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Assessment of cadmium-induced hepatotoxicity and protective effects of zinc against it using an improved cell-based biosensor

Jie Zhou[a], Chengxiong Wu[a], Jue Tu[b], Yun Ling[b], Ning Hu[a], Yongsheng Zhang[c], Kaiqi Su[a], Ping Wang[a,*]

[a] Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, China
[b] Experimental Animal Research Center, Zhejiang Chinese Medical University, Hangzhou 310053, China
[c] Pharmaceutical Science College, Zhejiang Chinese Medical University, Hangzhou 310053, China

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ABSTRACT

Cadmium (Cd) is a toxic environmental pollutant which creates risks to human health. Liver is one of the major sites of Cd accumulation in the organism and target organs under chronic and acute exposure of Cd. It was estimated that Zn^{2+} supplementation can prevent apoptosis induced by a variety of agents such as Cd^{2+}. In this paper, an improved electric cell-substrate impedance sensing (ECIS) assay was developed to investigate the Cd^{2+}-induced hepatotoxicity and the effects of Zn^{2+} at different concentrations on it *in vitro*. Normal liver L-02 cells were cultured onto interdigitated electrodes. With ECIS technology the dynamic responses of L-02 cells were monitored in a non-invasive and label-free manner. The results showed a clear dose- and time-depending toxic effect of Cd^{2+} on L-02 cells. The half-inhibition concentration of Cd^{2+} was 20 μM after 24 h of exposure. The results also revealed that enhanced Zn^{2+} consumption (10–20 μM) may be beneficial for preventing Cd^{2+}-induced hepatotoxicity. However, its excessive intake (40 μM) may intensify the hepatotoxicity. Furthermore, for improving the detection sensitivity of the system, a cell immobilization approach using self-assembly techniques was applied to bind cells on electrodes for improving the detection sensitivity of the system. This study demonstrated that the ECIS system holds promise as a utility platform for real-time studying cell status and evaluating chemical effect with high detection sensitivity.

1. Introduction

Cadmium (Cd) is a non-essential trace element, which is well known to be one of the most toxic heavy metals. The major sources of human exposure to cadmium contamination are environmental pollution [1]. Liver is one of the main target organs for Cd toxicity and accumulation, even moderate treatment with this metal may cause distinct pathological alteration in the liver [2,3]. Acute hepatotoxicity induced by Cd exposure provokes cell morphological changes such as hepatocellular swelling, pyknosis and karyrrhexis which result in cell apoptosis and necrosis [4,5]. Thus, it is necessary to explore efficient ways to provide protections for hepatic injury under exposure of Cd. Zn, which functions as a complex antioxidant could inhibit the oxidative stress induced by Cd or other chemicals with similar effects. Moreover, Zn is a well-known inhibitor of apoptosis and necrosis in cells for Zn prevents the production of reactive oxygen species (ROS) which are described as apoptosis mediators [6]. Recent studies suggested that Zn can reduce Cd-induced liver damage; several experiments were done *in vivo* [5,7] models to investigate Cd-induced hepatotoxicity and the effect of Zn supplementation on it. However, the major drawbacks of animal experiments are time consuming and labor-intensive. While cell-based assay which has been used as a suitable substitute method for animal experiments provides a high-throughput platform for toxicological testing [6,8,9].

The conventional approaches for *in vitro* cytotoxicity assays can provide deep insights into the mechanism of chemicals on cells. However, they often contain fluorescent, chemiluminescent, or radioactive labeling procedures which often involve destruction of cells [10,11]. Besides, most of them are based on single endpoints which can only detect very specific cellular changes at a given time point and do not provides dynamic information with regard to cell responding to toxicants. Thus, in order to accurately assess Cd-induced hepatotoxicity and protective effects of Zn on it, we need to develop a non-invasive and label-free method allowing for dynamic detection of physiological responses of liver cells to Cd or a mixer of Cd and Zn.

In the last 20 years, electrochemical impedance spectroscopy (EIS) has emerged as one of the most interesting label-free technology in biology field such as immunology [12–14], tissue [15–17] and cell physiology [18,19], etc. Among these applications, electric cell-based impedance sensing (ECIS) has been recognized as a

* Corresponding author. Tel.: +86 571 87952832; fax: +86 571 87952832.
E-mail address: cnpwang@zju.edu.cn (P. Wang).

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quantitative mean for monitoring live cell status in a non-invasive and real-time manner. By culturing biological cells on the chip surface, ECIS can directly sense detailed information about cellular activities occurring on electrodes by measuring the induced resistance and capacitance changes. Recently, it has gained a great deal of attentions for investigating chemical-induced changes on cell morphology and viability [20,21].

In the present work, we report on the application of ECIS for monitoring activities of L-02 cells when exposed to Cd^{2+}, Zn^{2+} or a mixer of Cd^{2+} and Zn^{2+}. Also, in order to improve the biocompatibility of gold electrodes which act as the sensing part of the impedance sensor and the sensitivity of the ECIS system, we covalently immobilize the adhesive polypeptide poly-L-lysine (PLL) to promote the cell adhesion and growth on electrodes. And this is achieved by using the self-assemble monolayers (SAM) technique which offers attachment points on electrodes for adhesive substance such as PLL [22–24].

2. Experimental

2.1. Chip fabrication and surface modification

The fabrication of the chip begins with sputtering a layer of Cr (20 nm thick) on a sterilized Pyrex glass 7740 (Corning) and then Au (200 nm thick). Subsequently, the interdigitated electrodes, interconnects and pads were patterned from this composited metallic layer by conventional lithography and etching techniques. The sensing chip consists of eight pairs of round-shaped interdigitated electrodes (shown in Fig. 1a). The circle-on-line electrode array with high coverage rate on chip surface helps improve the sensitivity of detection system [18]. The diameter of the circle is 90 μm. Since some organic solvents used in the process of surface treatment were corrosive to chambers, chips were packaged with PCB board and perspex chamber after the surface modification procedure.

