Graphene Oxide-Based Nanosensing Method for the Detection of Hydrogen Peroxide (H_2O_2) in Bacterial Cells

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ABSTRACT

Reactive oxygen species (ROS) are pivotal mediators in numerous pathophysiological processes. An imbalance between ROS generation and the cellular antioxidant defense system can lead to a wide spectrum of disorders. Accurate and quantitative assessment of ROS is therefore essential for evaluating cellular oxidative status. Among ROS, hydrogen peroxide (H_2O_2) is widely regarded as a reliable biomarker of oxidative stress due to its relative stability, membrane permeability, and well-characterized role as an intracellular signaling molecule. In bacterial systems, elevated ROS levels contribute to antibiotic-induced cell death and may act as secondary mediators of cytotoxicity. Catalase, a primary antioxidant enzyme, preserves redox homeostasis by catalyzing the decomposition of H_2O_2 into water and molecular oxygen. Accordingly, the simultaneous quantification of H_2O_2 concentration and catalase activity is critical for a precise characterization of cellular redox balance. Conventional spectroscopic methods for measuring H_2O_2 and catalase are often limited by labor-intensive protocols, extensive sample preparation, and reduced sensitivity in complex biological matrices. In this study, we developed a graphene oxide–based nanosensor platform for the rapid and sensitive detection of intracellular H_2O_2. Graphene oxide, a two-dimensional carbon nanomaterial functionalized with oxygen-containing groups, possesses a high surface area and favorable electron transfer properties, making it an effective substrate for signal amplification. Its intrinsic oxidase-mimetic activity facilitates H_2O_2-dependent reactions, thereby enhancing analytical sensitivity. Moreover, the impact of silver nanoparticles on oxidative stress induction and intracellular H_2O_2 generation in Escherichia coli was investigated. Exposure to silver nanoparticles increased intracellular H_2O_2 levels, which were accurately quantified by the graphene oxide nanosensor through fluorescence emission. These results demonstrate that the proposed platform represents an efficient analytical tool for investigating oxidative stress mechanisms in microbial systems and provides a foundation for the development of redox-based diagnostic strategies in biomedical research and personalized medicine.

Keywords: Graphene oxide, silver nanoparticles, Escherichia coli, hydrogen peroxide, catalase

INTRODUCTION

Oxidative stress is a physiological condition arising from an imbalance between the production of reactive oxygen species (ROS) and the capacity of cellular antioxidant defense systems. Disruption of redox homeostasis can result in damage to proteins, lipids, and nucleic acids, thereby contributing to the pathogenesis of diverse disorders, including cancer, cardiovascular diseases, and inflammatory conditions [1]. ROS encompass molecules such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet-}$), and hydroxyl radical (•OH), each of which exhibits distinct roles in cellular signaling and oxidative damage [2]. Among these species, H_2O_2 is particularly significant due to its relative stability and ability to diffuse across biological membranes, functioning both as a marker of oxidative stress and as a critical intracellular

signaling mediator [3]. Catalase, a principal antioxidant enzyme, catalyzes the decomposition of H_2O_2 into water and molecular oxygen, thereby mitigating ROS-induced cellular damage [4]. Accurate quantification of catalase activity and intracellular H_2O_2 levels is therefore essential for evaluating oxidative status and elucidating cellular defense mechanisms. Conventional analytical approaches, including spectrophotometric and electrochemical assays, provide high precision; however, they are often time-consuming and require specialized instrumentation and technical expertise. In complex biological matrices, these methods are frequently constrained by matrix interferences, high detection limits, and dependence on colorimetric reagents, which limit their accuracy in quantifying low H_2O_2 concentrations in cellular or environmental samples. Consequently, nanomaterial-based systems have emerged as rapid and sensitive alternatives. Owing to their high surface-to-volume ratios and efficient electron transfer capabilities, these systems enhance analytical sensitivity and lower detection thresholds [5]. A range of nanomaterials has been developed to address these limitations, including metallic nanoparticles (e.g., silver and gold) and carbon-based nanomaterials such as graphene oxide (GO). These materials are widely employed in electrochemical H_2O_2 sensors due to their high surface reactivity and superior electron transfer properties [6]. Graphene oxide, a two-dimensional material rich in oxygen-containing functional groups, facilitates uniform deposition of metallic nanoparticles and, owing to its high electrical conductivity, large specific surface area, and intrinsic oxidative activity, serves as an effective platform for H_2O_2 detection [7]. Silver nanoparticles (AgNPs) have been extensively investigated for their antimicrobial activity and ability to generate ROS. In the presence of H_2O_2 and molecular oxygen, AgNPs release Ag^+ ions, inducing oxidative stress in bacterial cells. ROS-mediated damage compromises cellular membranes, DNA, and essential proteins, accelerating bacterial cell death. The synergistic mechanisms of silver ion release and ROS generation are recognized as principal contributors to the antimicrobial efficacy of AgNPs [8]. Escherichia coli serves as a widely utilized model organism in microbiology, and its response to H_2O_2-induced oxidative stress has been extensively characterized. This bacterium possesses regulatory systems, such as OxyR, which are activated at micromolar H_2O_2 concentrations and coordinate DNA repair pathways to mitigate oxidative damage. Precise quantification of intracellular H_2O_2 in E. coli is critical, as this molecule functions both as a metabolic byproduct and as a signaling mediator that regulates cellular stress response pathways [9]. Graphene oxide–based nanomaterials have demonstrated substantial advances in electrochemical H_2O_2 detection. Sensors constructed from hydrothermally reduced AgNP/GO composites can detect H_2O_2 across micromolar to millimolar concentrations with high reproducibility and stability. These results underscore the potential of GO-based composites as reliable analytical platforms for diverse biological and environmental applications [10].

