Microchim Acta
DOI 10.1007/s00604-017-2179-2

REVIEW ARTICLE

A review on nanomaterial-based electrochemical sensors for H_2O_2, H_2S and NO inside cells or released by cells

Hongying Liu [1,2] · **Lingyan Weng** [1] · **Chi Yang** [1,3]

Received: 23 September 2016 / Accepted: 11 March 2017

Abstract The incorporation of nanomaterials into electrochemical sensors is an attractive approach towards the improvement of the sensitivity of amperometry and also can provide improved sensor selectivity and stability. This review (with 137 references) details the current state of the art and new trends in nanomaterial-based electrochemical sensing of hydrogen peroxide (H_2O_2), hydrogen sulfide (H_2S) and nitric oxide (NO) in cells or released by cells. The article starts with a discussion of the significance of the three analytes, and this is followed by three sections that summarize the electrochemical detection schemes for H_2O_2, H_2S and NO. Each section first summarizes the respective physiological roles, and then reviews electrochemical sensors based on the use of carbon nanomaterials, noble metal nanomaterials, metal oxide nanomaterials, and layered doubled hydroxides. The materials are compiled in three tables along with figures of merit for the various sensors.

Keywords Cellular signalling · Cellular stress · Amperometry · Carbon nanomaterial · Noble metal nanoparticles · Metal oxide nanoparticles · Layered double hydroxide · Reactive oxygen species · Reactive nitrogen species · Reactive hydrogen sulfide species

✉ Chi Yang
yangchi@seu.edu.cn

[1] Yale-NUIST Center on Atmospheric Environment, Nanjing University of Information Science and Technology, Nanjing, Jiangsu 210044, China

[2] College of Life Information Science & Instrument Engineering, Hangzhou Dianzi University, Hangzhou 310018, China

[3] State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, China

Introduction

Biologically relevant small molecules including hydrogen peroxide (H_2O_2), hydrogen sulfide (H_2S) and nitric oxide (NO), endogenously produced molecules and play key roles in many biological processes. The expression levels of these molecules in cells are of great significance, abnormal expression levels in biological system are associated with many diseases, such as Parkinson's disease and cancer [1]. For example, H_2O_2 is an oxidative stress marker in age-related diseases including Alzheimer's disease, cardiovascular disorders and cancer [2, 3]. Similarly, H_2S is a biologically generated, gaseous signaling molecule that interacts with a wide range of physiological functions and is linked to numerous pathologies ranging from neurodegenerative diseases to hypertension and diabetes [4, 5]. Further, Unusual concentration of NO can boost the generation of reactive nitrogen species which can break down biomolecules like lipids, proteins and DNA [6]. Therefore, from these perspectives, it is worthy to develop sensitive, specific and fast techniques for these molecules detection from cells.

Currently, research into NM-based sensors has been used to improve the sensitivity or selectivity. For example, metal oxide NMs have been widely used for the detection of small molecules due to their special shapes and extraordinary chemical and physical properties [7]. They not only possess high surface areas favorable biocompatibilities, and good chemical stabilities, but also exhibit fast electron transfer. Semiconductor NMs have also received considerable interest as electrochemical sensors due to their low costs, variety of morphologies, high specific surface areas, excellent electrocatalytic activities, and the possibility of promoting electron transfer reactions at lower overpotentials [8]. Additionally, it has been observed that the large surface area and fast electron transfer of graphene make it an excellent carrier for active

Published online: 28 March 2017

probes and active domains as well as a fast conductor between electrodes and probes [9].It seems that the NMs make possible the potential development of (bio) sensor application in biologically relevant small molecules in cell. To date, several analytical approaches have been introduced for the determination of these biologically relevant small molecules in cells, such as chemiluminescence [10, 11], colorimetric assays [4, 5, 12], fluorescence [13–15], and electrochemical methods [16–18]. Among them, electrochemical techniques [19–23] are extremely attractive for the development of simple, inexpensive methods for the rapid and on-site monitoring of biologically relevant small molecules in cells. Thus, the combination of NMs with electrochemical sensors may provide a promising platform for the biosensing of biologically relevant small molecules in cells. The intent of this review is to impart insights into NM-based electrochemical sensors, and to illustrate their potential benefits in sensors for H_2O_2, H_2S and NO detection in cells.

In this review, we aim to present a universal and critical review of the recent advances in NM-based electrochemical sensors for biologically relevant small molecules in cells. New and significant developments in NM-based on electrochemical biosensors for the detection of H_2O_2, H_2S, and NO from cells are first summarized in sections 2, 3 and 4, respectively. Finally, the future research directions for NM-based electrochemical sensors for sensing systems are discussed. This review will help further developments in this hot area.

Electrochemical detection of H_2O_2 from cells

Physiological roles of H_2O_2

H_2O_2 is the most common representative and stable ROS in living organisms, and it plays a critical role in normal cellular function and proliferation [24]. It is a product of a wide range of biological and chemical processes and it is produced by external stimuli and intracellular signaling molecules [25]. The long lifetime and chemical stability allow it to penetrate into other cellular compartment easily, which gives it many important physiological functions [26]. First, as an intracellular signaling molecule, it regulates DNA damage, protein synthesis, and cell apoptosis, as well as many other functions [27]. These functions lead to diverse physiological and biochemical processes, such as immune system, stem cell and cancer effects. Second, it can produce intracellular thermogenesis, which is the basic foundation for life processes [28]. Third, the over production and accumulation of H_2O_2 in cells give rise to internal sabotage activities and lead to oxidative stress, which subsequently damages cellular constituents and causes various diseases such as cardiovascular disease and cancer [24]. Thus, the dynamic monitoring of H_2O_2 release from living cells is of great significance. This information leads to a perfect understanding of its regulating signal transduction pathways and provide reliable and new therapeutic strategies for related diseases.

Sensing of H_2O_2 from cells

Considerable efforts have been devoted to developing various sensing methods and among them, electrochemical biosensors that contain enzymatic and non-enzymatic electrochemical H_2O_2 sensors have received considerable attention. Nanomaterials, which have excellent electrochemical behaviors, have been used in promising immobilization matrices, transduction platforms and mediators in enzymatic and non-enzymatic electrochemical sensors.

Enzymatic H_2O_2 electrochemical sensors

Enzymatic electrochemical sensors have proven to be an effective method for detecting H_2O_2. Many redox proteins including cytochrome *C*, myoglobin, hemoglobin, ferredoxin, and horseradish peroxide (HRP) have been used to fabricate enzymatic H_2O_2 electrochemical sensors [2]. Their operation principles are based on direct electron transfer from proteins. The redox state of the analyte or associated species was altered by intermediately storing the redox equivalents in a redox protein-integrated prosthetic group or the transfer of electrons between a suitable co-substrate within the active site. Thus, these enzymatic electrochemical sensors exhibit low detection limits, fast response times, good long-term stabilities and low costs.

In particular, the enzymes HRP and cytochrome c have received considerable interest. They are the most commonly used enzymes in enzymatic H_2O_2 electrochemical sensors due to their efficient catalysis of the oxidation reactions of several substances with H_2O_2. Furthermore, tremendous efforts have been made to detect extra-cellular or intra-cellular H_2O_2. However, only a few sensors satisfy the abovementioned requirements for H_2O_2 sensing in cells due to their insufficient stabilities, lower reproducibility and oxygen limitations. For example, in 2005, Kasai et al. reported a H_2O_2 electrochemical sensor array that is fabricated by modifying a 64-channel ITO electrode array with HRP and an electron transfer mediator [29]. In this way, the H_2O_2 release from a rat hippocampal slice culture at multiple positions was monitored using this H_2O_2 electrochemical sensor.

To overcome this problem, various strategies were employed to immobilize enzymes on electrode surfaces, such as polymer entrapment, electropolymerization, sol-gels, layer-by-layer techniques and covalent linking [30–33]. However, the sensor cannot be applied for tracing the extracellular H_2O_2 released from cells due to the limited electron transfer reactivity and activity of the immobilized enzyme, which is attributed

to the lack of orientation of the immobilized enzymes on the electrode surface. Li et al. presented an amperometric biosensor for the detection of hydrogen peroxide released from human breast cancer cells based on a sequence-specific peptide [34]. The favorable orientation of the immobilized HRP on the electrode surface due to the strong affinity between the peptide and the HRP promotes the catalytic activity of the immobilized enzyme towards the reaction of o-phenylenediamine and H_2O_2. The amperometric current is highly linear over the range from 0.1 to 100 μM with an LOD of 0.03 μM. Finally, the biosensor was used to monitor extracellular H_2O_2 released from MCF-7 human breast cancer cells. Similarly, Suarez et al. developed a novel third generation biosensor based on one-shot adsorption of chemically modified cytochrome c on bare gold electrodes, as shown in Fig. 1 [35]. The introduction of thiol derivatives on the cytochrome c leads to quasireversible electrochemical behavior and fast direct electron transfer. The electrode showed good analytical performance for sensing H_2O_2 and, thus, was applied to detect and non-invasively detect H_2O_2 produced by phytoplanktonic cells in response to Cd(II) exposure non-invasively and in real time.

The stabilities of directly used enzymes in solution are poor. Thus, another effective method is selecting an appropriate carrier for immobilizing enzymes. NMs have exceedingly large surface areas, which make them excellent carriers for immobilizing enzymes. They will preserve the intrinsic structures of the enzyme through higher number of active site exposed to NMs [36] and enhance the efficiency of the biosensor. Due to these properties, various NMs have been employed to immobilize enzyme. Cai's group reported a new amperometric H_2O_2 biosensor based on HRP-attapulgite that was prepared by self-assembling HRP on the surfaces of natural mineral attapulgite nanostructures [36]. The nanosize of the attapulgite greatly facilitates direct electron transfer between the HRP and the electrode surface. Therefore, the biosensor was employed to measure the H_2O_2 flux from RAW 264.7 macrophage cells. Similarly, they also presented an electrochemical biosensor based on HRP-hydroxyapatite nanohybrids for the detection of extracellular H_2O_2 released from RAW 264.7 murine macrophage cells. A rapid response (less than 2 s), a low detection limit (0.1 μM), and a wide linear range (5 μM-0.82 mM) were presented. Tian et al. fabricated cytochrome *c*/hexagonal ZnO nanosheet electrochemical biosensors via an electrodeposition and immersing method [37]. The resulting ZnO nanosheets not only retain the intrinsic activity of cytochrome c but also exhibited a more positive formal redox potential than previously reported metal-oxide and zeolite-surface-modified biosensors. Experimental data demonstrate that the biosensor possesses an excellent selectivity. Thus, it is a promising method for the detection of extracellular H_2O_2 released from human hepatoma cell line HepG2.

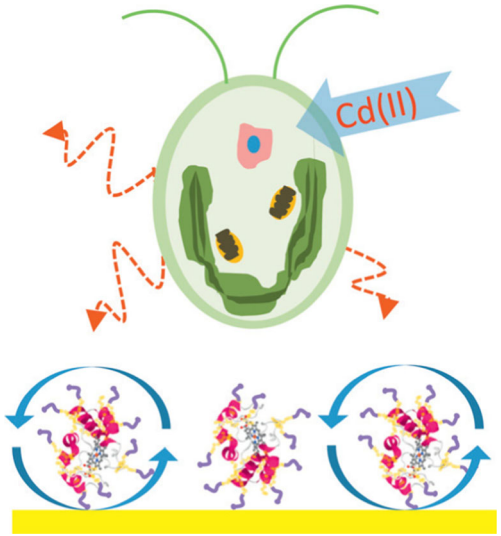

Fig. 1 Schematic representation of the extracellular detection of H_2O_2 released by *C. reinhardtii* upon Cd(II)-induced oxidative stress using the MPA-cyt *c*/Au biosensor

As society progresses, the miniaturization, integration and portability of chemical analysis equipments are some of the most crucial trends. For this purpose, Tian's group explored miniature microarrays that were successively decorated with Au-TiO_2 micropatterns, *L*-cysteine, and cytochrome *c* [38]. The construction process of the biosensor is shown in Fig. 2. Experimental results revealed that the enzymatic activity of cytochrome *c* was maintained and that the direct electron transfer of cytochrome *c* was enhanced. Under the optimum conditions, it was used to electrochemically monitor cellular H_2O_2 in the range of 10^{-9} to 10^{-2} M in real-time.

