Biosensors and Bioelectronics 100 (2018) 453–461

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Flexible nanohybrid microelectrode based on carbon fiber wrapped by gold nanoparticles decorated nitrogen doped carbon nanotube arrays: *In situ* electrochemical detection in live cancer cells

Yan Zhang[a], Jian Xiao[a], Yimin Sun[b], Lu Wang[a], Xulin Dong[a], Jinghua Ren[c], Wenshan He[c], Fei Xiao[a,*]

[a] Key laboratory of Material Chemistry for Energy Conversion and Storage, Ministry of Education, Hubei Key Laboratory of Material Chemistry and Service Failure, School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan 430074, PR China
[b] Hubei Key Laboratory of Plasma Chemistry and Advanced Materials, School of Materials Science and Engineering, Wuhan Institute of Technology, Wuhan 430073, PR China
[c] Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, PR China

ARTICLE INFO

Keywords:
Flexible fiber microelectrode
Nitrogen doped carbon nanotube arrays
Gold nanoparticles
Electrochemical biosensors
in situ detection
Cancer cells

ABSTRACT

The rapidly growing demand for *in situ* real-time monitoring of chemical information *in vitro* and *in vivo* has attracted tremendous research efforts into the design and construction of high-performance biosensor devices. Herein, we develop a new type of flexible nanohybrid microelectrode based on carbon fiber wrapped by gold nanoparticles decorated nitrogen-doped carbon nanotube arrays, and explore its practical application in *in situ* electrochemical detection of cancer biomarker H_2O_2 secreted from live cancer cells. Our results demonstrate that carbon fiber material with microscale size and fascinating mechanical properties can be used as a robust and flexible microelectrode substrate in the electrochemical biosensor system. And the highly ordered nitrogen-doped carbon nanotube arrays that grown on carbon fiber possess high surface area-to-volume ratio and abundant active sites, which facilitate the loading of high-density and uniformly dispersed gold nanoparticles on it. Benefited from the unique microstructure and excellent electrocatalytic properties of different components in the nanohybrid fiber microelectrode, an effective electrochemical sensing platform based on it has been built up for the sensitive and selective detection of H_2O_2, the detection limit is calculated to be 50 nM when the signal-to-noise ratio is 3:1, and the linear dynamic range is up to 4.3 mM, with a high sensitivity of 142 μA cm^{-2} mM^{-1}. These good sensing performances, coupled with its intrinsic mechanical flexibility and biocompatibility, allow for its use in *in situ* real-time tracking H_2O_2 secreted from breast cancer cell lines MCF-7 and MBA-MD-231, and evaluating the sensitivity of different cancer cells to chemotherapy or radiotherapy treatments, which hold great promise for clinic application in cancer diagnose and management.

1. Introduction

Cancer is the second largest life-threatening disease that has over 200 types identified all over the world and leads to more than 1500 deaths occurring each day (Jayanthi et al., 2017). In spite of recent technological advancement towards the treatments of cancer, the patient's survival rate is still low due to the poor prognosis and limitation of cancer diagnosis and therapy at the late stage (Pan et al., 2017; Wang et al., 2017). Therefore, early diagnosis of cancer is helpful for its effective treatment and decreasing the mortality rate. However, the conventionally available methods in cancer diagnosis are primarily based on the phenotypic properties of the tumors using ultrasound, X-ray, magnetic resonance imaging, and biopsy (Hossain et al., 2012; Prével et al., 2016; Yang et al., 2017), which are lack of sensitivity for early-stage cancer detection until the cancer cells have spread surrounding tissues and metastasized throughout the body. In recent times, tremendous research efforts have been focused on developing cancer biosensors for their excellent analytical performances in effective measurement of cancer biomarkers to improve the early diagnosis (Jayanthi et al., 2017; Liu et al., 2017a; Mittal et al., 2017). H_2O_2 is a general endogenously enzymatic byproduct of oxidases that plays an increasingly important role in a variety of biological processes and can function as an indicator in tracing many biochemical reactions (Xi et al., 2015; Xiao et al., 2012). Recent researches have demonstrated

* Corresponding author.
E-mail address: xiaofei@hust.edu.cn (F. Xiao).

