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Study on detection methods for H_2O_2 in biological samples

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Abstract
Hydrogen peroxide (H_2O_2) plays significant roles in regulating diverse biological processes such as immune cell activation, vascular remodeling, apoptosis, stomatal closure and root growth. Many studies have suggested that an excessive amount of H_2O_2 will induce various kinds of biological damage, leading to aging, neurodegeneration, as well as many other diseases. Thus, determination of H_2O_2 in trace level in biological and various water samples is of great importance. In this article the studies of detection methods for H_2O_2 in biological samples in recent years are reviewed.

Keywords: hydrogen peroxide; H_2O_2; determination; detection; sensor

1. Introduction
Hydrogen peroxide (H_2O_2) is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress related state. It is a by-product of several enzymatic reactions that can be used as diagnostic tools for detection of the onset of various biological conditions. It is not only involved in phagocytosis but also acts as insulin that helps the transport of sugar through the body [1-3]. The role of H_2O_2 in the processes of human life is closely related to its amount. Therefore, highly sensitive determination of H_2O_2 is necessary and vital, and various H_2O_2 detection and measurement methods have been explored [4-5]. In this paper, the attributes of different analytical technique for the determination of H_2O_2 in biological samples in recent years are reviewed.

2. Analytical Methods
2.1. Chemiluminescence method. Chemiluminescence (CL) is a well-known and popular analytical method because of its high sensitivity, low detection limit, wide linear working range, and its rapidity, as well as the fact that it can be performed with relatively simple and inexpensive instrumentation, as an excitation source and optical filters are not needed. It has been widely applied in various fields, including clinical diagnosis, biotechnology, pharmacology, food safety, and environmental chemistry [6].
Xie *et al.* [7] synthesized Co_3O_4 nanoparticles with a hydrothermal method, which were used to amplify a weak CL of a luminol-H_2O_2 system. The results of UV-visible absorption and CL spectra showed that the CL luminophor was 3-aminophthalate, indicating that the CL enhancement of the luminol- H_2O_2 system was attributed to the intrinsic catalytic effect of Co_3O_4 nanoparticles acting as the electron transfer accelerators and radical generation proliferators. Based on the H_2O_2 concentration dependence of the catalytic activity of Co_3O_4 nanoparticles, they constructed a simple, sensitive and relatively selective CL assay for H_2O_2. Under the optimized conditions, a linear relationship was obtained between the CL intensity and H_2O_2 concentration in the range of 1.0×10^{-8}–1.0×10^{-5} mol L^{-1} with a detection limit of 1.1×10^{-9} mol L^{-1}.
Lebiga *et al.* [8] reported the design and characterization of a disposable light shielded paper–plastic microfluidic device that could detect nanomolar levels of H_2O_2 using a smartphone camera and a light sealed accessory. CL reaction of H_2O_2 with bis (2,4,6-trichlorophenyl) oxalate in the presence of rubrene and imidazole was confined to a paper reaction site where the individual reagents were delivered via plastic microfluidic channels. The net photon emission from the CL reactions, detected by using a smartphone, was correlated with H_2O_2 concentrations. With a total reagent volume of 25 μL, the sensor system was able to detect H_2O_2 concentrations as low as 250 nM. The smartphone based CL sensing system had great potential as a point of care diagnostic tool for monitoring nanomolar levels of H_2O_2 in biological samples.

2.2. Colorimetric method. Colorimetry has commonly been used for routine analysis due to its simplicity, low-cost and practicability. It does not require any expensive or sophisticated instruments and the color changes can be even directly observed by the naked eye. Recently, some colorimetric systems have been set up to detect various kinds of substances such as DNA, biologically relevant molecules, metal ions, viruses and micro-organisms and so on [9-11].

Chen *et al.* [12] developed a simple and highly sensitive colorimetric assay of H_2O_2 using triangular Ag nanoplates. In the presence of H_2O_2, the Ag nanoplates were etched from triangle to round, which led to the vanishment of the surface plasmon resonance absorption at 400 nm and significant decrease the absorbance of the band at 670 nm. The color change of the reaction system from the initial blue to mauve could be observed. This proposed method could determine H_2O_2 with a dynamic range of 50nM to 1μM and a low detection limit of 1.36 nM. This sensing system was further extended to the detection of glucose by H_2O_2 generated from the enzymatic oxidation of glucose, and thus had great promises in clinical applications.

