized on the ITO electrode noncovalently bonded with the SWCNTs. In this way, the SWCNTs improved the conductivity of the electrode and catalysed the reaction of \( \text{Ru(bpy)}_3^{2+}/\text{TPrA} \) to provide a strong ECL signal. After the electrochemical characterization, this biosensor for \( \text{Hg}^{2+} \) detection showed a low detection limit of 22 pM, with a linear range from 50 pM to 5 nM. Other researchers have also applied this method for the detection of thrombin; they also obtained satisfactory results.

In 2014, Geim won the Noble Prize for his preparation of a single graphene layer. This material is very popular, and its applications are being explored in almost all scientific fields.\(^{63,64}\) Our group constructed a highly selective and reliable \( \text{Hg}^{2+} \) biosensor by designing a novel 3D graphene/gold (G/Au) film.\(^{65}\) The preparation process of this material is shown in Fig. 7b. After hydrothermal growth, the 3D-interlaced graphene framework with porous layer-by-layer structures was tightly attached onto the gold electrode, which was pretreated with a self-assembled monolayer. At the same time, \( \text{AuNPs} \) were formed on the graphene framework during the reduction of graphene rather than by using additional reductants. This is a facile and green method for \( \text{in situ} \) and one-step synthesis of 3D G/Au films which possess large specific areas, excellent electrical conductivity and good binding strengths.
due to the synergistic cooperation of graphene and AuNPs. This unique structure greatly enhanced the electro-analysis performance of the as-prepared biosensor. As a result, this sensing platform for Hg$^{2+}$ detection possessed a wide linear range from 0.1 fM to 0.1 pM as well as an ultralow detection limit of 50 aM.

Tu et al. utilized graphene for the construction of an ultra-sensitive Hg$^{2+}$ FET array biosensor. In this work, graphene enhanced the current response and sensitivity, providing a large surface area with high electron mobility and a tunable ambipolar field effect. Specifically, graphene was synthesized on a copper foil firstly via chemical vapor deposition (CVD). After that, the as-prepared graphene was transferred to the SiO$_2$ capping layer with modification of polymethylmethacrylate (PMMA). This biosensor possessed a fast response time below one second, with a low detection limit of 40 pM and a wide linear range between 100 pM and 100 nM.

Carbon material-based electrochemical Hg$^{2+}$ biosensors are summarized in Table 1. Although carbon materials can have high conductivity, which is beneficial for electron transfer, weak catalysis is an obstacle to their further performance enhancement in mercury detection. Therefore, these materials are often improved through combination with other catalytic materials to form various hybrid materials.

### 3.3 Hybrid materials

In the past few years, various hybrid materials have been developed with numerous significant applications in catalysis, gas separation and biosensing. The hybrid material is often composed of both inorganic and organic materials connected through certain chemical bonds, including van der Waals forces, hydrogen bonds, and covalent and ionic bonds. Therefore, hybrid materials can be enhanced through the synergistic effects of their inorganic and organic components to integrate high catalysis, conductivity and stability.

In Hg$^{2+}$ recognition, most hybrid materials contain noble elements in order to strengthen the response signal. Our group has synthesized a metal–organic hybrid microarray on an Au electrode to construct a novel electrochemical Hg$^{2+}$ biosensor. N(en)$_3$Ag$_2$I$_4$, a material with excellent conductivity, was employed as the recognition core. However, without structure control, its single crystals can freely grow to sizes of several millimetres; these are difficult to directly modify on the substrate, as shown in Fig. 8a(ii). In order to address the above problem, we introduced a 1,4-benzenendithiol monolayer as an anchor to limit growth behaviour during the initial nucleus formation period. One terminal –SH group of 1,4-benzenendithiol can be anchored onto the surface of the Au electrode by an Au–S bond, while another terminal –SH group links the Ag atom of N(en)$_3$Ag$_2$I$_4$. Due to the rigid structure of the 1,4-benzenendithiol monolayer, the growth of N(en)$_3$Ag$_2$I$_4$ was preferentially constrained in the vertical direction to build a hexagonal prism nanostructure with a diameter of 1 μm, which corresponds to the image in Fig. 8a(i). This regular shape has the ability to accelerate electron transfer and provide abundant attracting sites for DNA immobilization. As a result, its performance in Hg$^{2+}$ detection can achieve a wide linear range from 15 pM to 500 nM with a low recognition limit of 5 pM.