MATERIALS AND METHODS

GO Characterization

Bacterial Culture and Determination of Minimum Inhibitory Concentration

Escherichia coli DH5α cells were initially cultured in 5 mL of LB medium (0.5% yeast extract, 1% tryptone, 1% NaCl) at 37°C overnight. Following incubation, the cells were diluted 1:100 in 50 mL of fresh LB medium and incubated with silver nanoparticles at concentrations of 50, 75, and 100 µg/mL at 37°C and 180 rpm for 24 hours. Bacterial growth over time was monitored by measuring the optical density (OD) at 600 nm using a spectrophotometer (UV-1800, SHIMADZU).

Cell Extraction for H2O2 Assay

For H_2O_2 analysis, 1.5 mL of both the silver nanoparticle-treated bacterial culture and control sample were centrifuged at 13,000 rpm for 15 minutes. The resulting pellet was resuspended in 5 mL of 0.1% (w/v) trichloroacetic acid on ice, sonicated for 10 minutes at 22 kHz, and centrifuged again under the same conditions. The supernatant was collected in a new tube for subsequent H_2O_2 measurement. For the GO-based H_2O_2 assay, 100 µL of graphene oxide (GO) and 100 µM 2',7'-dichlorofluorescein diacetate (DCFH-DA, used as substrate) were combined with 20 µL of bacterial extract. After incubation at room temperature for 10 minutes, fluorescence spectra were recorded using a fluorescence spectrometer (JASCO FP-750, Rev. 1.00) (Figure 1).

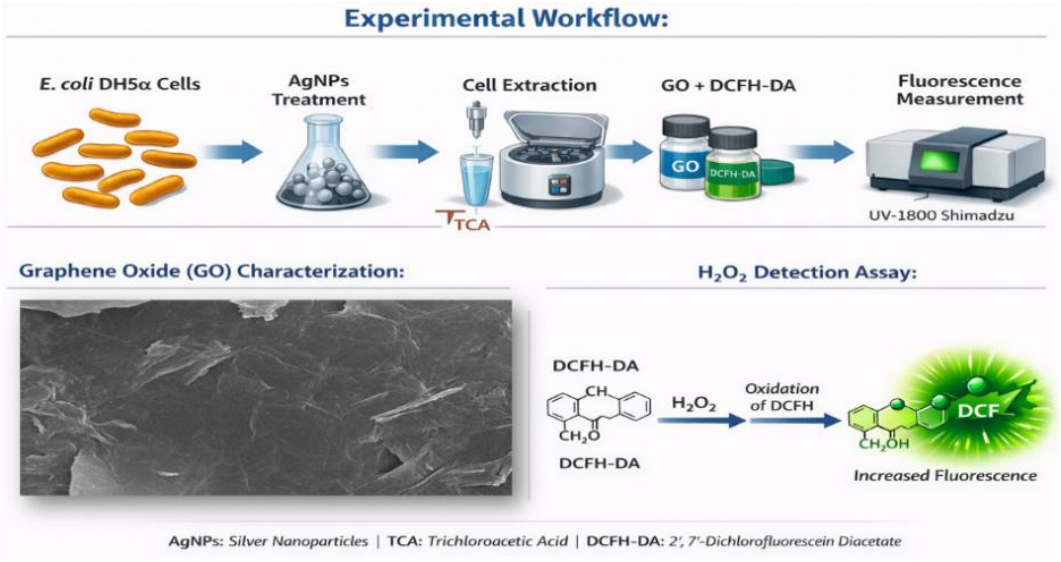

Fig. 1. *Schematic representation of the experimental workflow and H_2O_2 detection strategy. E. coli DH5α cells were treated with silver nanoparticles, followed by cellular extraction and quantification of H_2O_2 using a graphene oxide–based DCFH-DA fluorescence assay*

RESULT AND DISCUSSION

Fluorescence analysis using 2',7'-dichlorofluorescein diacetate (DCFH-DA) in the presence of graphene oxide (GO) demonstrated a concentration-dependent increase in fluorescence intensity, reflecting corresponding hydrogen peroxide (H_2O_2) levels. As illustrated in Figure 2, fluorescence intensity increased progressively with H_2O_2 concentrations ranging from 0 to 1.5 μM/mL, reaching approximately 60 fluorescence units at 1.5 μM/mL. Lower H_2O_2 concentrations produced proportionally smaller fluorescence responses, with 1 μM/mL and 0.8 μM/mL yielding approximately 45 and 28 fluorescence units, respectively. The resulting calibration curve exhibited a strong linear correlation between H_2O_2 concentration and fluorescence intensity ($y = 28.888x – 0.4158$, $R^2 = 0.9595$), confirming the sensitivity and reliability of the GO-based assay for quantitative H_2O_2 determination. Subsequently, Escherichia coli DH5α cells were exposed to silver nanoparticles (AgNPs) at concentrations of 50, 75, and 100 μg/mL, and intracellular H_2O_2 production was assessed. Fluorescence measurements of cell extracts following treatment with GO and DCFH-DA revealed a significant increase in fluorescence intensity relative to control samples, indicating induction of oxidative stress by the AgNPs.

