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Recent advances in nanostructures and nanocrystals as signal-amplification elements in electrochemical cytosensing

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ABSTRACT

Considering the vital role of cells in life science and human health, cytosensors have become a hot research topic. Electrochemical cytosensors attract much attention. In this review, we discuss some recent efforts to construct novel and improved electrochemical cytosensors based on graphene, carbon nanotubes, some metal-nanoparticle composites, quantum dots, nanofibers and nanowires. In addition, we summarize examples of nanostructure applications in electrochemical cytosensing reported in the literature from 2009 to date, with their advantages and limitations, and stress their potential for future development. Also, we focus on the current surface-modification strategies with some nanostructures, the influence of nanostructure properties on cytosensor performance and the applications of these materials in conjunction with different transducers to the detection of cells.

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Contents

1. Introduction

There have been thousands of sensor papers published in the past two years, and electrochemical sensors represent the most rapidly growing class. Compared to other methods, such as spectroscopy and chromatography, electrochemical measurements are much cheaper, simpler and easier to miniaturize, so they are more suitable for point-of care (POC) detection, particularly for delivering benefits to resource-limited areas in developed and developing countries. Besides that, a wide variety of strategies are used to improve the efficacy of sensing. Signal amplification has been achieved for detection utilizing nanostructures as carriers or tracers, catalysts, and electronic conductors, with synergistic effects on catalytic activity, conductivity and biocompatibility. In recent years, electrochemical methods were shown to be sensitive, selective, and cost effective for the studies of type, concentration, activity, proliferation, and apoptosis of cells, and the distribution of specific molecules. Also, electrochemical methods attracted considerable attention for developing cytosensing systems, since they can decrease the cost and the time required for cell detection with simple instrumentation.

New developments in nanotechnology, materials science and custom engineering of biorecognition components have advanced the progress of useful, reliable electrochemical (bio)sensors. The materials and biomaterials with rich nanostructures not only improve

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the electronic properties and increase the effective electrode surface for transferring electrochemical signal but also produce detectable signals for indirect detection of targets. Thus, the resulting methods possess high sensitivity and good specificity. However, the synergy of multifunctional materials, recognition elements and electrochemical methods is improving selectivity, stability and reproducibility, thus promoting the development of sensors for assays and bioassays. A variety of nanomaterials and nanostructures including carbon nanotubes (CNTs), noble-metal nanoparticles (NPs), graphene (G), quantum dots (QDs) and nanofibers (NFs) have been used as sensing platforms for cytosensors, and can improve sensitivity through signal amplification. Therefore, developing highly sensitive cytosensors based on nanomaterials and nanostructures will have a great impact in healthcare.

Considering the vital role of cells in life sciences and human health, cytosensors have become a hot research topic. In the context of cytosensing, researchers require the ability to track molecules within their native environments. Thus, the efficiency of sensing systems critically relates to the outcome of the detection process in terms of response time, signal-to-noise characteristics, sensitivity and selectivity of the system. The combination of nanotechnology with chemistry, biology, physics, engineering and medicine has emerged as a key solution. It may revolutionize the research on cell adhesion, glycobiology, cell trafficking, signal transduction, structural biology, transcriptional regulation, and disease diagnosis and treatment. Nanomaterial-based techniques, combined with nanofluidics, single-molecule detection and multiplexing methodologies, excitingly provide new possibilities for ultrasensitive cytosensing.

Cytosensing modalities include optical fluorescence, molecular magnetic resonance imaging, surface-enhanced Raman scattering (SERS), colorimetry, scanometric detection and electrochemistry. Many cytosensors are electrochemical. Since a living cell can be properly described as an electrochemical dynamic system, the use of cell-based biosensor (cytosensor) platforms has attracted considerable attention [1], with remarkable advantages in the electrochemical monitoring of cell viability and proliferation, such as low cost, convenient operation, rapid detection and good sensitivity.

An electrochemical cytosensor is used to analyze and to evaluate cells based on electrochemical detection signals, such as current, impedance and capacitance. Notably, electrochemical cytosensing plays a more important role in analysis and detection of target cells due to inherent advantages, such as miniaturization, easy operation, rapid response, satisfactory sensitivity, high selectivity, affordability, and real-time and non-destructive analysis [2–7]. Recent advances in electrochemical cytosensors included detection of cell type and number, cellular physiological parameters, crucial molecules on the cell surface or inside cells, and pharmaceutical evaluation and screening.