Chen *et al.* [13] reported a facile green approach for in situ growth of silver nanoparticles (AgNPs) on the surface of graphene quantum dots (GQDs). The GQDs/AgNPs hybrid exhibited a superior absorbance fading response toward the reduction of H_2O_2. They proposed a simple colorimetric procedure for ultrasensitive detection of H_2O_2 without additional chromogenic agent. It provided a record detection limit of 33 nM for the detection of H_2O_2 by the AgNPs-based sensing system. This colorimetric sensing system was further extended to the detection of glucose in combination with the specific catalytic effect of glucose oxidase for the oxidation of glucose and formation of H_2O_2, giving rise to a detection limit of 170 nM. The observation described in the present study provided a new avenue for the development of a highly sensitive biosensing system.

2.3. Photoelectrochemical method. As a newly emerged and dynamically developing technique for the detection of biomolecules, photoelectrochemical (PEC) detection method has attracted substantial research interest for its desirable sensitivity and hence better analytical performances. In a typical PEC system, excitation light is introduced to induce the photoactive species on the electrode species and current is used as the detection signal. Coupling photo-irradiation with electrochemical catalytic reactions, PEC sensors leads to the possibility to detect certain substances that cannot be detected using common optical property based analytical methods. Photoactive semiconductors such as WO_3, TiO_2, CdS have demonstrated the use of semiconductor for PEC determination of DNA, proteins, or other biomolecules [14-16].

Li *et al.* [17] demonstrated for the first time the use of a p-type semiconductor, Cu_2O, as the core unit of a photocathode to set up a new photocathodic analysis platform. With the help of a facile protection strategy, the Cu_2O photocathode presented efficient photoelectrochemical performance for H_2O_2 sensing with a detection limit of 0.15 μM, which allowed the new photocathodic analysis platform to detect H_2O_2 released from living tumorigenic cells, thus demonstrating its potential application as a sensitive cancer detection probe.

Yue *et al.* [18] developed a new H_2O_2 sensor without any redox mediator by fixing CdS-FePt nanodimers on gold electrodes with dithiol compounds. Under illumination, electron transfer from gold electrode to CdS-QDs would occur by electron tunneling process. Because of the catalytic properties of FePt-nanoparticles associated with the CdS, photoinduced electrons could be further transferred to H_2O_2 resulting in a catalytic photocurrent. The results showed that the amplitude of photocurrent changed with the H_2O_2 concentration linearly in detection range and the properties of photoelectrochemical signals were influenced by the applied potential.

2.4. Fluorescence methods. In recent years, fluorescence measurements have received more attention owing to their operational simplicity, high sensitivity, good reproducibility and real-time detection. A series of fluorescence probes have been designed for the detection of biomolecules and metal ions. For example, gold nanoclusters (AuNCs), which exhibit molecule-like properties including discrete electronic states and size-dependent luminescence have received great attention. Fluorescent silicon nanoparticles (SiNPs), which have a zero-dimensional silicon-based nanostructure, have been widely used in biology, owing to their good biocompatibility, low cytotoxicity, and antiphotobleaching capability. Colloidal quantum dots (QDs) which exhibit broad absorption profiles and narrow emission with high quantum yields and allow the chemical modification of functional groups on their surfaces make QDs naturally suitable for serving as fluorescent platforms for sensing and imaging in biology [19, 20].

Wu *et al.* [21] developed a FRET-based ratiometric fluorescent probe for detecting H_2O_2 *in vitro* and *in vivo*. In this nanoprobe, carbon dots served as the energy donor and carrier for the H_2O_2 recognition element. This nanoprobe exhibited fast-response, low toxicity, high sensitivity (with a detection limit of 0.5 mM) and selectivity towards H_2O_2 over other reactive oxygen or nitrogen species. The successful detection of H_2O_2 by the nanoprobe *in vivo* might support its eventual use in clinical applications.

Ge *et al.* [22] synthesized highly fluorescent core/shell SiO2@CdTe nanoparticles (NPs) conveniently and efficiently via a hydrothermal method. The as-prepared SiO2@CdTe NPs were uniform with good fluorescence preservation. The SiO2@CdTe NPs could be used for the rapid detection of H_2O_2 with good sensitivity within several minutes. Excellent linear relationships existed between the quenching degrees of the SiO2@CdTe NPs and the concentration of H_2O_2 in the range of 0.005 mM to 0.1 mM. The detection limit for H_2O_2 was 10 nM. The excellent performance of SiO2@CdTe NPs could facilitate their applications in chemistry or biology for detection of H_2O_2.