Non-enzymatic H_2O_2 electrochemical sensors

As described above, to meet the requirements of H_2O_2 sensing in cells, various electrochemical biosensors based on the immobilization of enzymes on electrodes were used. However, the insufficient stability and lower reproducibility limited the wide application of enzyme sensors. Thus, non-enzymatic electrochemical H_2O_2 sensors were ideal substitutes [39]. Nanomaterials (NMs) are now being applied in more and more fields [40–44]. The excellent electrochemical behaviors of NMs indicate that NMs are promising electrocatalysts and can be used as electrode materials in the fabrication of non-enzymatic electrochemical sensors. Several classes of NMs, such as metals, metal oxides, alloys, carbon-based materials, and layered double hydroxides, have been employed as electrocatalysts in the fabrication of non-enzymatic electrochemical H_2O_2 sensors based on their strong electrocatalytic abilities. In this section, we present the application of these NMs in non-enzymatic electrochemical H_2O_2 sensors.

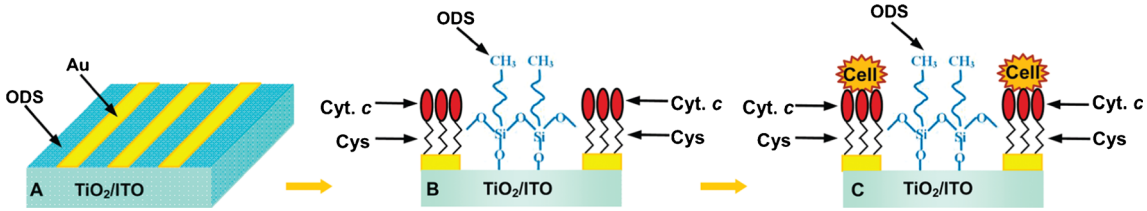

Fig. 2 Illustration of miniature enzyme-based electrodes

Carbon-based sensors

Carbon-based NMs with different dimension have a unique place in theoretical scientific communities for practical applications due to their exceptional electrical, thermal, chemical and mechanical properties. The advances in electrochemical sensors based on graphene NMs for the detection of H_2O_2 are summarized in Table 1. Since they were discovered by Iijima in 1991, carbon nanotubes have been the most widely studied NMs for electrochemical sensors [45]. Their superior electron transfer capabilities make them excellent electrocatalysts for non-enzymatic H_2O_2 electrochemical sensors. Rawson's group presented a novel, non-enzymatic, vertically aligned single-walled carbon nanotube (SWCN) sensor integrated onto an indium tin oxide conducting substrate for the detection of glucose [46]. Here, osmium bipyridine (Osbpy) was modified on the surface of SWCN and used as a bioelectrocatalyst. It offers unprecedented detection of local intracellular H_2O_2 'pulses' on a short second time scale in response to bacterial endotoxin stimulation. Furthermore, the results in this paper have the benefit of shedding new light on the effects of ROS in cell function.

In addition to carbon nanotubes, graphene is common, and a pioneering approach was introduced by Geim's group in 2004 [47]. These materials have attracted extensive interest and shown great promise for energy storage and conversion, biomedical devices and biosensors [48–53] due to their unique properties including high Young's moduli, high surface areas, excellent thermal and electrical conductivities, outstanding optical transparencies, and strong thermal conductivities [54–57]. In particular, their large surface areas and fast electron transfers make graphene materials excellent carrier for more active probes and active domains as well as fast conductors between electrodes and probes for the electrochemistry community [9]. These features will facilitate signal amplification and thus achieve high sensitivity for electrochemical biosensors. Advances in electrochemical sensors based on graphene for the detection of H_2O_2 in cells are summarized in Table 1.

Chemical doping with heteroatoms, such as nitrogen (N), boron (B) or sulfur (S) atoms, has been proven to be an effective strategy for modulating the chemical and physical properties of graphene and thus enhancing the performance of doped graphene in electrochemical biosensors [73]. In 2011, Cai et al. presented an electrochemical biosensor for measuring the dynamic process of H_2O_2 release from living cells based on N-doped grapheme, which suggesting a pronounced electrocatalytic activity of N-graphene for H_2O_2 reduction. [26]. At −0.4 V, the biosensor revealed a good relationship in the range of 0.5–1200 μM with a detection limit of ca. (0.05 ± 0.01) mM ($S/N = 3$). Zhu's group proceeded to synthesize N and B co-doped graphene with a hierarchical framework via a microwave-assisted strategy [58]. Compared with graphene solely doped with N or B atoms, the N and B co-doped graphene showed an improved electrochemical performance due to a synergistic effect between the heteroatoms. Thus, it was used to fabricate an electrochemical biosensor for real-time quantitative detection of H_2O_2 from living cells at the nanomolar level.

The combination of graphene and inorganic NMs was another promising approach to improve the signals from electrochemical biosensors. Jiang et al. presented a novel Copper Sulfide (CuS)-decorated reduced graphene oxide (rGO)-based sensor for the reliable detection of H_2O_2 [8] (see Fig. 3). The innovative electrocatalyst CuS/rGO was prepared by heating the mixture of $CuCl_2$ and Na_2S aqueous solutions in the presence of poly(vinyl pyrrolidone)(PVP)-protected rGO at 180 °C,which possess good electrocatalytic activity towards the redox of H_2O_2. As shown in Fig. 3, satisfactory results were presented in monitoring the concentration of H_2O_2 in biological samples including human serum, urine samples and H_2O_2 released from human cervical cancer cells. Similarly, Pt nanoparticles [8, 59], Fe_3O_4 nanoparticles [60], Co_3O_4 nanowires [61], Au@Pt@Au core-shell nanoparticles [62], MnO_2 nanowires [64] and their decorated rGO can promote the electron transfer reactions of H_2O_2 at a low overpotential. To improve the sensitivity of the H_2O_2 electrochemical sensors, slices of the metal-metal oxide nanostructures were introduced into graphene systems. A new type of flexible electrochemical sensor fabricated by depositing high-density Pt nanoparticles on freestanding rGO paper carrying MnO_2 nanowire networks has been reported by Duan's group [65], as shown in Fig. 4. The sensitivity was greatly improved to 129.5 μA cm^{-2} mM^{-1} based on the metal-metal oxide design, which integrates the mechanical and electrical properties of rGO, the large surface area of MnO_2 networks, and the catalytic activity of well-dispersed small Pt nanoparticles.

Table 1 Summary of graphene-based and metal (oxide)-based electrochemical sensors for H_2O_2

Electrode composition	Linear range (μM)	Detection limit (μM)	Sensitivity μA cm^{-2} mM^{-1}	Working potential	Year	Ref
N-graphene	0.5–1200	0.05		-0.4 V	2011	[26]
NB-G	0.5–5000	0.05	-	-0.25 V	2013	[58]
CuS/rGO	5–1500	0.27	26.5	-0.27 V	2013	[23]
rGO − Pt	0.5–3475	0.2	459	-0.08 V	2014	[59]
Fe_3O_4/rGO	1–20,000	0.17	387.6	-0.3 V	2014	[60]
Co_3O_4–rGO	15–675	2.4	1140	-0.19 V	2014	[61]
GO/Au@Pt@Au	0.05–17,500	0.02		+0.5 V	2015	[62]
	500–110,000	0.25		-0.3 V		
Pt/PG	1–1477	0.5	341.14	-0.1 V	2015	[63]
MnO_2–ERGO	0.1–45.4 mM	10	59.0	-0.5 V	2015	[64]
Pt–MnO_2/rGOP	2–13,330	1	129.5	-0.15 V	2012	[65]
rGO/AuFe_3O_4/Pt	0.5–11.5	0.1	184.43	0 V	2015	[66]
Au/MnO_2/ERGO/CF	50–1000	2	167	-0.4 V	2015	[67]
rGO-PMS@AuNPs	0.5–50,000	0.06	39.2	-0.75 V	2014	[68]
PtPd/IL–rGOP	0.1–37.6	0.01		0.2 V	2015	[69]
Pt/rGO–CNT paper	0.1–25	0.01	1.41	-0.25 V	2015	[70]
rGO–Au–PTBO	5–1077.1	0.2	63.39	-0.3 V	2013	[71]
	1474.5–2536.2	24.52				
3D graphene/pDA/TH	0.4–660	0.08	169.7	- 0.25 V	2013	[72]

Other metal-metal oxides, such as AuFe_3O_4/Pt [66], and Au/MnO_2 [67], were also explored.

In addition, the modification of graphene with small molecules and polymers opens a new avenue for enhancing the electrochemical signals from graphene-based biosensors. Wang's group explored a facile electrochemical biosensor fabricated via the integration of graphene with gold nanoparticles and poly-(toluidine blue O) films (rGO–Au–PTBO) to assess the oxidative stress elicited by H_2O_2 in cells [71]. As shown in Fig. 5, the rGO–Au–PTBO-modified electrode was presented by simultaneously depositing AuNP and PTBO films on graphene via layer-by-layer electrochemical deposition. The experimental results revealed that linear response range for the rGO–Au–PTBO films was greatly increased, which provides an opportunity for the evaluation of intracellular

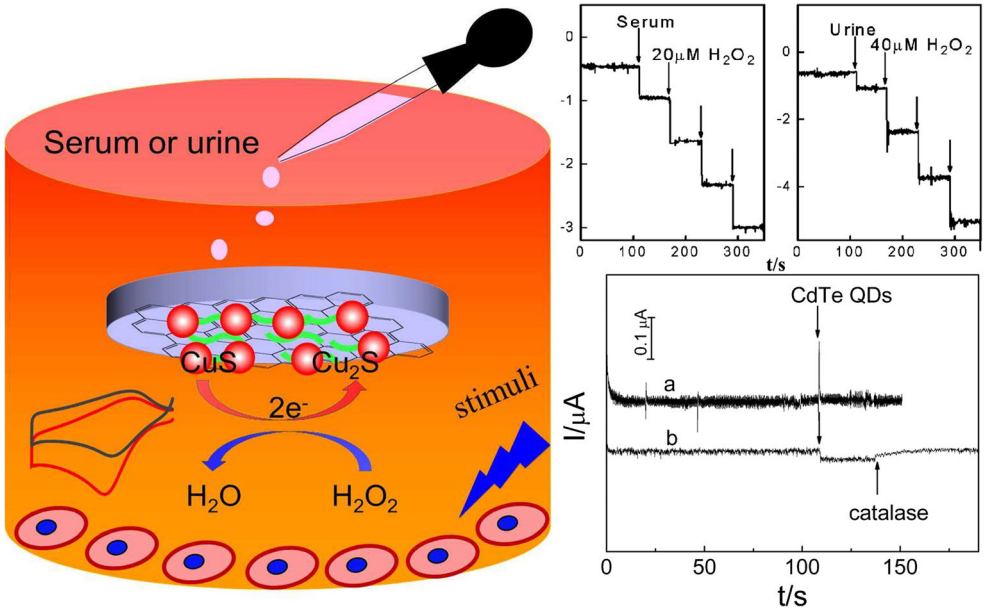

Fig. 3 Schematic illustration of copper sulfide-decorated reduced graphene oxide composites for enhanced detection of H_2O_2 in biological environments