Wang et al. developed an ultrasensitive electrochemical biosensor based on a hybrid material composed of gold nanoparticles, bovine serum albumin and reduced graphene oxide (AuNPs–BSA–rGO). This hybrid material was synthesized through simple mixing of an AuNPs dispersion and a BSA/reduced GO solution. In this hybrid material, the BSA–rGO film could greatly limit the aggregation of AuNPs to sizes of 10 nm. The small AuNPs with high contact areas could link more thiol-modified DNA probes via the Au–S bond. As a result, the as-prepared biosensor for the mercury assay possessed a linear range between 0.1 and 130 nM, with a low detection limit of 0.03 nM.

Some hybrid materials possess both electrochemical and optical properties; therefore, they can also be applied in the construction of photovoltaic (PEC) or electrochemiluminescence (ECL) biosensors. Jiang et al. designed a signal-switchable ECL biosensor with a target recycling amplification strategy for Hg$^{2+}$ detection. An ABEI-functionalized silver nanoparticles-decorated graphene oxide nanocomposite (GO-AgNPs-ABEI) was fabricated via a simple one-step method of mixing AgNO$_3$, GO and ABEI in a room temperature reaction, as shown in Fig. 8b. The synergistic effect of the hybrid nanoparticles with average sizes of 50 nm resulted in great enhancement of the ECL signal and electrocatalytic activity. As a noble material, palladium (Pd) shows outstanding catalytic activity for the development of electrochemical detection. Hu et al. designed an electrochemiluminescence (ECL) biosensing platform for Hg$^{2+}$ detection based on host–guest interactions. In their work, β-cyclodextrin-modified Pd nanoparticles (β-CY-PdNPs) were prepared via mixing H$_2$PdCl$_4$ and β-CD in DMSO solution under reduction by NaBH$_4$. The as-prepared β-CY-PdNPs with small diameters of about 3 to 5 nm were applied to enhance the signal intensity due to their large surface areas and excellent electrocatalytic performance. Moreover, β-CY-PdNPs could bind with ferrocene which was functionalized on the termini of ssDNA through supramolecular interactions. This Hg$^{2+}$ biosensor exhibited a low detection limit of 0.0015 ng mL$^{-1}$ with high specificity as well as a linear range between 0.003 and 600 ng mL$^{-1}$.

As a metallic oxide, ZnO has been attracted great attention for its large energy band gap and exciton binding energy, which lead to outstanding optical performance. Due to the poor conductivity of ZnO, there are many restrictions to its electrochemical or photoelectrochemical applications. To solve this
problem, many ZnO-based hybrid materials are being continuously designed. Zhang et al. prepared a GO-ZnO-CdS hybrid material by a hydrothermal method to construct a novel photo-electrochemical mercury biosensor. After mixing GO and ZnO in distilled water, the S\(^2\) ions were initially adsorbed on the ZnO surface by a Lewis acid–base interaction. In this case, CdS NPs were strongly linked with the GO-ZnO film to form a lamellar structure with particles. As a result, both ZnO and the CdS nanoparticles could achieve uniform distribution on the surface of the GO sheets. CdS nanoparticles with sizes of 50 to 70 nm were anchored on the surface of ZnO which possessed a width of around 100 nm and a length of around 300 nm. In order to achieve better detection performance, this group further developed an Fe\(^{3+}\) doped ZnO–Ag photocatalyst (Fe\(^{3+}/\text{ZnO–Ag}\)) which greatly improved the photosresponse and catalytic ability of ZnO. The Fe\(^{3+}\)-doped ZnO–Ag hybrid materials were fabricated via an atmospheric self-induction synthesis method. Briefly, ZnAc\(_2\), AgNO\(_3\), and Fe(NO\(_3\))\(_3\) were dissolved in alcohol, and C\(_2\)H\(_4\)O\(_2\)H was added to the mixture later. Once uniformly mixed and completely dry, the product was calcined at 600 \(^\circ\)C to obtain Fe\(^{3+}/\text{ZnO–Ag}\). The resulting hybrid material consisted of rod-like structures with diameters of 200 nm and lengths of 700 nm. The response signals were linearly related to Hg\(^{2+}\) concentration from 0.5 nM to 100 nM, with a low detection limit of 100 pM.