http://dx.doi.org/10.1016/j.bios.2017.09.038
Received 7 September 2017; Accepted 20 September 2017
Available online 22 September 2017
0956-5663/

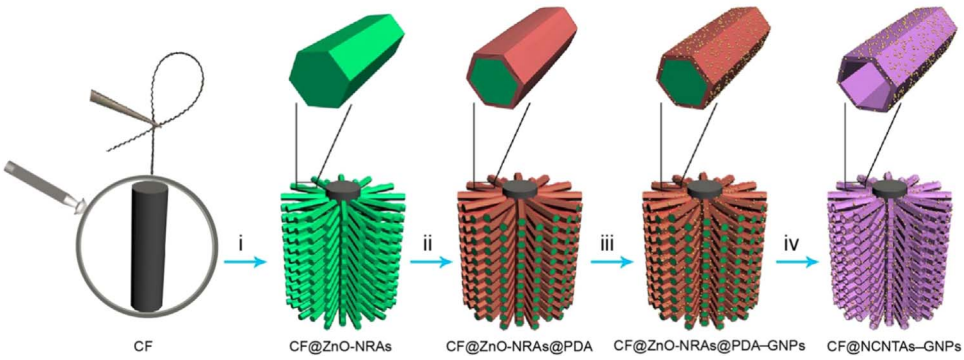

Fig. 1. Schematic illustration of the fabrication procedure of CF@NCNTAs–GNPs nanocomposite. Step i: Growth of ZnO-NRAs on CF *via* hydrothermal process; Step ii Coating PDA layer on ZnO-NRAs *via* polymerization of DA; Step iii: Decorating GNPs on PDA layer *via* chemical reduction of $KAuCl_4$ precursor by catechol groups of PDA. Step iv: Carbonizating and removing ZnO-NRAs template.

that H_2O_2 is excreted by live cells and can reach various cellular regions by diffusing out through the membranes to keep its concentration at the same level (Kim et al., 2017; Lin et al., 2016; Zhu et al., 2016). An appropriate level of H_2O_2 is benefit for body normal function and signal transduction of cells. Nevertheless, it is also noteworthy that the excess of H_2O_2 can destroy the orderliness of the body and lead to different diseases, including Parkinson's, heart attack, Alzheimer's and so on (Bai and Jiang, 2013; Wang et al., 2016a). Especially, emerged evidences have proved that cancer cells can generate more H_2O_2 than normal cells due to an elevation of H_2O_2 production capacity in abnormal growth of tumor (Ju and Chen, 2015; Liu et al., 2015). As a result, H_2O_2 can be used as a specific molecular probe, and the detection of H_2O_2 in biological system is significant for the evaluation of oxidative stress capability distinctions in different live cell as well as the identification of cancer cells.

Nowadays, a wide spectrum of analytical techniques, including spectrophotometry, titrimetry (Liu et al., 2017b), chemiluminescence (Ravi et al., 2003), chromatography (Xu et al., 2016), and electrochemistry (Rui et al., 2010; Sun et al., 2015, 2016; Xi et al., 2016), have been applied for the determination of H_2O_2. Among them, electrochemical methods have sparked great research interests for their explicitly capable in *in situ* real-time analysis of H_2O_2 due to their rapid response, high sensitivity and accuracy, facile operation, user-friendliness, as well as excellent reproducibility (Beitollahi et al., 2014, 2015; Bojdi et al., 2016a, 2014; Karimi-Maleh et al., 2012; Mazloum-Ardakani et al., 2011; Taleat et al., 2008). The electrochemical H_2O_2 sensors are usually based on catalytic redox of H_2O_2 by natural enzyme, *e.g.,* horseradish peroxidase (Chen et al., 2017). However, the inevitable disadvantages, such as complicated immobilization procedure, insufficient stability, and high costs, have seriously limited its application in practice. In comparison with enzymatic sensors, the nonenzymatic H_2O_2 sensors based on noble metals (*e.g.,* Pt, Au, Pd, Ag *etc.)* and their alloy nanoparticles (NPs) have been developed as an alternative for their high specific electrocatalytic activity and outstanding electron transfer ability toward direct electrochemical redox of H_2O_2 (Chen et al., 2012, 2014a; Wang et al., 2016a; Xi et al., 2015; Xiao et al., 2012, 2013; Zhang et al., 2014, 2015). More importantly, loading noble metal NPs on high surface-to-volume ratio support, such as tubular structure, can effectively enhance their electrocatalytic efficiency (Bojdi et al., 2016b; Hosseini et al., 2014), which is anticipated to exhibit good analytical performance in rapid and accurate detection of low-level H_2O_2 in real samples. In parallel with the development of nanocatalyst on electrode, the design of flexible electrode substrates have also attracted tremendous research efforts. In our previous works, several flexible one-dimension (1D), two-dimension (2D) and three-dimension (3D) carbon materials, such as 1D carbon fiber (CF) (Wang et al., 2016), 2D graphene paper (Sun et al., 2015, 2016; Xiao et al., 2013, 2012) and 3D graphene foam (Dong et al., 2015), have been developed in constructing electrochemical biosensors for their special features including high chemical stability, good biocompatibility, and intrinsic flexibility and mechanical strength. Consequently, the resultant modified electrodes exhibit good sensing performances for *in vitro* and *in vivo* tracking biological information.