An explosion of interest has occurred in the use of various kinds of zero-, one-, two-, and three-dimensional nanomaterials for electrochemical cytosensing [e.g., semiconductor QDs, metallic NPs, CNTs, nanostructured conductive polymers or nanocomposites thereof]. The significant roles of nanomaterials in cytosensing lie in their ability to address some key issues, including design of the cell-compatible interface, facilitation of the electron transfer in electrochemical reactions, achievement of efficient transduction of the biorecognition event, increases in sensitivity and selectivity, and improvement of response times [8]. The voltammetric responses of the redox centers in living cells usually show irreversible electron transfer related to the oxidation of guanine, but the presence of nanomaterials on electrodes for cell immobilization can significantly reinforce the electrochemical response and maintain cell viability [9].

In this review, we summarize selected research articles from 2009 to January 2015 on electrochemical cytosensors. In the following sections, we review some recent efforts to construct novel and improved electrochemical cytosensors, involving G, CNTs and some metal-NP composites, QDs, NFs and nanowires (NWs). This article reviews recent advances in the electrochemical cytosensors of various analytes. In addition, we summarize below examples of nanostructure applications in electrochemical cytosensing reported so far in the literature, with their advantages and limitations and stress their potential for future developments in this field. Also, we focus on current surface-modification strategies using some nanostructures, the influence of nanostructure properties on cytosensor performance and the applications of these materials in conjunction with different transducers for the detection of cells.

2. Types of nanostructure-based electrochemical cytosensors

2.1. Noble metals covering gold and silver nanostructure-based electrochemical cytosensors

Gold NPs (AuNPs) have become highly valuable nanomaterials in biological science, mainly because of their biocompatibility, easy conjugation to biomolecules, and tunable optical properties depending on their size, shape, and surface layer [10,11]. The unique ability of AuNPs to facilitate electron transfer between the NP surface-immobilized proteins and electrode surfaces has led to their intensive use of AuNPs and related nanostructures for the construction of electrochemical biosensors with enhanced analytical performance compared with other biosensor designs [12]. Characteristics of AuNPs, such as high surface-area-to-volume ratio, high surface energy, ability to decrease protein-metal-particle distance, and functioning as electron-conducting pathways between prosthetic groups and the electrode surface facilitate electron transfer between redox proteins and electrode surfaces [13]. Most cells can be easily coupled to AuNPs and they do not lose their biological activity. AuNPs have also constituted useful interfaces for the electroanalytical detection of different cells (especially cancer cells) involved in many significant biochemical reactions. This sub-section summarizes recent advances in the construction of AuNP-based electrochemical cytosensors.

Pioneer work has been done by Ding and co-workers [14]. An electrochemical cytosensor has been designed based on the specific recognition of mannosyl on a cell surface to concanavalin A (Con A) and the signal amplification of AuNPs. In this report, the Ding group prepared this electrochemical cytosensor by sandwiching a cancer cell between a gold electrode modified with Con A and the AuNPs with Con A and 6-ferrocenylhexanethiol (Fc). Then, the cell number and the amount of cell-surface mannose moieties were quantified by cyclic voltammetry analysis of the ferrocene (Fc) loaded on the surface of the AuNPs. Since a single AuNP could be loaded with hundreds of Fc, amplification for the detection of target cell was significant. Results of this work showed that, in using K562 leukemic cells (K562 cells), the electrochemical response was proportional to the cell concentration in the range 10^2–10^7 cells/mL. Also, the limit of detection (LOD) for cell concentration was calculated to be 73 cells/mL. The signal amplification could be further used to evaluate the cell-surface mannose moieties, and the amount of mannose moieties on a single living K562 cell was detected to correspond to 4.7×10 molecules of free mannose. From this work, it was found that this strategy presented a promising platform with a highly sensitive cytosensor and convenient estimation of cell-surface carbohydrate. Also, it showed that AuNPs are a good basis for signal amplification.

The alpha isoform folate receptor (FR) is a cancer-associated antigen. It is cancer-specific and is more abundant on cancer cells than on normal cells, so it can be used as a marker for cancer cells. The high affinity between the FR and folic acid (FA) enables FA to direct drugs toward cancer cells and is an attractive property for

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