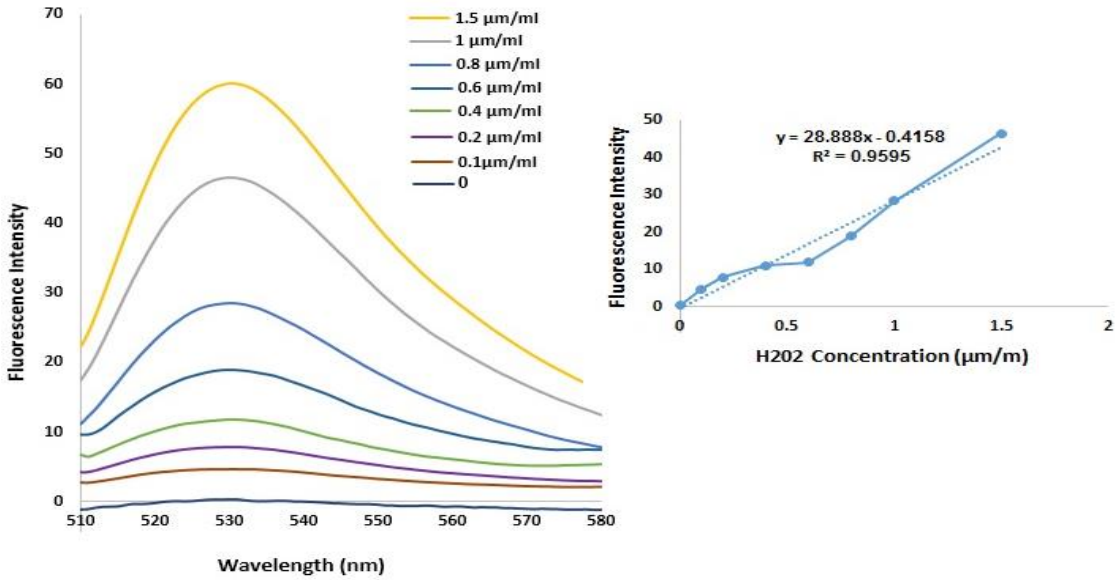

Fig. 2. *Standard calibration curve for H_2O_2 measured using a graphene oxide–based nanosensor*

Fluorescence intensity exhibited a dose-dependent response: 50 µg/mL AgNPs produced an intensity of 0.091, 75 µg/mL yielded 0.217, and 100 µg/mL resulted in the highest intensity of 0.687 (Figure 3). These findings indicate that exposure to AgNPs stimulates the generation of reactive oxygen species within E. coli cells, with intracellular H_2O_2 levels increasing proportionally to nanoparticle concentration.

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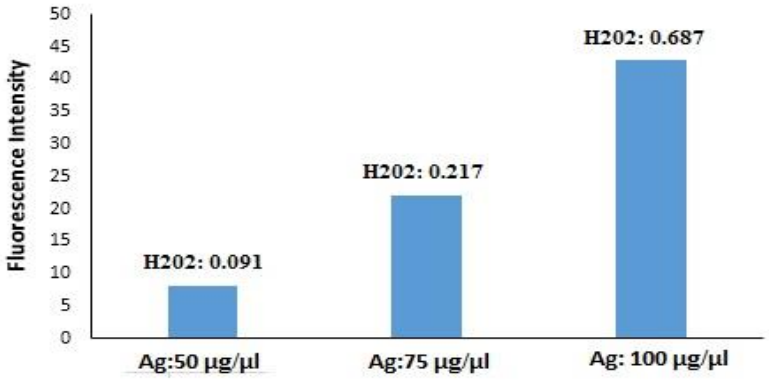

Fig. 3. *Graph depicting the cellular extract levels of H_2O_2 in the bacterial sample*

CONCLUSION

The present study demonstrates that a graphene oxide-based nanosensor platform enables rapid and sensitive quantification of intracellular H_2O_2 levels in bacterial systems. The results indicate that exposure to silver nanoparticles significantly elevates H_2O_2 production in Escherichia coli, and these alterations were reliably detected by the nanosensor. The oxidase-mimetic properties of graphene oxide, coupled with its signal amplification capabilities, contribute critically to the observed enhancement in analytical sensitivity. Collectively, these findings suggest that such nanosensor platforms represent effective tools for elucidating oxidative stress mechanisms in microorganisms and for the development of redox-based diagnostic strategies in both biomedical research and personalized medicine.

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