Various hybrid material-based electrochemical Hg\(^{2+}\) biosensors are summarized in Table 1. The different structures and types of hybrid materials show unique performance with a combination of optical and electrical properties. Generally, hybrid material-based biosensors have higher detection limits and narrower detection ranges than noble metal material and carbon material-based biosensors due to the worse conductivity of the latter materials in combination with organic components.

3.4 Quantum dots

In the last decade, scientists have sought to decrease the dimensions of materials from three to zero, thus harvesting special properties for performance enhancement. Quantum dots (QD) are considered to be typical zero-dimensional materials which are defined as semiconducting. Recently, these materials have attracted increasing attention due to their excellent optical and electrical responses, outstanding biocompatibility and large specific surface areas. QDs were firstly used in optical sensors for their high luminescent efficiency. Photoelectrochemical applications have been developed to enhance the sensitivity and stability of the as-prepared biosensors.

Wang et al. designed a signal-on photoelectrochemical strategy for the detection of Hg\(^{2+}\) based on the principle of ion exchange. ZnS quantum dots (ZnS QDs) were applied in this work, where they acted as a source of S element. The mercaptoacetic acid (TGA)-capped ZnS QDs were prepared through an aqueous synthesis method. With an average particle size of about 4 nm, the QDs were assembled on the electrode through electrostatic interactions. When detecting Hg\(^{2+}\), HgS formed on the surface of ZnS via ion exchange. As a result, the cathodic photocurrent increased under visible light. In this strategy, ZnS functioned as an n-type semiconductor with a low basic cathodic photocurrent, leading to a low background signal as well as a low detection limit. In contrast, HgS functioned as a p-type semiconductor, which enabled the generation of cathodic photocurrent. The ion-exchange process could quantitatively detect Hg\(^{2+}\). This biosensor possessed a low detection limit of 2 pM with a linear range from 10 pM to 1 \(\mu\)M.

Shi et al. developed a “signal-on” ECL aptasensor for the detection of Hg\(^{2+}\) with the CdTeSe@CdS QDs–DNA bioconjugates and enzyme-catalysis signal amplification strategy. In their work, CdTeSe QDs were first synthesized through mixing NaHTe and NaHSe in alkaline CdCl\(_2–\)MPA solution under nitrogen atmosphere for 1 h. However, the CdTeSe QDs showed low fluorescence efficiency due to defects and unsaturated suspension keys on their surfaces. On this basis, with the aid of microwave radiation, CdTeSe@CdS QDs were prepared to improve their optical properties in similar solutions and were then modified with carboxyl groups to immobilize the DNA probe. The mean size of the as-prepared core–shell QDs was about 5 nm; they demonstrated excellent fluorescence and electrochemical performance as well as good stability. This constructed ECL aptasensor possessed a wide linear range from 10 pM to 1 \(\mu\)M with a detection limit of 3.5 pM.

As shown in Table 1, QDs are a popular new type of nanomaterial in biosensor construction. They possess superior optical properties that benefit PEC and ECL applications. However, due to their extremely small sizes, the prevention of particle aggregation and activity loss is still a challenge for their further application.

4. Applications of Hg\(^{2+}\) biosensors in real samples

To evaluate the reliability and stability of nanomaterials-based Hg\(^{2+}\) biosensors, many studies have applied as-prepared biosensors for the detection of real water samples. Due to the specificity of the T–Hg\(^{2+}\)–T mismatch, most of these biosensors demonstrate excellent performance with high selectivity in the detection process. Following are some practical application results of reported Hg\(^{2+}\) biosensors which adopt real samples for detection, such as water from rivers and lakes.

The Au–graphene-based Hg\(^{2+}\) biosensor constructed by Zhang et al. was tested in tap water, river water and landfill leachate samples, respectively. The detection results corresponded with those detected via atomic fluorescence spectrometry (AFS). Even in a sample whose Hg\(^{2+}\) concentration was below the detection limit of AFS, this biosensor could still function with good accuracy and reliability. The AuNPs–rGO modified Hg\(^{2+}\) biosensor prepared by Zhang et al. was applied to detect Hg\(^{2+}\) in tap water and river water. The recovery values were around 97% and the relative standard deviations (RSD) were nearly 3%. These results indicate that this Hg\(^{2+}\) biosensor shows excellent performance in practical samples. An ECL biosensor based on AuNPs was adopted in the detection of Hg\(^{2+}\) in water from Yanhu Lake on the campus of the Chengdu University of Technology. After the pretreatment process, the results were in good agreement with those obtained...
by AFS and ICP-MS. Moreover, the sensitivity of the proposed ECL assay is comparable to that of ICP-MS, which is much higher than that of AFS. Miao et al. constructed an Fe₃O₄@Au-based Hg²⁺ biosensor and evaluated its possible applications in real samples, including Taihu Lake water, drinking water from Suzhou, orange juice and red wine. The obtained results showed satisfactory recoveries and acceptable accuracies, which also confirmed their good concordance with the atomic absorption spectroscopy (AAS) results.