For surface modification, the chip was firstly washed with acetone, ethanol, and DI water respectively to remove the organic residues off the substrate. Subsequently, the electrodes were reacted with a 10 mM carboxyl-terminated alkanethiolates 16-mercaptoundecanoic acid (16-MHA, Sigma) overnight (>18 h) to form an alkanethiolates SAM on gold electrodes. Among different kinds of SAM, the SAM of alkanethiolates on gold forms a Au—S covalent bond which is stable for a period of several months in various solutions, making it the most widely used class of SAMs [25,26]. Then the chip was immersed in a mixer of 15 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Sigma) and 75 mM N-hydroxy- succinimide (NHS, Sigma) for 1 h. In this process, the NHS ester intermediate activated carboxylate groups of the alkanethiol SAM to covalently bond primary amino groups of PLL.

After each step of the surface modification, the chip was washed with its original solvent and DI water by gently shaking for 30 s 3 times, followed by air drying with a stream of nitrogen. As a result, NHS ester-terminated alkanethiol SAM formed a biocompatible layer for PLL on gold electrodes.

Subsequently, chips were packaged on a PCB board. The electric contact was established by bonding the pads of the chip to pads on PCB board with a row of metallic clips. The clips were welded on PCB board for better electric contact. Then the perspex chamber with eight wells was bonded onto each chip to separate electrodes

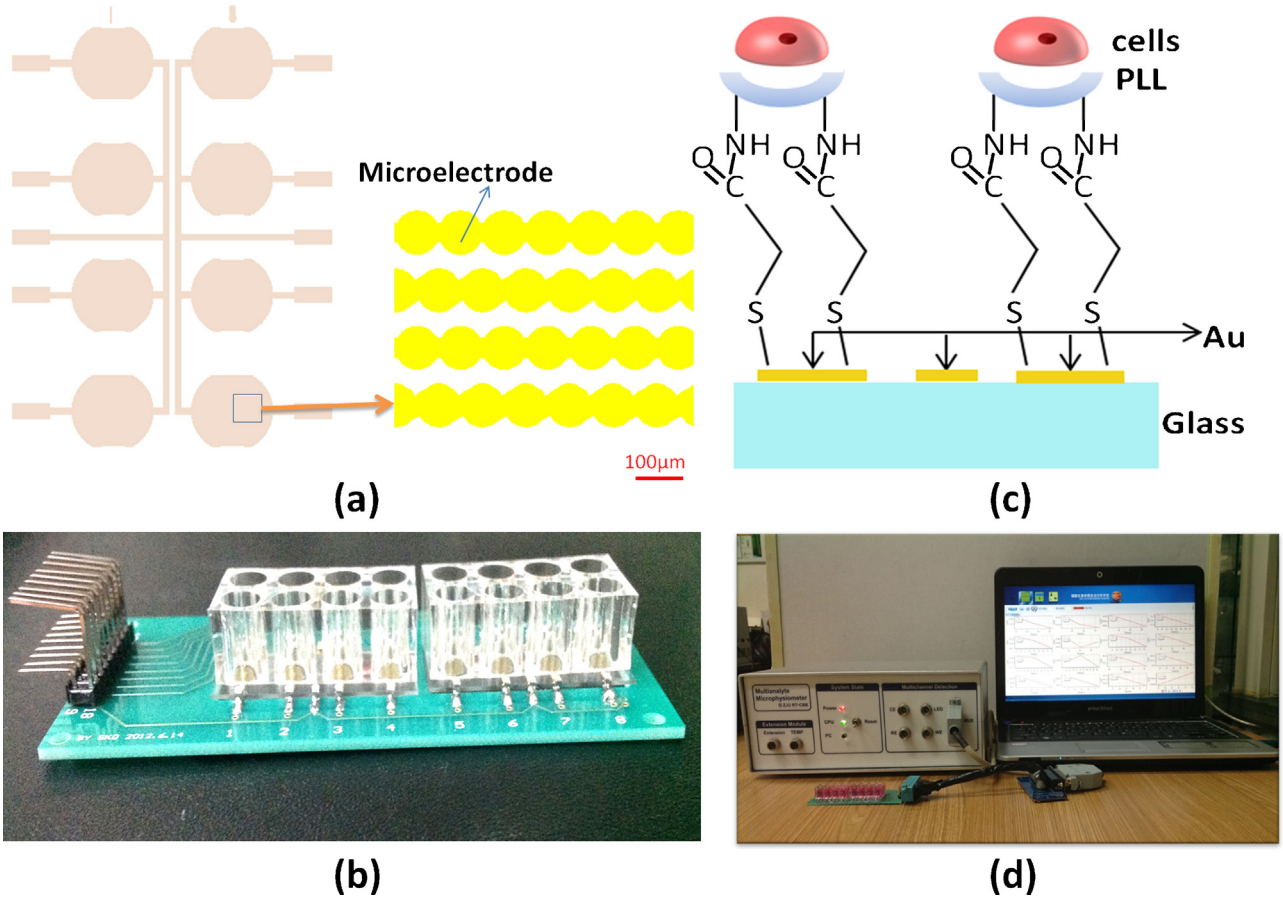

Fig. 1. (a) The layout of the sensing chip. It consists of eight pairs of round-shaped interdigitated electrodes. (b) The packaged device of the chip. Two chips were fixed onto one PCB board. (c) The schematic representation of modified chip's surface for guiding cell adhesion. (d) The picture of the ECIS system.