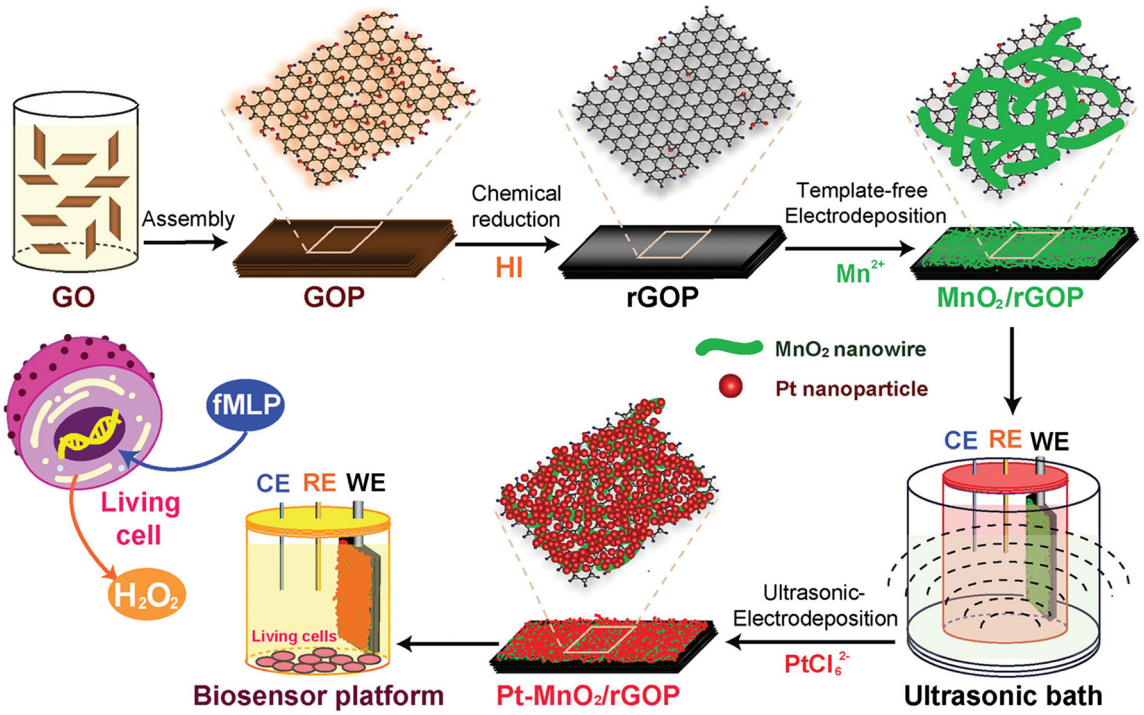

Fig. 4 Schematic illustration of metal–metal oxide nanostructures on freestanding graphene paper for flexible biosensors

oxidative stress elicited by H_2O_2 from tumor cells. At the same time, Chen et al. presented a novel three-dimensional electrochemical sensor, which is fabricated by immobilizing thionine molecules on graphene via polydopamine linkers [72]. Under stimulation of *N*-formyl-*L*-methionyl-*L*-leucyl-phenylalanine (fMLP), a bacteria-derived potent pro-inflammatory stimulant, the biosensor can detect the dynamic release of H_2O_2 in real-time from MD-435 cells. Ionic liquids (ILs) also were used to modify graphene. Duan's group fabricated a new type of flexible and versatile nanohybrid paper electrode by ultrasonically-electrodepositing PtPd alloy nanoparticles on freestanding ILs [69]. The electroactive surface area of the graphene-based nanohybrid paper electrode was increased, and the adhesion and dispersion of the metal nanoparticles on the paper surface was improved. It was also used for real-time tracking of H_2O_2 secretion in live macrophages cells.

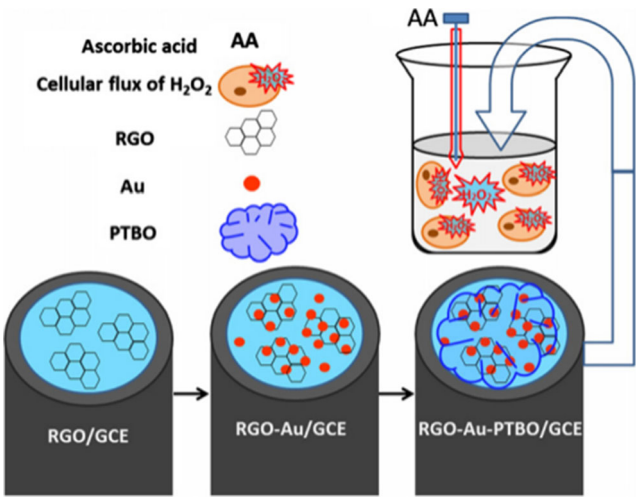

Fig. 5 Schematic of the LBL assembly of graphene, Au and poly (toluidine blue O) films sensor for tracking H_2O_2 flux from cells stimulated with AA

Grapheme quantum dots(GQDs), i.e., the zero dimensional NMs composed of graphene oxide, possess better performance than micrometer-sized graphene. This material has been recognized as a favorable candidate for sensing materials considering its better characteristics as an electron transporter and acceptor. In Zhang's work [74], GQDs were covalently assembled on Au electrodes, and then the dynamic H_2O_2 release in human breast adenocarcinoma cell line MCF-7 was monitored using the prepared GQDs/Au electrode. The H_2O_2 release was triggered by injecting phorbol myristate acetate, a compound that can induce H_2O_2 generation in cells. Due to the high peroxidase-like activity and small size, the sensor exhibited a wide linear range, low detection limit, and fast amperometric response to H_2O_2. Subsequently, an electrochemical biosensor based on rGO QDs/ZnO hybrids was reported by Yang et al. [75]. In this work, rGO QDs/ZnO hybrids were synthesized via the combination of simple electrospinning and thermal treatment processes (see Fig. 6).Then the release flux of H_2O_2 from cells was measured with the use of the rGO QDs/ZnO hybrids electrode. The results show that the electrodes with rGO

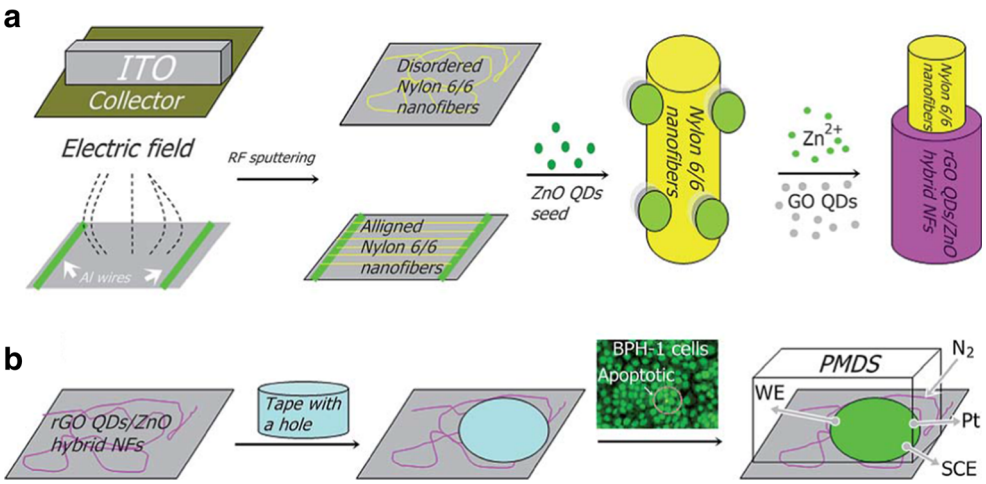

Fig. 6 (a) Illustration of the synthetic route of rGO QDs/ZnO hybrid nanofibers and (b) fabrication of the cell-based intracellular H_2O_2 sensors

QDs/ZnO have high electrocatalytic activities. The main reason for the high electrocatalytic activity of rGO QDs/ZnO composite is synergistic electrocatalytic reduction of H_2O_2 by rGO QDs, ZnO(Oi^-/Oi^{2-} on the surface of ZnO) and inferred the following reaction:

$$Oi^- \Big/ Oi^{2-} + H_2O_2 \rightarrow O_2(g) + H_2O + e^- \Big/ 2e^-$$
$$rGO\text{-}(C\text{-}OH)n + H_2O_2 \rightarrow O_2(g) + H_2O + rGO$$

Similar strides have been made for surfactant-free Au nanoparticles on nitrogen-doped graphene quantum dots (NGQDs) [76]. And used for electrocatalytic reduction and sensing of H_2O_2, showing a low detection limit of 0.12 μM and sensitivity of 186.22 μA/mM cm^2. Importantly, the Au-NGQDs-based electrochemical biosensor has shown great potential applications for detection of H_2O_2 released from human cervical cancer cells with satisfactory results.

In addition to the above-mentioned carbon materials, other carbon materials such as mesoporous carbon nanocomposite have also been applied in the field of H_2O_2 electrochemical biosensors. MnO nanoparticle@mesoporous carbon composites were grown on conducting substrates via a two-step coating/calcination approach [77]. The biosensor was reported to measure H_2O_2 produced by living cells.

Noble metal, non-noble metal NMs and their hybrid nanomaterial-based sensors

Noble metal nanomaterials (NMs) have attracted considerable interest in many fields from fundamental research to clinical applications due to their unique physicochemical properties [78]. Their physical and chemical properties of the hybrid noble metal NMs can be accurately tuned by controlling their size, shape, architecture, and composition [79]. This property will enhance their potential applications, especially in biosensors. Thus, considerable effort has been devoted to integrating noble metal NMs (Pt, Pd, Ag, and Au) and their alloys into biosensors because of their high catalytic activities [80]. This approach will improve the sensitivity and selectivity of biosensors. In this case, Pt NMs, which possess high-index facets, complex morphologies or multi-compositions, were regarded as the most widely used catalyzer considering their high catalytic activities toward small molecule oxidation [81]. For instance, Huang et al. discovered a novel sensitive electrochemical sensor by electrochemically depositing Pt nanoparticles on the surface of carbon fiber microdisk electrodes [82] in 2009. Nafion was chosen as the molecular template for the deposition and dispersion of Pt nanoparticles, which makes the formation of nanoparticles uniform. With the aid of the microelectrochemical sensors, oxidative bursts from single plant protoplasts were monitored in real-time. The group subsequently reported a self-supported nanoporous gold microelectrode decorated with well-dispersed and tiny platinum nanoparticles that was applied to monitor the H_2O_2 released from single isolated human breast cancer cells in real time [83]. The detection limit was as low as 0.3 nM. Similarly, Yu and co-workers used the same electrochemical device based on a Pt nanosphere-paper working electrode to determine the flux of H_2O_2 from SK-BR-3 cancer cells [84].

Another noble metal, silver, was discovered to be the most valued and precious metal throughout history. Silver nanoparticles(AgNPs) have been particularly interesting and extensively applied as biosensors, antibacterial agents and catalysts since Evanoff and Chumanov reported the Lee-Meisel method for the synthesis of AgNPs in 1982 [85]. AgNPs are usually stable. However, Loza et al. noted that the dissolution of AgNPs can be enhanced by adding strong oxidizing species such as H_2O_2 [86]. The AgNPs modified electrode can be used as the sensing

interface and H_2O_2 then decompose and generates hydroxyl radical and superoxide anion, which finally turn to O_2 and H_2O. The mechanism is as follows:

$$Ag_n(solid) + 3H_2O_2 + 2H^+ \rightarrow Ag_{n-2}(solid) + 4H_2O + 2Ag^+ + O_2$$

By taking advantage of this behavior, an electrochemical biosensor modified with AgNPs was explored for tracking H_2O_2 secretion in live cells [87].

In comparison with monometallic noble metal NMs, bimetallic NMs have been drawing significant attention and have been used as electrocatalysts for H_2O_2 due to their bifunctional effects and ligand and/or electronic effect [88]. For example, ultrathin AuCu nanowires were prepared via a facile wet chemical route at room temperature and then used as the modifying layer for electrodes [89]. The archived biosensor exhibits excellent electrocatalytic activity (the sensitivity was up to 2,710,000 μA cm^{-2} mM^{-1}, which is greater than any other reported previously) because of the synergetic effect of Cu and Au atoms. These excellent properties make this platform use for the detection of trace concentrations of H_2O_2 released by cells.