2.5. Electrochemical method. Since the early 70s electrochemistry has been used as a powerful analytical technique for monitoring electroactive species in living organisms. H_2O_2 can be electrochemically oxidized or reduced. Nevertheless, the detection of H_2O_2 by measuring the current from the reduction of H_2O_2 is preferential for biological samples as the detection is free from interferences caused by bio-molecules, such as ascorbic acid and uric acid, which are present in large quantity in biological samples. On the other hand, to develop the electrochemical H_2O_2 sensor with high sensitivity, a suitable electrocatalytic method, which can effectively catalyze the reduction of H_2O_2 is required. Much effort for H_2O_2 detection has been devoted to design the modified electrodes to improve the catalytic properties, sensitivity, and selectivity of electrochemical sensors. Numerous materials, such as metal nanoparticles, polymers, carbon nanotubes, fullerenes, graphenes, and enzymes, have been used as modifiers to construct highly sensitive and selective H_2O_2 biosensors [23, 24].

Wang *et al.* [25] synthesized bimetallic AuCu nanowires via a facile water solution method at room temperature. They observed enhanced electrocatalytic activity toward the oxidation of H_2O_2. So they developed a highly sensitive H_2O_2 biosensor on the base of the as-prepared AuCu nanowires catalyst. A very low real determination limit (2.0 nM) was reached, and a linear range as wide as 5 orders of magnitude was demonstrated. In addition, a trace amount of H_2O_2, which was released from Raw 264.7 cells, was selectively detected, hinting at the possible applications for real-time quantitative detection of H_2O_2 in a biological environment.

Kong *et al.* [26] synthesized an efficient catalyst based on artificial bionic peroxidase for electrocatalyst. They prepared a poly-(ethyleneimine)/Au nanoparticle composite (PEI-AuNP) and then linked it to hemin via a coupling reaction between carboxyl groups in hemin and amino groups in PEI without the activation of a carboxyl group by carbodiimide. PEI-AuNP–hemin exhibited intrinsic peroxidase like catalytic activities. The PEI-AuNP–hemin deposited on a glass carbon electrode had strong sensing for H_2O_2 with a well-defined linear relationship between the amperometric response and H_2O_2 concentration in the range from 1 μM to 0.25 mM. The detection limit was 0.247 nM with a high sensitivity. The study suggested that PEI-AuNP–hemin might have promising application prospects in biocatalysis and bioelectronics.

2.6. Other methods. In addition to these main approaches mentioned above for H_2O_2 detection, still a few special techniques with high sensitivity have been applied. Wang *et al.* [27] prepared graphene oxide/gold nanoparticle (GO/GN) composites that exhibited a strong resonance RS (RRS) peak at 370 nm. When KIO_3 and H_2O_2 were added respectively, they reacted with KI to form I_3^- ions that adsorbed on the GO/GN surfaces and the RRS intensity decreased. So, a simple and sensitive GO/GN surface plasmon resonance Rayleigh scattering-energy transfer analytical platform was fabricated and could be utilized to detect trace KIO_3 and H_2O_2. Shan *et al.* [28] developed a rapid, reproducible, cost-effective approach for the detection of H_2O_2 based on the change of localized surface plasmon resonance (LSPR) peak of Au nanorods (NRs). H_2O_2 with high oxidation potential could decompose Au NRs. As a result, Au NRs could be shortened through an oxidation reaction by H_2O_2. After shortening Au NRs, the LSPR peaks showed blue shift. The LSPR peak of Au NRs displayed the dependence of spectral shift on concentration of H_2O_2. It provided a more simple and sensitive method for detecting H_2O_2.

3. Conclusions

The design and development of high-performance methods and sensors for H_2O_2 determination is vital, given the constant importance and presence of this key analyte in biological fluids, with implications in redox processes and human health. This review has highlighted the significant developments in rapid and alternative techniques for the detection of H_2O_2 in recent years. We believe the development of H_2O_2 sensors with better sensitivity and specificity, lower cost, simplicity, along with *in vivo* analytical technique is still the future effort.

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