In this work, we develop a new type of flexible nanohybrid microelectrode based on CF modified by gold nanoparticles (GNPs) decorated highly ordered nitrogen doped carbon nanotube arrays (NCNTAs), and explore its practice application in *in situ* real-time detection of H_2O_2 secreted from live cancer cells. CF is a fascinating flexible substrate in microscale regard due to its small size (5–30 μm in diameter), elasticity modulus, and high tenacity, which can be used in electrical signal transduction of electrochemical biosensor system. In order to increase its active surface area, NCNTAs were directly synthesized on CF by a facile and template-based procedure using ZnO nanorod arrays (NRAs) as the template, where ZnO-NRAs were grown on CF through hydrothermal synthesis and used as the sacrificial template to construct the hollow structure of nitrogen doped carbon (NC), with dopamine (DA) as the carbon source and nitrogen source. DA is a biomolecule that can self-polymerize at alkaline pH values and spontaneously form polydopamine (PDA) films on virtually any surface as well as secondary surface-mediated reaction (Liu et al., 2011; Wang et al., 2016b), and the reduction properties of catechol groups of PDA facilitate the growth of highly dense and well disperse GNPs on the PDA coated support from $KAuCl_4$ precursor. After carbonizating and removing ZnO-NRAs template, the CF wrapped by GNPs decorated NCNTAs (CF@NCNTAs–GNPs) was obtained (Fig. 1). In virtue of the structural merits and electrocatalytic properties of different components in CF@NCNTAs–GNPs, a highly sensitive, selective and reliable sensing platform has be built up for nonenzymatic electrochemical detection of H_2O_2 and *in situ* real-time tracking H_2O_2 secreted from breast cancer cell lines MCF-7 and MBA-MD-231. The results show that the proposed electrochemical sensor based on flexible CF@NCNTAs–GNPs microelectrode can determine H_2O_2 secreted from MDA-MB-231 and MCF-7 cells stimulated by extracellular matters, and evaluate the sensitivity of different cancer cells to chemotherapy or radiotherapy treatments, which is of great clinic significance for the development of effective strategies in the diagnosis and treatment of different cancer diseases.

2. Material and methods

2.1. Reagents and apparatus

Zinc acetate, ethylenediamine, ethylene glycol methyl ether, zinc nitrate hexahydrate and hexamethylene tetramine were purchased from Aladdin Chemistry Co., Ltd (China) and have been used for the preparation of ZnO-NRAs. DA, $KAuCl_4$, tris(hydroxymethyl) aminomethane and HCl (mass fraction is 37.5%) were obtained from Sigma-Aldrich Co., Ltd (USA). Ascorbic acid (AA), β-D(+)-glucose, uric acid (UA), and H_2O_2 were obtained from Sinopharm Chemical Reagent Co., Ltd (China). N-formylmethionyl-leucyl-phenyl-alanine (fLMP, ≥99.5%) and catalase (come from bovine liver, Lyophilized, ≥ 3000 units mg^{-1}) were obtained from Sigma-Aldrich Co., Ltd (USA). Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), trypsine-EDTA