On the other hand, non-noble metals and their corresponding oxides or sulfides with different compositions and micro/nanostructures have also attracted extensive interest in H_2O_2 electrochemical biosensors [22] (Table 2) . Their unique properties including high surface areas and fast electron transfer rates make them useful as electrocatalysts. Recently, ZnO nanowires, an inorganic metal oxide NMs, was discovered to fabricate a sensor based on the piezotronic effect for sensing H_2O_2 released by Raw264.7 cells at a detection limit of 2 nM [90]. Du's group synthesized monodisperse $Cu_{2-x}S$ NCs and found that $Al_2(SO_4)_3$ can control the shape and size of $Cu_{2-x}S$ NCs [95]. The synthesized $Cu_{2-x}S$ NCs have activities for the sensitive detection of H_2O_2 that are comparable with noble metals and peroxidase enzymes. They have the best catalysis for sensing H_2O_2. The detection limit of 0.18 μM is useful for the detection of H_2O_2 release from MKN-45 cells.

Moreover, the hybrid NMs by encapsulating noble metal and non-noble metal presents a new approach to develop new-style material with high quality and to improve the capabilities of nanocatalysts for H_2O_2 sensing. Wang and co-workers synthesized ultrafine Pd nanoparticles encapsulated in microporous Co_3O_4 and investigated their catalytic activities, as illustrated in Fig. 7 [91]. The Pd@Co_3O_4 hollow nanospheres exhibited greater activity than Pd or Co_3O_4 nanoparticles even when the Pd content is as low as 1.14 wt% because of the synergy between the permeable microporous Co_3O_4 shell and the active Pd NPs. Based on these findings, an electrochemical biosensor was fabricated to detect the secretion of H_2O_2 in living human cells. Similarly, dumbbell-like PtPd − Fe_3O_4 nanoparticles [92], core/shell Au/MnO nanoparticles [93], and quasi-core/shell-structured TiO_2@Cu_2O [94] were also reported for the electrode modification in cells released H_2O_2 detection.

Electrochemical detection of H_2S from cells

Physiological roles of H_2S

H_2S was recognized as a colorless gas with a pungent rotten egg taste 300 years ago [3]. In the 1990s, it was regarded as the

Table 2 Summary of metal and metal oxide-based non-enzymatic electrochemical for H_2O_2

Electrode composition	Linear range (μM)	Detection limit (μM)	Sensitivity μA cm^{-2} mM^{-1}	Working potential	Year	Ref
Pt NPs/CFME	0.01–0.1	0.005	-	0.6 V	2009	[82]
NPG/Pt NPs	-	0.0003	-	-0.2 V	2015	[83]
Pt nanosphere					2014	[84]
AgNPs	1–300	0.5	-	-	2015	[87]
AuCuNWs	0.003–0.36	0.002	2,710,000	0.484 V	2015	[89]
ZnO NW	0.01–60	0.002	0.5 μA M^{-1} S^{-1}	-	2015	[90]
CuS	5–11,300	5	-0.35 V	2015	88	
Pd@Co_3O_4	0.5–1200	0.1	240	0.6 V	2015	[91]
PtPd-Fe_3O_4		0.005				[92]
Au/MnO	0.02–0.1	0.008	17,850	-0.28 V	2014	[93]
	0.1–1111.1		208			
	1111.1–15,110		530			
TiO_2@Cu_2O	1–15	0.15	2229.1	-0.2 V	2015	[94]
	15–330		453.7			
	330–16,600		181.1			

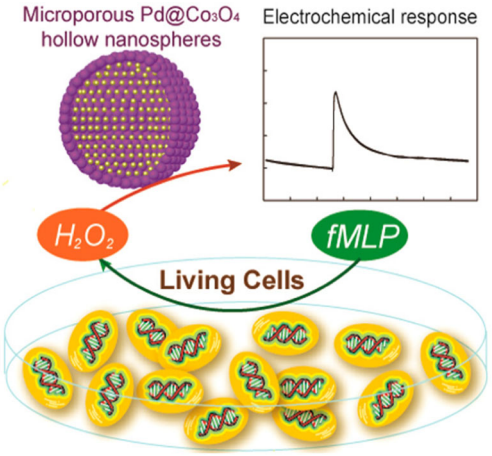

Fig. 7 Schematic diagram of a Pd@Co_3O_4 hollow nanosphere modified electrode. Most recently, a new type hybrid NMs of layered doubled hydroxides (LDHs) as a class of multi-metal anionic clays with lamellar structures has attracted considerable interest from researchers due to their relatively low costs, high redox activities, and environmentally friendly nature [96]. Their extraordinary intercalation features make them excellent carriers for entrapping nanoparticles. Liu's group prepared a series of MgAl LDH nanohybrids loaded with MnO_2 nanoparticles with fixed Mg/Al via a facile co-precipitation method (Fig. 8) [97]. The NMs were used to fabricate an electrochemical biosensor for real-time tracking of H_2O_2 secreted by live cells. Due to synergistic effect of the good catalytic ability of MnO_2 and the conductivity of MgAl LDH, a wide linear range of 0.05–78 mM and lowest detection limit of 5 mM was presented

third endogenous gas signal molecule (following nitric oxide and carbon monoxide) that mediates the central nervous system, cardiovascular system and other biological systems under both physiological and pathological conditions [98]. The production of H_2S in the central nervous system can be catalyzed by cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST) [99]. It has important physiological regulation functions in the nervous system, vascular system, metabolism, erythrocytes, and digestive system [100]. At physiological concentrations, H_2S plays a key role in the induction of long-term hippocampal potentiation and has some antioxidant, anti-inflammatory, and anti-apoptotic effects. It induces the formation of calcium waves to mediate the signal transmission between neurons and astrocytes. Finally, H_2S is also related to many clinical diseases including Alzheimer's disease, Parkinson's disease, ischemic stroke, and traumatic brain injury [101].

Sensing of H_2S from cells

The above studies highlight the potential value of developing useful methods to monitor the concentration of H_2S in biological samples. For this purpose, several avenues were explored. Compared with other methods, electrochemical methods are comparatively sensitive, fast and simple. Over the past 20 years, scientists presented many electrochemical strategies including ion sensitive electrodes, polarographic H_2S sensors, and enzyme-based electrodes for sensing H_2S [102–109]. In the chemical industry, solid-electrolyte sensors were the most widely used method for monitoring H_2S, while ion sensitive electrodes and amperometric H_2S sensors were used in biological samples. Zhang et al. has made an effort to summarize up-to-date research on the electrochemical detection of H_2S in biological samples, such as plasma, blood, tissues, and mitochondria [110]. However, few references focused on electrochemical sensors for H_2S released from cells, especially in combination with NMs. We found that Yu [111] built up a photoelectrochemical sensor for H_2S determination. Cd^+ was adsorbed and immobilized onto TiO_2 nanotubes by modified thioglycolic acid. CdS would be formed on the surface of TiO_2 nanotubes after treating with cell-released H_2S. Photocurrent is generated by the grafting of covalently CdS nanoparticles on the surface of TiO_2 nanotubes, which is associated with the H_2S released from cancer cells.

Electrochemical detection of NO from cells

Physiological roles of NO

NO, as a short-lived regulatory and soluble gas, is synthesized in the body from *L*-arginine with the aid of the family of enzymes named nitric oxide synthase (NOS), which were discovered in the 1980s [112]. It was identified as the endothelium-derived relaxation factor (EDRF) by Furchgott, Ignarro, and Murad, who were awarded the Nobel Prize in

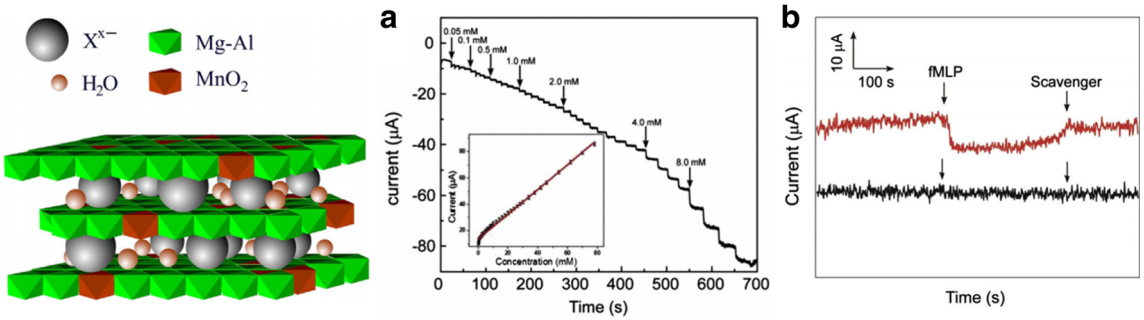

Fig. 8 Schematic diagram of the MgAl LDH biosensor for the detection of H_2O_2 released from cells

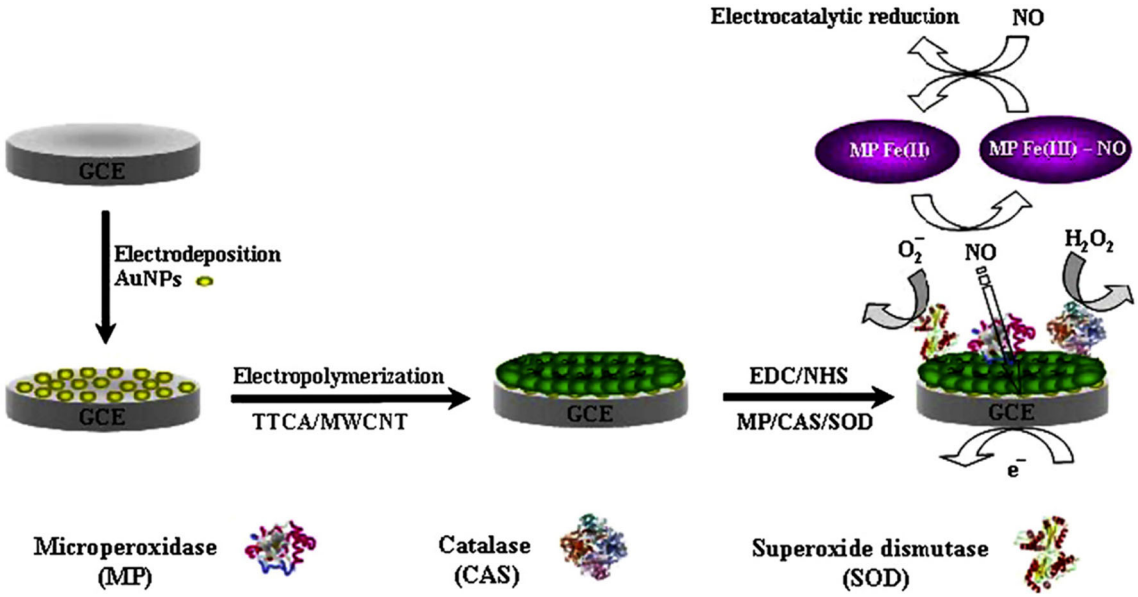

Fig. 9 Schematic for the overall detection process of NO using the CAS/SOD/MP/MWCNT-PTTCA/AuNPs biosensor

physiology in 1998 [113]. Many scientists have claimed that it is an important intracellular and intercellular secondary messenger in the human body that is involved in a wide variety of biological and cellular functions, such as the regulation of diverse physiological and pathophysiological mechanisms of the cardiovascular system and immune system [114]. The biological roles of NO have shown strong links to numerous clinical disorders, including asthma, rheumatoid arthritis, tuberculosis, and Alzheimer's disease [115]. Interestingly, NO is double-edged, i.e., beneficial or harmful based on its level of production. For instance, several studies have demonstrated that a low level of NO was good for tumor growth via