A GO–ZnO–CdS nanohybrid-based biosensor prepared by Zhang et al. was used for the photoelectrochemical detection of river water, lake water, tap water, domestic sewage, and coalmine wastewater samples from the city of Taiyuan. The results showed great feasibility compared with a fluorescence biosensor. A CdTeSe@CdS QDs-based ECL biosensor was applied for testing mercury ions in fly ash. After a pretreatment process, the solution was determined by both the ECL biosensor and AAS. The results showed that the Hg²⁺ concentration from this biosensor matched the data detected by AAS. For the ECL biosensor, a linear regression equation was obtained to calculate the concentrations of mercury in real samples.

Our group has developed several Hg²⁺ biosensors based on different nanomaterials. The Pt nanotube arrays, tunable vertical SWCNTs, vertical metal–organic hybrid microarrays and 3D graphene/gold-based biosensors were all used in tap water and Taihu Lake samples. The results showed good agreement with the results obtained from ICP-MS. The tunable vertical SWCNTs and the 3D graphene/gold modified biosensors were also applied in the assay of human serum; the results indicated that these two nanomaterials demonstrate excellent biocompatibility and selectivity.

5. Conclusions and perspective

We have summarized the recent progress of advanced nanomaterials for the construction of various Hg²⁺ biosensors. The working principles of different detection strategies, namely enzyme inhibition, DNA probe and ASV, have been carefully discussed to evaluate their advantages and deficiencies. Novel nanomaterials with different categories for Hg²⁺ recognition have been introduced to exhibit their special morphologies and different preparation methods. Through nanostructure control, uniform and regular materials can be obtained that show positive effects on detection performance due to their excellent conductivity and electrocatalysis. Additionally, some of these materials possess both optical and electric properties, which can enhance promote their sensitivities, LODs and linear detection ranges in the PEC and ECL methods. Compared to the current widely applied Hg²⁺ analytical techniques, these advanced nanomaterials-based electrochemical biosensors not only exhibit high selectivity and sensitivity but can also realize rapid, inexpensive and portable detection of Hg²⁺. Similarly, these detection strategies can be extended to other heavy metal ions through the use of different enzymes and DNA probes.

For further practical applications, in situ and on-site monitoring of Hg²⁺ pollution is more meaningful to evaluate water quality and hazard levels. To achieve this goal, the stability and reusability of these nanomaterials require further study to enable long-term application.

Despite the abundant great progress that has already been made, several challenges remain to be solved in the future, and some of them are highlighted as follows. (1) Sensing materials: with the aid of advanced nanomaterials, the performance of electrochemical biosensors is promising to obtain much greater improvement via exact nanostructure control. Nanomaterials function as a bridge between the electrode and bioactive substances for signal generation and transfer. Further study is required to control the morphologies of nanomaterials and to enhance the binding forces between the nanomaterials and the electrode. (2) Detection targets: although currently proposed electrochemical biosensors show great performance in the detection of Hg²⁺, other mercury species which exist in the form of organic mercury are difficult to detect in real water samples. Thus, effective pretreatment procedures are often required to transform organic mercury into free Hg²⁺. Additionally, the design of other effective detection strategies is strongly encouraged in order to assay the total mercury element according to practical applications. (3) Component interference: in real water systems, various ions and organics can easily cause great damage to DNA probes. In order to meet the requirements of in situ and on-site detection, the stability and reliability of Hg²⁺ biosensors functioning in complex solution systems must be taken into consideration. (4) Device development: the common three-electrode system is complex and impractical to operate in real detection processes. More attention should be paid to developing portable devices and sensing chips for the immediate recognition of mercury leakage.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 21706116 and 21727818), the Innovative Research Team Program by the Ministry of Education of China (No. IRT_17R54), the Top-notch Academic Programs Project of Jiangsu Higher Education Institutions (TAPP) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Notes and references
