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A novel electrochemical method to evaluate the cytotoxicity of heavy metals

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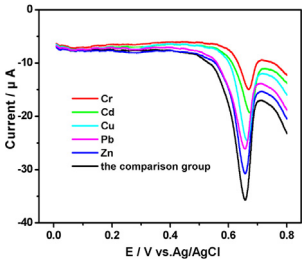
HIGHLIGHTS

- The electrochemical behaviors of HeLa cells on a highly sensitive graphene modified electrode were proposed for the first time.
- A novel label-free electrochemical method based on the electrochemical response of HeLa cells was developed.
- Cytotoxicity of five heavy metals was tested with the electrochemical method.

GRAPHICAL ABSTRACT

A novel label-free electrochemical method based on the direct voltammetric response of human cervical carcinoma (HeLa) cells on a highly sensitive graphene modified electrode was developed. Five heavy metals were tested with this method. This work will be beneficial in providing a novel simple method for cytotoxicity evaluation of hazardous pollutants in environment.



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ABSTRACT

There is an ongoing search to develop techniques for detection of heavy metals which are highly toxic and can cause damaging effects even at very low concentrations. In this present study, we report a label-free electrochemical method based on the direct voltammetric response of human cervical carcinoma (HeLa) cells on a highly sensitive graphene modified electrode. Five heavy metals were tested with the method and the results were validated by the traditional methyl tetrazolium (MTT) assay. The results revealed that the most toxic metal was Cr, followed by Cd, Cu, Pb and Zn. A good correlation between the two methods was observed. This work will be beneficial in providing a novel monitoring method to detect hazardous pollutants in the field of environmental toxicology.

1. Introduction

Heavy metals are known to be potentially cytotoxic to biota and present a serious health threat to the public. Thus, the establishment of a sensitive biological monitoring approach for early detection and ecotoxicological evaluation is required [1,2]. The *in vitro* toxicity testing techniques, which are relatively rapid, cost-effective, readily reproducible and can be used in automated

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high-throughput screening techniques [3], have been developed and validated as an alternative to whole animal tests [4]. For investigating the *in vitro* toxicity, cell viability was evaluated as one of the most important parameters, and the most common analytical techniques currently used include 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, flow cytometry experiment and lactate dehydrogenase (LDH) release assay, etc. [5–7]. However, these marking approaches have some disadvantages, such as the complexity, requirement of pretreatment steps, time consumption and high cost.

Recently, the emerging electrochemical method has shown great promise in the detection of the cell viability owing to its simple instrumentation, high sensitivity, rapidness, low-cost, label-free, non-toxicity and reutilization of samples [8]. The electrochemical equipments could be used to detect the electrical signals of biological active analytes generated by cellular life activities. Then the obtained signals could be used to objectively reveal the information about cell growth and metabolism [9,10]. Nevertheless, the *in vitro* electrochemical methods were employed mostly for pharmaceutical screening by using MFC-7 [11], PC-3 [12], or K562 cell [13] as a model. Therefore, to develop a sensitive and high specific cell model for toxicity evaluation of heavy metals is necessary.

Compared with fish cells, plant cells, insect cells, germ cells, etc., which have been used to measure the cytotoxicity of heavy metals [14–19], HeLa cells have some incomparable advantages. As the first type of human cells successfully cultivated in laboratory, they cannot only replicate themselves rapidly, easily and cheaply, but also express and regulate genes, produce proteins, communicate with one another, and are susceptible to infections as normal cells [20,21]. Additionally, HeLa cells have been proved to be good model organisms in the field of biology and environmental toxicology [22]. However, to the best of our knowledge, they have never been used for cytotoxicity evaluation of heavy metals, whose mechanisms of electrochemical response have rarely been studied. Here, we chose HeLa cells as the model for electrochemical detection.

Based on the chosen good cell model, constructing a sensitive electrode to improve the sensitivity of electrochemical responses is also important [23,24]. Some efforts launched in this area have been focused on constructing nanomaterial and biomaterial modified electrode. Some materials including carbon nanotubes [11], nanoparticle [25], aniline [13], etc., have been employed. Recently, graphene has attracted great attention in the field of biosensors due to its strong mechanical strength, excellent conducting nature and highly specific electrocatalytic ability [26–29]. In this paper, we developed a highly sensitive graphene modified electrode. The electrochemical behavior of HeLa cells on the modified electrode was proposed. A simple electrochemical method based on the electrochemical responses of HeLa cells was firstly developed. The cytotoxicity of five heavy metals was evaluated with the electrochemical method and the results were consistent with those of the MTT assay. This study provides a new insight into risk assessment of hazardous pollutants in environment.