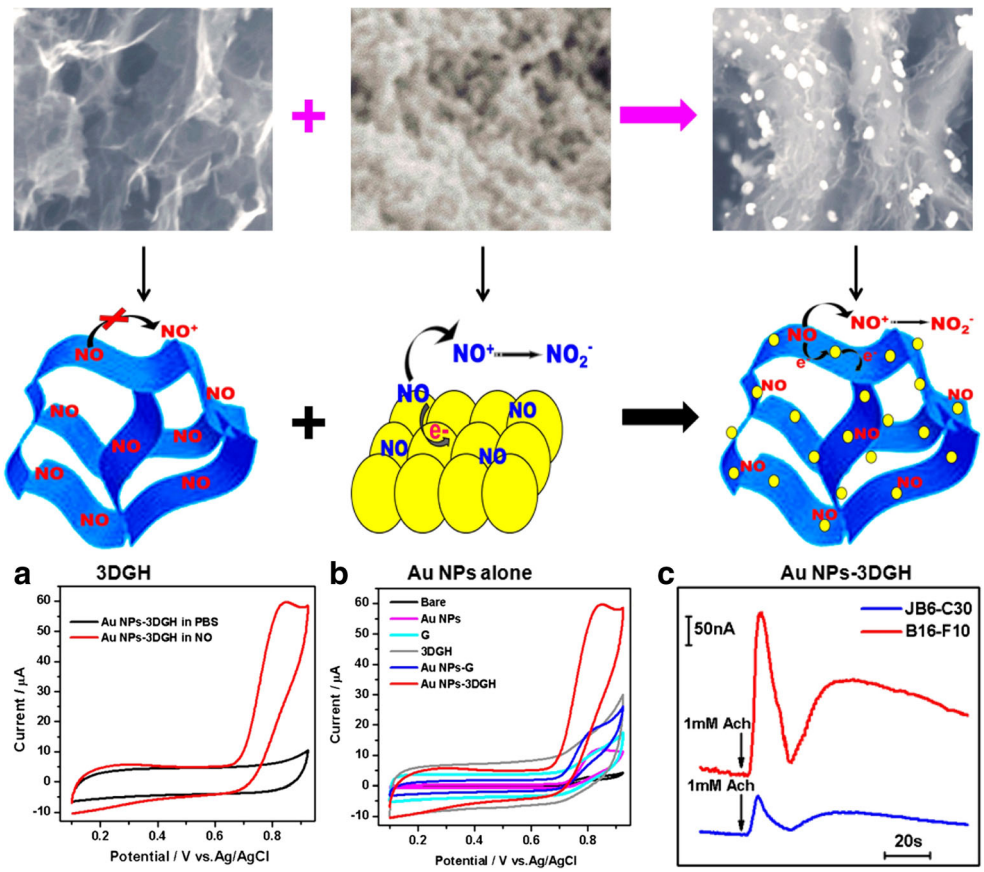

Fig. 10 Diagram of the catalytic mechanism of the Au NPs − 3DGH nanocomposite toward NO

angiogenesis, while high levels are damaging to tumor cells [116]. Due to the above mentioned functions, monitoring NO in biological samples is extremely important. However, this measurement is an extremely challenging task because of the short half-life, high reactivity, and low expression of the molecule.

Sensing of NO from cells

Several attempts have been made to propose new tricks for the rapid and precise detection of NO, such as chemiluminescence [117, 118], paramagnetic resonance spectrometry [119, 120], and electrochemical methods [121–124]. Electrochemistry has aroused received considerable interest due to its high sensitivity, instrumental simplicity, short analysis time, and low cost. Up to now, three electrochemical sensor styles including Shibuki-style, solid permselective, and solid catalytic have been used to detect NO. The electrode materials play a crucial role in the sensitivity and selectivity of the sensor. In recent years, NMs ranging from carbon NMs to metal NMs have opened new approaches for sensing NO from cells using electrochemical sensors due to their "small" sizes, large surface areas, and unique chemical properties [125].

Carbon nanomaterial-based electrochemical sensors

Since their discovery in 1991, carbon nanotubes (CNT) have been widely applied in electrochemical sensors due to their remarkable chemical, physical, electrical, and structural properties. Their high conductivities and strong electrocatalytic activities make them attractive substrates for electrochemical sensors. New CNT based nanomaterials (NMs) were introduced to measure NO from cells, as shown in Table 3. A single-walled carbon nanotube and Nafion membrane-modified carbon fiber microdisk was successfully applied for the measurement of NO release from a single isolated human umbilical vein endothelial cell [126]. The detection limit was 4.3 nM, which is lower than those of bare sensors and most of the earlier reported NO electrochemical sensors. The interference effects from nitrite and ascorbic acid are reduced due to the introduction of the Nafion membrane. Another water-soluble multi-walled carbon nanotube and polyazocarmine B nanofilm-modified electrode was fabricated via a new noncovalent approach coupled with an electropolymerization procedure and then used to monitor NO release from rat liver cells [127]. Subsequently, Shim et al. reported a highly sensitive and selective electrochemical method for NO detection via covalently immobilizing three enzymes including microperoxidase, catalase, and superoxide dismutase onto the MWCNT-poly-5,2′,5′,2″-terthiophene-3-carboxylic acid (PTTCA) nanocomposite layer, as illustrated in Fig. 9 [128]. The co-immobilized enzymes can remove the interference of H_2O_2 and O_2^- during NO detection. The detection limit was down to 4.3 nM and can monitor NO release from real samples containing rat liver, stomach (AGS), and intestinal (HT-29) cancer cells.

Graphene is also a promising carbon-based material for improving the sensitivity of sensors due to its large surface area and biocompatibility. Li's group has made many contributions in this field. In 2012, they constructed an RGD-peptide functionalized graphene biomimetic live-cell sensor for real-time detection of NO released from live-cells [129]. Here, the existence of RGD can provide favorable conditions for the attachment and growth of cells.

Their next task was preparing Au nanoparticle − 3D graphene hydrogel (Au-3DGH) hybrid through a facile one-step approach via the in situ reduction of Au^{3+} on a 3DGH [130]. Fig. 10 shows that 3DGH exhibits poor electrocatalytic activity owing to its carbon nature, but it provides large active surface area, allowing Au to load with a larger amount, smaller size, and more uniform distribution for active sites and effective electron transport toward NO oxidation. So, the unique Au-3DGH hybrid formation can permit much NO molecules to absorb on the large amount of Au active centers, followed by rapid electron loss to form NO^+ (eq. 1). Owning to the highly porous Au-3DGH hybrids, a high mass transport of NO^+ meet OH^- and yield NO^{2-} (seen in eq. 2).

$$NO - e^- \rightarrow NO^+ \quad (1)$$

$$NO^+ + OH^- \rightarrow HNO_2 \rightarrow H^+ + NO_2^- \quad (2)$$

Due to the strong synergistic effects from the large surface area of the porous 3DGH and high electrocatalytic activity of the AuNP, the sensor has excellent selectivity, a fast response, and a low detection limit. Experimental data also revealed that the NO released form B16-F10 tumor cell is 5 times greater than the normal JB6-C30 cells. Subsequently, shape-controlled ceria-reduced graphene oxide nanocomposites [131] and gold nanoparticles decorated with reduced graphene oxide [132] were introduced for the detection of NO released from cells by Li's group. Huang's group has made contributions to this project, especially at the single cell levels, that improved the sensitivity to picomolar concentrations [133]. They have presented a reusable biomimetic micro-electrochemical sensor array for monitoring NO released from human endothelial cells based on metalloporphyrin and 3-aminophenyl boronic acid co-functionalized rGO. The sensor has three advantages: First, the involvement of a metalloporphyrin improved the sensitivity of the sensor to sub-nanomolar levels; second, the existent of 3-aminophenylboronic acid can enhance the cytocompatibility of the microsensor without reducing the sensitivity; finally, the reversible reactivity between 3-aminophenylboronic acid and cell membrane carbohydrates allows practical reusability.

Table 3 Sensing of NO from cells by NM-based and metal (oxide)-based electrochemical sensors

Electrode	Linear range (μM)	Detection limit (nM)	Sensitivity (μA/μM)	Working potential (V)		Ref
SWNTs/CFMDE	0.1–0.8	4.3	-	0.8	2008	[126]
MWNTs-ACB/PACB/GCE	0.22–120	28	0.25	0.75	2008	[127]
CAS/SOD/MP/MWCNT -PTTCA/AuNPs/GCE	1–40	4.3	1.1	-0.8	2010	[128]
RGD-graphene	0.1–100 100–1000	25	-	0.75	2012	[129]
AuNP − 3DGH/GCE	0.2–6	9	-	0.81	2015	[130]
rGO-CeO_2/GCE	0.018–5.6	9.6	1.67606	0.8	2015	[131]
AuNP-ERGO/GCE	0–200	133	5.38	0.821	2013	[132]
APBA/FGHNs /ITO	0–200	0.09	37.6	0.75	2015	[133]

Metal nanomaterial-based electrochemical sensors

Metal nanomaterials (NMs), especially noble metal (Au and Pt) NMs, have been used in electrochemical sensors due to their unique electrochemical, electronic, and catalytic properties. Varatharajan and co-workers have demonstrated that the combination of gold nanocomposites and hydroxypropyl-β-cyclodextrin encapsulated soluble ferrocene significantly improves the sensitivity of the estimation of NO in biological systems including peripheral blood mononuclear cells [134]. Duan's group reported a new type of flexible electrochemical biosensor based on graphene paper loaded with closely packed Au@Pt core-shell nanoparticles as a freestanding cell culture substrate that was used for the real-time monitoring of NO cell secretions [135]. The sensor displayed high sensitivity, a wide linear range, a low detection limit, and good biocompatibility. On the other hand, micro-array electrodes have aroused increasing attention due to their high signal-to-noise (S/N) ratios because of the smaller the active electrode area, the higher the S/N ratio [136]. Nano-array electrodes are the newest development in this field. Jin et al. reported a novel microporous aluminium anodic oxide film-modified Pt nano-array electrode for the direct measurement of NO release from myocardial cells [137].

Conclusion

As nanotechnology has advanced, various NMs including carbon-based NMs, noble metal NMs, metal oxide NMs, and layered doubled hydroxides with different structures (particles, wires, rods, pores, and tubes) have been presented and used in a wide variety of clinical systems. Due to their inherent advantages from their electrochemical properties, such as high surface areas, moderate conductivities, and excellent biocompatibilities, NMs have been used to fabricate electrochemical sensors for a wide range of applications. However, research on NM-based electrochemical sensors for H_2O_2, H_2S and NO from cells is still in its infancy, and researchers are making rapid progress in this area. In this paper, we critically reviewed the most significant achievements reached in the field of NM-based electrochemical sensors for H_2O_2, H_2S and NO in cells. General descriptions of the physiological roles of H_2O_2, H_2S and NO were presented. Then, examples of electrochemical sensors for sensing H_2O_2, H_2S and NO in cells were summarized according to the type of NMs. Most research is focused on the detection of H_2O_2 and NO. NM-based electrochemical probes to detect H_2S are very rare and research in this area is still in progress. There can be no doubt that respective electrochemical sensors for H_2O_2, H_2S and NO in cells have a very bright and exciting future.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (61404075, and 21405029), China Postdoctoral Science Foundation funded project (No.2016 M601694), Jiangsu Planned Projects for Postdoctoral Research Funds (No.1601090B), Science and Technology Program of Zhejiang Province of China (2016C37042), Young Talent Cultivation Project of Zhejiang Association for Science and Technology (2016YCGC007), and the social development project of Hangzhou (20160533B70). The authors extend their appreciation to Prof. Otto S. Wolfbeis for his help.

Compliance with ethical standards The author(s) declare that they have no competing interests.

References

1. Bedioui F, Griveau S (2014) Electrochemical detection of nitrite oxide: assessement of twenty years of strategies. Electroanalysis 26:1277–1286
2. Rhee SG, Chang TS, Jeong W, Kang D (2010) Methods for detection and measurement of hydrogen peroxide inside and outside of cells. Mol Cells 29:539–549. doi:10.1007/s10059-010-0082-3
3. Yagati AK, Choi J (2014) Protein based electrochemical biosensors for H_2O_2 detection towards clinical diagnostics. Electroanalysis 26:1259–1276. doi:10.1002/elan.201400037
4. Li J, Yin C, Huo F (2015) Chromogenic and fluorogenic chemosensors for hydrogen sulfide: review of detection

mechanisms since the year 2009. RSC Adv 5:2191–2206. doi:10.1039/C4RA11870G
5. Lippert AR (2014) Designing reaction-based fluorescent probes for selective hydrogen sulfide detection. J Inorg Biochem 133: 136–142. doi:10.1016/j.jinorgbio.2013.10.010
6. Taha ZH (2003) Nitric oxide measurements in biological samples. Talanta 61:3–10. doi:10.1016/S0039-9140(03)00354-0
7. Shi X, Gu W, Li B, Chen N, Zhao K, Xian Y (2014) Enzymatic biosensors based on the use of metal oxide nanoparticles. Microchim Acta 181:1–22. doi:10.1007/s00604-013-1069-5
8. Bai J, Jiang X (2013) A facile one-pot synthesis of copper sulfide-decorated reduced graphene oxide composites for enhanced detecting of H_2O_2 in biological environments. Anal Chem 85:8095–8101. doi:10.1021/ac400659u
9. Chen D, Feng H, Li J (2012) Graphene oxide: preparation, functionalization, and electrochemical applications. Chem Rev 112:6027–6053. doi:10.1021/cr300115g
10. Moriguchi K, Ohno N, Ogawa T, Hirai K (1999) Highly specific detection of H_2O_2-dependent luminol chemiluminescence in stimulated human leukocytes using polyvinyl films. J Electron Microsc 48:177–179. doi:10.1093/oxfordjournals.jmicro.a023665
11. Planchet E, Kaiser WM (2006) Nitric oxide (NO) detection by DAF fluorescence and chemiluminescence: a comparison using abiotic and biotic NO sources. J Exp Bot 57:3043–3055. doi:10.1093/jxb/erl070
12. Yao D, Vlessidis AG, Evmiridis NP (2004) Determination of nitric oxide in biological samples. Microchim Acta 147:1–20. doi:10.1007/s00604-004-0212-8
13. Wang Y, Li S, Feng L, Nie C, Liu L, Lv F, Wang S (2015) Fluorescence ratiometric assay strategy for chemical transmitter of living cells using H_2O_2-sensitive conjugated polymers. ACS Appl Mater Interfaces 7:24110–24118. doi:10.1021/acsami.5b07172
14. Wang B, Li P, Yu F, Song P, Sun X, Yang S, Lou Z, Han K (2013) A reversible fluorescence probe based on se-BODIPY for the redox cycle between HClO oxidative stress and H_2S repair in living cells. Chem Commun 49:1014–1016. doi:10.1039/C2CC37803E
15. Kirejev V, Kandoth N, Gref R, Ericson MB, Sortino S (2014) A polymer-based nanodevice for the photoregulated release of NO with two-photon fluorescence reporting in skin carcinoma cells. J Mater Chem B 2:1190–1195. doi:10.1039/c3tb21414a
16. Xu T, Scafa N, Xu L, Su L, Li C, Zhou S, Liu Y, Zhang X (2014) Electrochemical sensors for nitric oxide detection in biological applications. Electroanalysis 26:449–468. doi:10.1002/elan.201300564
17. Bedioui F, Griveau S (2013) Electrochemical detection of nitric oxide: assessement of twenty years of strategies. Electroanalysis 25:587–600. doi:10.1002/elan.201200306
18. Griveau S, Bedioui F (2013) Overview of significant examples of electrochemical sensor arrays designed for detection of nitric oxide and relevant species in a biological environment. Anal Bioanal Chem 405:3475–3488. doi:10.1007/s00216-012-6671-6
19. María-Hormigos R, Gismera MJ, Sevilla MT (2015) Straightforward ultrasound-assisted synthesis of bismuth oxide particles with enhanced performance for electrochemical sensors development. Mater Lett 158:359–362. doi:10.1016/j.matlet.2015.05.165
20. Qi S, Zhao B, Tang H, Jiang X (2015) Determination of ascorbic acid, dopamine, and uric acid by a novel electrochemical sensor based on pristine graphene. Electrochim Acta 161:395–402. doi:10.1016/j.electacta.2015.02.116
21. Aung NN, Crowe E, Liu X (2015) Development of self-powered wireless high temperature electrochemical sensor for in situ corrosion monitoring of coal-fired power plant. ISA Trans 55:188–194. doi:10.1016/j.isatra.2014.09.003
22. Bathany C, Han JR, Abi-Samra K, Takayama S, Cho YK (2015) An electrochemical-sensor system for real-time flow measurements in porous materials. Biosens Bioelectron 70:115–121. doi:10.1016/j.bios.2015.03.002
23. de Oliveira Farias EA, dos Santos MC, de Araujo DN, Quelemes PV, de Souza Almeida Leite JR, Eaton P, da Silva DA, Eiras C (2015) Layer-by-layer films based on biopolymers extracted from red seaweeds and polyaniline for applications in electrochemical sensors of chromium VI. Mater Sci Eng B 200:9–21. doi:10.1016/j.mseb.2015.05.004
24. Rhee SG (2006) H_2O_2, a necessary evil for cell signaling. Science 312:1882–1883. doi:10.1126/science.1130481
25. Winterbourn CC (2008) Reconciling the chemistry and biology of reactive oxygen species. Nat Chem Biol 4:278–286. doi:10.1038/nchembio.85
26. Wu P, Cai Z, Gao Y, Zhang H, Cai C (2011) Enhancing the electrochemical reduction of hydrogen peroxide based on nitrogen-doped graphene for measurement of its releasing process from living cells. Chem Commun 47:11327–11329. doi:10.1039/c1cc14419g
27. Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y, Hayashi J (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320:661–664. doi:10.1126/science.1156906
28. Douglass WC (2003) Hydrogen peroxide: medical miracle. Rhino Publishing, Panama
29. Kasai N, Han C, Torimitsu K (2005) Hydrogen peroxide distribution and neuronal cell death in a rat hippocampal slice. Sensor Actuat B-Chem 108:746–750. doi:10.1016/j.snb.2004.11.096
30. Liu X, Feng H, Zhang J, Zhao R, Liu X, Wong DK (2012) Hydrogen peroxide detection at a horseradish peroxidase biosensor with a au nanoparticle-dotted titanate nanotube|hydrophobic ionic liquid scaffold. Biosens Bioelectron 32:188–194. doi:10.1016/j.bios.2011.12.002
31. Mathew M, Sandhyarani N (2011) A novel electrochemical sensor surface for the detection of hydrogen peroxide using cyclic bisureas/gold nanoparticle composite. Biosens Bioelectron 28: 210–215. doi:10.1016/j.bios.2011.07.020
32. Peng Y, Jiang D, Su L, Zhang L, Yan M, Du J, Lu Y, Liu YN, Zhou F (2009) Mixed monolayers of ferrocenylalkanethiol and encapsulated horseradish peroxidase for sensitive and durable electrochemical detection of hydrogen peroxide. Anal Chem 81: 9985–9992. doi:10.1021/ac901833s
33. Xi F, Liu L, Chen Z, Lin X (2009) One-step construction of reagentless biosensor based on chitosan-carbon nanotubes-nile blue-horseradish peroxidase biocomposite formed by electrodeposition. Talanta 78:1077–1082. doi:10.1016/j.talanta.2009.01.023
34. Zhao J, Yan Y, Zhu L, Li X, Li G (2013) An amperometric biosensor for the detection of hydrogen peroxide released from human breast cancer cells. Biosens Bioelectron 41:815–819. doi:10.1016/j.bios.2012.10.019
35. Suárez G, Santschi C, Martin OJ, Slaveykova VI (2013) Biosensor based on chemically-designed anchorable cytochrome c for the detection of H_2O_2 released by aquatic cells. Biosens Bioelectron 42:385–390. doi:10.1016/j.bios.2012. 10.083
36. Wu P, Cai Z, Chen J, Zhang H, Cai C (2011) Electrochemical measurement of the flux of hydrogen peroxide releasing from RAW 264.7 macrophage cells based on enzyme-attapulgite clay nanohybrids. Biosens Bioelectron 26:4012–4017. doi:10.1016/j.bios.2011.03.018
37. Rui Q, Komori K, Tian Y, Liu H, Luo Y, Sakai Y (2010) Electrochemical biosensor for the detection of H_2O_2 from living cancer cells based on ZnO nanosheets. Anal Chim Acta 670:57–62. doi:10.1016/j.aca.2010.04.065
38. Li X, Liu Y, Zhu A, Luo Y, Deng Z, Tian Y (2010) Real-time electrochemical monitoring of cellular H_2O_2 integrated with in situ

selective cultivation of living cells based on dual functional protein microarrays at au-TiO_2 surfaces. Anal Chem 82:6512–6518. doi:10.1021/ac100807c