2. Materials and methods

2.1. Chemicals

Phosphate buffer saline (PBS, pH 7.4) containing 136.7 mM NaCl, 2.7 mM KCl, 9.7 mM $Na_2HPO_4{\cdot}12H_2O$, 1.5 mM KH_2PO_4 was used in the experiments. Xanthine, guanine and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were purchased from Sigma. Five metal salts of analytical grade including $K_2Cr_2O_7$, $CuSO_4{\cdot}5H_2O$, $3CdSO_4{\cdot}8H_2O$, $ZnSO_4{\cdot}7H_2O$, and $Pb(NO_3)_2$ were purchased from J&K. Stock solutions of these metal salts were prepared respectively and stored at 4 °C for no more than a week before use. For the cytotoxicity assays, eight serial dilutions of each salt stock were made in the cell culture medium to final concentrations of 1, 5, 10, 20, 40, 80, 100 μM for Cr and Cd, and 5, 10, 20, 40, 100, 150, 200 μM for Cu and Pb, and 10, 20, 40, 100, 150, 200, 300 μM for Zn, respectively. The high purity multiwalled carbon nanotubes (MWCNTs, 10–20 nm in diameter, Shenzhen Nanotech Port Co., China) were purified by refluxing in 30% nitric acid at 100 °C for 24 h. After separation from the mixture, the sediment was washed with double-distilled water until the pH reached 7.0, and then followed by filtering, rinsing with double-distilled water and drying. Finally, a 1.0 mg/mL MWCNTs suspension was obtained. The graphene oxide (GO) was synthesized by using Hummer's method [30]. All other chemicals were of analytical grade and used as received.

2.2. Cell culture and collection

HeLa cells were obtained as a gift from the Basic Medical Science College, Harbin Medical University (Harbin, China). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) containing 10% fetal calf serum, 100 μg/mL penicillin (Gibco) and 100 μg/mL streptomycin (Gibco) in an incubator (5% CO_2, 37 °C). These cells were sub-cultured every 2–3 d according to the standard procedure and harvested at exponential growth phase for cytotoxicity tests. Prior to heavy metal exposure, the growth medium was discarded and replaced with the medium containing varied concentrations of heavy metal. The same culture conditions were provided for both the control and the experimental groups. *In situ* cell collection method developed by our group recently [31] was applied in this work. Additionally, in our previous study [32], we found that the origin of the voltammetric response of the intact cell suspension was given by purines secreted by the living cells from the cytoplasm. The voltammetric behavior of the intact cell suspension was weaker than that of the fragmentized cell suspension, and heat inactivation was superior to ultrasonication in improving the voltammetric response by increasing the release of the intracellulare guanine and xanthine into the cell eluent as a result of fragmenting the cell membrane. Hence, the heat inactivation method was applied in the present study. The collected cells were kept in the culture dishes with the medium removed. The still adherent cells were washed with the sterile pH 7.4 PBS for three times. Then some amount of pH 7.4 PBS was added to the cells, and they were heated in 50 °C water bath for 30 min. The HeLa cell suspension was obtained finally. Cell concentrations were determined by a CBC DRM-700 cell counting plate (China).

2.3. Production of the ERGO/GCE

The glassy carbon electrode (GCE, 3 mm in diameter) was first polished with 0.05 mm alumina slurry on a polishing cloth to produce a mirror-like surface. Subsequently, it was rinsed with doubly distilled water and ethanol respectively to remove any physically adsorbed substances. After the MWCNTs suspension were fully dispersed by sonication, 10 μL of 1.0 mg/mL MWCNTs suspension obtained was cast onto the surface of the GCE, and then the electrodes were allowed to dry under an infrared lamp to give the MWCNTs/GCE. After 0.5 mg/mL GO dispersion was ultrasonicated, 5 μL of the dispersion was cast onto the GCE surface, and then the electrodes were allowed to dry under an infrared lamp to give the GO/GCE. Finally, the GO/GCE was placed in a 0.1 M PBS (pH 5.0), and electrochemically reduced with potential sweep −1.5 V at 100 mV/s for 720 s. Prior to the cyclic voltammetric measurement, the MWCNTs/GCE and ERGO/GCE were electrochemically treated with several cycles between 0.0 and +0.8 V in pH 7.4 PBS to obtain stable background lines.