39. Chen X, Wu G, Cai Z, Oyama M, Chen X (2014) Advances in enzyme-free electrochemical sensors for hydrogen peroxide, glucose, and uric acid. Microchim Acta 181:689–705. doi:10.1007/s00604-013-1098-0
40. Yao Z, Yang X, Wu F, Wu W, Wu F (2016) Synthesis of differently sized silver nanoparticles on a screen-printed electrode sensitized with a nanocomposites consisting of reduced graphene oxide and cerium (IV) oxide for nonenzymatic sensing of hydrogen peroxide. Microchim Acta 183(10):2799–2806. doi:10.1007/s00604-016-1924-2
41. MeiL ZP, Chen J, Chen D, Quan Y, Gu N, Cui R (2016) Non-enzymatic sensing of glucose and hydrogen peroxide using a glassy carbon electrode modified with a nanocomposite consisting of nanoporous copper, carbon black and nafion. Microchim Acta 183(4):1359–1365. doi:10.1007/s00604-016-1764-0
42. Wu Q, Sheng Q, Zheng J (2016) Nonenzymatic amperometric sensing of hydrogen peroxide using a glassy carbon electrode modified with a sandwich-structured nanocomposite consisting of silver nanoparticles, Co_3O_4 and reduced graphene oxide. Microchim Acta 183(6):1943–1951. doi:10.1007/s00604-016-1829-0
43. Yang Z, Qi C, Zheng X, Zheng J (2016) Sensing hydrogen peroxide with a glassy carbon electrode modified with silver nanoparticles, AlOOH and reduced graphene oxide. Microchim Acta 183(3):1131–1136. doi:10.1007/s00604-016-1743-5
44. Shahid MM, Rameshkumar P, Huang NM (2016) A glassy carbon electrode modified with graphene oxide and silver nanoparticles for amperometric determination of hydrogen peroxide. Microchim Acta 183(2):911–916. doi:10.1007/s00604-015-1679-1
45. Iijima S (1991) Helical microtubules of graphitic carbon. Nature 354:56–58. doi:10.1038/354056a0
46. Rawson FJ, Hicks J, Dodd N, Abate W, Garrett DJ, Yip N, Fejer G, Downard AJ, Baronian KH, Jackson SK, Mendes PM (2015) Fast, ultrasensitive detection of reactive oxygen species using a carbon nanotube based-electrocatalytic intracellular sensor. ACS Appl Mater Interfaces 7:23527–23537. doi:10.1021/acsami.5b06493
47. Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV, Grigorieva IV, Firsov AA (2004) Electric field effect in atomically thin carbon films. Science 306:666–669. doi:10.1126/science.1102896
48. Mao HY, Laurent S, Chen W, Akhavan O, Imani M, Ashkarran AA, Mahmoudi M (2013) Graphene: promises, facts, opportunities, and challenges in nanomedicine. Chem Rev 113:3407–3424. doi:10.1021/cr300335p
49. Bitounis D, Ali-Boucetta H, Hong BH, Min DH, Kostarelos K (2013) Prospects and challenges of graphene in biomedical applications. Adv Mater 25:2258–2268. doi:10.1002/adma.201203700
50. Luo B, Liu S, Zhi L (2012) Chemical approaches toward graphene-based nanomaterials and their applications in energy-related areas. Small 8:630–646. doi:10.1002/smll.201101396
51. Chung C, Kim YK, Shin D, Ryoo SR, Hong BH, Min DH (2013) Biomedical applications of graphene and graphene oxide. Accounts Chem Res 46:2211–2224. doi:10.1021/ar300159f
52. Wei D, Liu Y (2010) Controllable synthesis of graphene and its applications. Adv Mater 22:3225–3241. doi:10.1002/adma.200904144
53. Kim J, Cote LJ, Huang J (2012) Two dimensional soft material: new faces of graphene oxide. Accounts Chem Res 45:1356–1364. doi:10.1021/ar300047s
54. Geim AK, Novoselov KS (2007) The rise of graphene. Nat Mater 6:183–191. doi:10.1038/nmat1849
55. Guo S, Dong S (2011) Graphene nanosheet: synthesis, molecular engineering, thin film, hybrids, and energy and analytical applications. Chem Soc Rev 40:2644–2672. doi:10.1039/c0cs00079e
56. Shao Y, Wang J, Wu H, Liu J, Aksay I, Lin Y (2010) Graphene based electrochemical sensors and biosensors: a review. Electroanalysis 22:1027–1036. doi:10.1002/elan.200900571
57. Zhu C, Dong S (2014) Energetic graphene-based electrochemical analytical devices in nucleic acid, protein and cancer diagnostics and detection. Electroanalysis 26:14–29. doi:10.1002/elan.201300056
58. Yang G, Zhou Y, Wu J, Cao J, Li L, Liu H, Zhu J (2013) Microwave-assisted synthesis of nitrogen and boron co-doped graphene and its application for enhanced electrochemical detection of hydrogen peroxide. RSC Adv 3:22597–22604. doi:10.1039/c3ra44284e
59. Zhang Y, Bai X, Wang X, Shiu KK, Zhu Y, Jiang H (2014) Highly sensitive graphene-Pt nanocomposites amperometric biosensor and its application in living cell H_2O_2 detection. Anal Chem 86:9459–9465. doi:10.1021/ac5009699
60. Fang H, Pan Y, Shan W, Guo M, Nie Z, Huang Y, Yao S (2014) Enhanced nonenzymatic sensing of hydrogen peroxide released from living cells based on Fe_3O_4/self-reduced graphene nanocomposites. Anal Methods 6:6073–6081. doi:10.1039/C4AY00549J
61. Kong L, Ren Z, Zheng N, Du S, Wu J, Tang J, Fu H (2015) Interconnected 1D Co_3O_4 nanowires on reduced graphene oxide for enzymeless H_2O_2 detection. Nano Res 8:469–480. doi:10.1007/s12274-014-0617-6
62. Li X, Xu M, Chen H, Xu J (2015) Bimetallic Au@Pt@Au core–shell nanoparticles on graphene oxide nanosheets for high-performance H_2O_2 bi-directional sensing. J Mater Chem B 3:4355–4362. doi: 10.1039/c5tb00312a
63. Liu J, Bo X, Zhao Z, Guo L (2015) Highly exposed Pt nanoparticles supported on porous graphene for electrochemical detection of hydrogen peroxide in living cells. Biosens Bioelectron 74:71–77. doi:10.1016/j.bios.2015.06.042
64. Dong S, Xi J, Wu Y, Liu H, Fu C, Liu H, Xiao F (2015) High loading MnO_2 nanowires on graphene paper: facile electrochemical synthesis and use as flexible electrode for tracking hydrogen peroxide secretion in live cells. Anal Chim Acta 853:200–206. doi:10.1016/j.aca.2014.08.004
65. Xiao F, Li Y, Zan X, Liao K, Xu R, Duan H (2012) Growth of metal-metal oxide nanostructures on freestanding graphene paper for flexible biosensors. Adv Funct Mater 22:2487–2494. doi:10.1002/adfm.201200191
66. Wang L, Zhang Y, Cheng C, Liu X, Jiang H, Wang X (2015) Highly sensitive electrochemical biosensor for evaluation of oxidative stress based on the nanointerface of graphene nanocomposites blended with gold, Fe_3O_4, and platinum nanoparticles. ACS Appl Mater Interfaces 7:18441–18449. doi:10.1021/acsami.5b04553
67. Abdurhman AA, Zhang Y, Zhang G, Wang S (2015) Hierarchical nanostructured noble metal/metal oxide/graphene-coated carbon fiber: in situ electrochemical synthesis and use as microelectrode for real-time molecular detection of cancer cells. Anal Bioanal Chem 407:8129–8136. doi:10.1007/s00216-015-8989-3
68. Maji SK, Sreejith S, Mandal AK, Ma X, Zhao Y (2014) Immobilizing gold nanoparticles in mesoporous silica covered reduced graphene oxide: a hybrid material for cancer cell detection through hydrogen peroxide sensing. ACS Appl Mater Interfaces 6(16):13648–13656. doi:10.1021/am503110s
69. Sun Y, Zheng H, Wang C, Yang M, Zhou A, Duan H (2016) Ultrasonic-electrodeposition of PtPd alloy nanoparticles on ionic liquid-functionalized graphene paper: towards a flexible and versatile nanohybrid electrode. Nano 8:1523–1534. doi:10.1039/c5nr06912b

70. Sun Y, He K, Zhang Z, Zhou A, Duan H (2015) Real-time electrochemical detection of hydrogen peroxide secretion in live cells by Pt nanoparticles decorated graphene-carbon nanotube hybrid paper electrode. Biosens Bioelectron 68:358–364. doi:10.1016/ j.bios.2015.01.017
71. Chang H, Wang X, Shiu KK, Zhu Y, Wang J, Li Q, Chen B, Jiang H (2013) Layer-by-layer assembly of graphene, au and poly(toluidine blue O) films sensor for evaluation of oxidative stress of tumor cells elicited by hydrogen peroxide. Biosens Bioelectron 41:789–794. doi:10.1016/j.bios.2012.10.001
72. Xi F, Zhao D, Wang X, Chen P (2013) Non-enzymatic detection of hydrogen peroxide using a functionalized three-dimensional graphene electrode. Electrochem Commun 26:81–84. doi:10.1016/j.elecom.2012.10.017
73. Panchakarla LS, Subrahmanyam KS, Saha SK, Govindaraj A, Krishnamurthy HR, Waghmare UV, Rao CNR (2009) Synthesis, structure, and properties of boron- and nitrogen-doped graphene. Adv Mater 21:4726–4730. doi:10.1002/adma.200901285
74. Zhang Y, Wu C, Zhou X, Wu X, Yang Y, Wu H, Guo S, Zhang J (2013) Graphene quantum dots/gold electrode and its application in living cell H_2O_2 detection. Nano 5:1816–1819. doi:10.1039/c3nr33954h
75. Yang C, Hu L, Zhu H, Ling Y, Tao J, Xu C (2015) rGO quantum dots/ZnO hybrid nanofibers fabricated using electrospun polymer templates and applications in drug screening involving an intracellular H_2O_2 sensor. J Mater Chem B 3:2651–2659. doi:10.1039/C4TB02134G
76. Ju J, Chen W (2015) In situ growth of surfactant-free gold nanoparticles on nitrogen-doped graphene quantum dots for electrochemical detection of hydrogen peroxide in biological environments. Anal Chem 87:1903–1910. doi:10.1021/ac5041555
77. Wang T, Peng Z, Wang Y, Tang J, Zheng G (2013) MnO nanoparticle@mesoporous carbon composites grown on conducting substrates featuring high-performance lithium-ion battery, supercapacitor and sensor. Sci Rep 3:2693–2701. doi:10.1038/srep02693
78. Doria G, Conde J, Veigas B, Giestas L, Almeida C, Assunção M, Rosa J, Baptista PV (2012) Noble metal nanoparticles for biosensing applications. Sensors-Basel 12:1657–1687. doi:10.3390/s120201657
79. Guo S, Wang E (2011) Noble metal nanomaterials: controllable synthesis and application in fuel cells and analytical sensors. Nano Today 6:240–264. doi:10.1016/j.nantod.2011.04.007
80. Arvizo RR, Bhattacharyya S, Kudgus RA, Giri K, Bhattacharya R, Mukherjee P (2012) Intrinsic therapeutic applications of noble metal nanoparticles: past, present and future. Chem Soc Rev 41:2943–2970. doi:10.1039/c2cs15355f
81. Jain PK, Huang X, El-Sayed IH, El-Sayed MA (2008) Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. Accounts Chem Res 41:1578–1586. doi:10.1021/ar7002804
82. Ai F, Chen H, Zhang SH, Liu SY, Wei F, Dong XY, Cheng JK, Huang WH (2009) Real-time monitoring of oxidative burst from single plant protoplasts using microelectrochemical sensors modified by platinum nanoparticles. Anal Chem 81:8453–8458. doi:10.1021/ac901300b
83. Xiao C, Liu YL, Xu JQ, Lv SW, Guo S, Huang WH (2015) Real-time monitoring of H_2O_2 release from single cells using nanoporous gold microelectrodes decorated with platinum nanoparticles. Analyst 140:3753–3758. doi:10.1039/c4an02056a
84. Liu F, Ge S, Yu J, Yan M, Song X (2014) Electrochemical device based on a Pt nanosphere-paper working electrode for in situ and real-time determination of the flux of H_2O_2 releasing from SK-BR-3 cancer cells. Chem Commun 50:10315–10318. doi:10.1039/c4cc04199b
85. Evanoff DD, Chumanov G (2005) Synthesis and optical properties of silver nanoparticles and arrays. ChemPhysChem 6:1221–1231. doi:10.1002/cphc.200500113
86. Loza K, Diendorf J, Sengstock C, Ruiz-Gonzalez L, Gonzalez-Calbet JM, Vallet-Regi M, Köller M, Epple M (2014) The dissolution and biological effects of silver nanoparticles in biological media. J Mater Chem B 2:1634–1643. doi:10.1039/c3tb21569e
87. Miao P, Wang B, Yin J, Chen X, Tang Y (2015) Electrochemical tracking hydrogen peroxide secretion in live cells based on autocatalytic oxidation reaction of silver nanoparticles. Electrochem Commun 53:37–40. doi:10.1016/j.elecom.2015.02.007
88. Xia P, Liu H, Tian Y (2009) Cathodic detection of H_2O_2 based on nanopyramidal gold surface with enhanced electron transfer of myoglobin. Biosens Bioelectron 24:2470–2474. doi:10.1016/j.bios.2008.12.029
89. Wang N, Han Y, Xu Y, Gao C, Cao X (2015) Detection of H_2O_2 at the nanomolar level by electrode modified with ultrathin AuCu nanowires. Anal Chem 87:457–463. doi:10.1021/ac502682n
90. Han Y, Gao C, Zhu H, Chen S, Jiang Q, Li T, Willander M, Cao X, Wang N (2015) Piezotronic effect enhanced nanowire sensing of H_2O_2 released by cells. Nano Energ 13:405–413. doi:10.1016/j.nanoen.2015.03.008
91. Xi J, Zhang Y, Wang N, Wang L, Zhang Z, Xiao F, Wang S (2015) Ultrafine Pd nanoparticles encapsulated in microporous Co_3O_4 hollow nanospheres for in situ molecular detection of living cells. ACS Appl Mater Interfaces 7:5583–5590. doi:10.1021/acsami.5b00600
92. Sun X, Guo S, Liu Y, Sun S (2012) Dumbbell-like PtPd-Fe_3O_4 nanoparticles for enhanced electrochemical detection of H_2O_2. Nano Lett 12:4859–4863. doi:10.1021/nl302358e
93. Zhu H, Sigdel A, Zhang S, Su D, Xi Z, Li Q, Sun S (2014) Core/Shell Au/MnO nanoparticles prepared through controlled oxidation of AuMn as an electrocatalyst for sensitive H_2O_2 detection. Angew Chem Int Edit 53:12508–12512. doi: 10.1002/anie.201406281
94. Li Z, Xin Y, Zhang Z (2015) New photocathodic analysis platform with quasi-core/shell-structured TiO_2@Cu_2O for sensitive detection of H_2O_2 release from living cells. Anal Chem 87:10491–10497. doi:10.1021/acs.analchem.5b02644
95. An L, Huang L, Liu H, Xi P, Chen F, Du Y (2015) High-quality copper sulfide nanocrystals with diverse shapes and their catalysis for electrochemical reduction of H_2O_2. Part Part Syst Charact 32:536–541. doi:10.1002/ppsc.201400209
96. Sajid M, Basheer C (2016) Layered double hydroxides: emerging sorbent materials for analytical extractions. TrAC, Trends Anal Chem 75:174–182. doi:10.1016/j.trac.2015.06.010
97. Asif M, Aziz A, Dao AQ, Hakeem A, Wang H, Dong S, Zhang G, Xiao F, Liu H (2015) Real-time tracking of hydrogen peroxide secreted by live cells using MnO_2 nanoparticles intercalated layered doubled hydroxide nanohybrids. Anal Chim Acta 898:34–41. doi:10.1016/j.aca.2015.09.053
98. Abe K, Kimura H (1996) The possible role of hydrogen sulfide as an endogenous neuromodulator. J Neurosci 16:1066–1071
99. Wang R (2012) Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev 92:791–896. doi:10.1152/physrev.00017.2011
100. Malinskin T (2002) Encyclopedia of electrochemistry: bioelectrochemistry. Wiley-VCH and Publishing House, Weinheim
101. Chen CQ, Xin H, Zhu YZ (2007) Hydrogen sulfide: third gaseous transmitter, but with great pharmacological potential. Acta Pharmacol Sin 28:1709–1716. doi:10.1111/j.1745-7254.2007.00629.x
102. Jeroschewski P, Steuckart C, Kühl M (1996) An amperometric microsensor for the determination of H_2S in aquatic environments. Anal Chem 68:4351–4357. doi:10.1021/ac960091b

103. Song Y, Muthuraman G, Zen J (2006) Trace analysis of hydrogen sulfide by monitoring as(III) at a poly(*L*-lactide) stabilized gold nanoparticles modified electrode. Electrochem Commun 8:1369–1374. doi: 10.1016/j.elecom.2006.06.014
104. Lawrence NS, Deo RP, Wang J (2004) Electrochemical determination of hydrogen sulfide at carbon nanotube modified electrodes. Anal Chim Acta 517:131–137. doi:10.1016/j.aca.2004.03.101
105. Vaishampayan MV, Deshmukh RG, Walke P, Mulla IS (2008) Fe-doped SnO_2 nanomaterial: a low temperature hydrogen sulfide gas sensor. Mater Chem Phys 109:230–234. doi:10.1016/j.matchemphys.2007.11.024
106. Cao Y, Jia D, Wang R, Luo J (2013) Rapid one-step room-temperature solid-state synthesis and formation mechanism of ZnO nanorods as H_2S-sensing materials. Solid State Electron 82:67–71. doi: 10.1016/j.sse.2012.12.015
107. Yin L, Chen D, Zhang H, Shao G, Fan B, Zhang R, Shao G (2014) In situ formation of Au/SnO_2 nanocrystals on WO_3 nanoplates as excellent gas-sensing materials for H_2S detection. Mater Chem Phys 148:1099–1107. doi: 10.1016/j.matchemphys.2014.09.025
108. Liu Y, Liu Z, Yang Y, Yang H, Shen G, Yu R (2005) Simple synthesis of $MgFe_2O_4$ nanoparticles as gas sensing materials. Sensor Actuat B-Chem B 107:600–604. doi:10.1016/j.snb.2004.11.026
109. Berahman M, Sheikhi MH (2015) Hydrogen sulfide gas sensor based on decorated zigzag graphene nanoribbon with copper. Sensor Actuat B-Chem 219:338–345. doi:10.1016/j.snb.2015.04.114
110. Xu T, Scafa N, Xu LP, Zhou S, Abdullah Al-Ghanem K, Mahboob S, Fugetsu B, Zhang X (2016) Electrochemical hydrogen sulfide biosensors. Analyst 141:1185–1195. doi:10.1039/c5an02208h
111. Ding LH, Ma C, Li L, Zhang LN , Yu JH (2016)A photoelectrochemical sensor for hydrogen sulfide in cancer cells based on the covalently and in situ grafting of CdS nanoparticles onto TiO_2 nanotubes. J Electroana Chem 783:176–181. doi:10.1016/j.jelechem.2016.11.025
112. Moncada S, Palmer RM, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43:109–142
113. Privett BJ, Shin JH, Schoenfisch MH (2010) Electrochemical nitric oxide sensors for physiological measurements. Chem Soc Rev 39:1925–1935
114. Trouillon R (2013) Biological applications of the electrochemical sensing of nitric oxide: fundamentals and recent developments. Biol Chem 394:17–30. doi:10.1515/hsz-2012-0245
115. Saldanha C, Lopes de Almeida JP, Silva-Herdade AS (2014) Application of a nitric oxide sensor in biomedicine. Biosensor 4:1–17. doi:10.3390/bios4010001
116. Hurst RD, Clark JB (2003) The utility of the nitric oxide electrochemical sensor in biomedical research. Sensors-Basel 3:321–329. doi:10.3390/s30800321
117. Kim S, Jung H, Lee C, Kim MH, Lee Y (2014) Biological application of RuO_2 nanorods grown on a single carbon fiber for the real-time direct nitric oxide sensing. Sens Actuators B 191:298–304. doi:10.1016/j.snb.2013.09.103
118. Wen W, Chen W, Ren QQ, Hu XY, Xiong HY, Zhang XH, Wang SF, Zhao YD (2012) A highly sensitive nitric oxide biosensor based on hemoglobin-chitosan/graphene-hexadecyltrimethylammonium bromide nanomatrix. Sens Actuators B 166-167:444–450
119. Pang J, Fan C, Liu X, Chen T, Li G (2003) A nitric oxide biosensor based on the multi-assembly of hemoglobin/montmorillonite/polyvinyl alcohol at a pyrolytic graphite electrode. Biosens Bioelectron 19:441–445. doi:10.1016/S0956-5663(03)00223-9
120. Haruyama T, Shiino S, Yanagida Y, Kobatake E, Aizawa M (1998) Two types of electrochemical nitric oxide (NO) sensing systems with heat-denatured Cyt C and radical scavenger PTIO. Biosens Bioelectron 13:763–769. doi:10.1016/S0956-5663(98)00040-2
121. Cancino J, Borgmann S, Machado SA, Zucolotto V, Schuhmann W, Masa J (2015) Electrochemical sensor for nitric oxide using layered films composed of a polycationic dendrimer and nickel (II) phthalocyaninetetrasulfonate deposited on a carbon fiber electrode. Microchim Acta 182(5–6):1079–1087. doi:10.1007/s00604-014-1425-0
122. Wang Y, Song B, Xu J, Hu S (2015) An amperometric sensor for nitric oxide based on a glassy carbon electrode modified with graphene, Nafion, and electrodeposited gold nanoparticles. Microchim Acta 182(3-4):711–718. doi:10.1007/s00604-014-1379-2
123. Govindhan M, Chen A (2016) Enhanced electrochemical sensing of nitric oxide using a nanocomposite consisting of platinum-tungsten nanoparticles, reduced graphene oxide and an ionic liquid. Microchim Acta 183(11):2879–2887. doi:10.1007/s00604-016-1936-y
124. ZhengD LX, ZhuS CH, ChenY HS (2015) Sensing nitric oxide with a carbon nanofiber paste electrode modified with a CTAB and nafion composite. Microchim Acta 182(15–16):2403–2410. doi:10.1007/s00604-015-1561-1
125. DangX HH, Wang S, Hu S (2015) Nanomaterial-based electrochemical sensors for nitric oxide. Microchim Acta 182(3–4):455–467. doi:10.1007/s00604-014-1325-3
126. Du F, Huang W, Shi Y, Wang Z, Cheng J (2008) Real-time monitoring of NO release from single cells using carbon fiber microdisk electrodes modified with single-walled carbon nanotubes. Biosens Bioelectron 24:415–421. doi:10.1016/j.bios.2008.04.020
127. Zheng D, Hu C, Peng Y, Yue W, Hu S (2008) Noncovalently functionalized water-soluble multiwall-nanotubes through azocarmine B and their application in nitric oxide sensor. Electrochem Commun 10:90–94. doi:10.1016/j.elecom.2007.10.027
128. Abdelwahab AA, Koh WC, Noh HB, Shim YB (2010) A selective nitric oxide nanocomposite biosensor based on direct electron transfer of microperoxidase: removal of interferences by co-immobilized enzymes. Biosens Bioelectron 26:1080–1086. doi:10.1016/j.bios.2010.08.070
129. Guo CX, Ng SR, Khoo SY, Zheng X, Chen P, Li CM (2012) RGD-peptide functionalized graphene biomimetic live-cell sensor for real-time detection of nitric oxide molecules. ACS Nano 6:6944–6951. doi:10.1021/nn301974u
130. Li J, Xie J, Gao L, Li CM (2015) Au nanoparticles-3D graphene hydrogel nanocomposite to boost synergistically in situ detection sensitivity toward cell-released nitric oxide. ACS Appl Mater Interfaces 7:2726–2734. doi:10.1021/am5077777
131. Hu FX, Xie JL, Bao SJ, Yu L, Li CM (2015) Shape-controlled ceria-reduced graphene oxide nanocomposites toward high-sensitive in situ detection of nitric oxide. Biosens Bioelectron 70:310–317. doi:10.1016/j.bios.2015.03.056
132. Ting SL, Guo CX, Leong KC, Kim D, Li CM, Chen P (2013) Gold nanoparticles decorated reduced graphene oxide for detecting the presence and cellular release of nitric oxide. Electrochim Acta 111:441–446. doi:10.1016/j.electacta.2013.08.036
133. Liu Y, Wang X, Xu J, Xiao C, Liu Y, Zhang X, Liu J, Huang W (2015) Functionalized graphene-based biomimetic microsensor interfacing with living cells to sensitively monitor nitric oxide release. Chem Sci 6:1853–1858. doi:10.1039/C4SC03123G
134. Varatharajan S, Kumar KS, Berchmans S, Amutha R, Kiruthiga PV, Devi KP (2010) Synergistic effect of hydroxypropyl-beta-cyclodextrin encapsulated soluble ferrocene and the gold nanocomposite modified glassy carbon electrode for the estimation of

NO in biological systems. Analyst 135:2348–2354. doi:10.1039/c0an00091d

135. Zan X, Fang Z, Wu J, Xiao F, Huo F, Duan H (2013) Freestanding graphene paper decorated with 2D-assembly of Au@Pt nanoparticles as flexible biosensors to monitor live cell secretion of nitric oxide. Biosens Bioelectron 49:71–78. doi: 10.1016/j.bios.2013.05.006
136. Uosaki K, Okazaki K, Kita H, Takahashi H (1990) Preparative method for fabricating a microelectrode ensemble: electrochemical response of microporous aluminum anodic oxide film modified gold electrode. Anal Chem 62:652–656. doi:10.1021/ac00205a023
137. Xian Y, Liu M, Cai Q, Li H, Lu J, Jin L (2001) Preparation of microporous aluminium anodic oxide film modified Pt nano array electrode and application in direct measurement of nitric oxide release from myocardial cells. Analyst 126:871–876. doi:10.1039/b010181h