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Detection of glutathione within single mice hepatocytes using microfluidic chips coupled with a laser-induced fluorescence system

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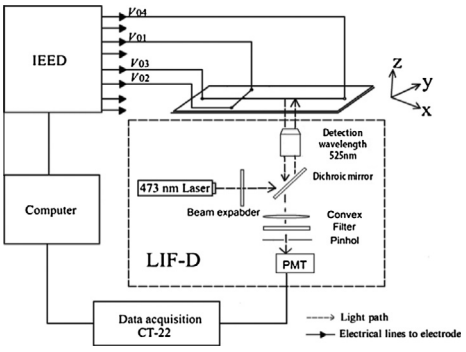
HIGHLIGHTS

- We established a rapid and accurate detection of GSH content in single cells.
- The derivatization of channel-surface proved to be necessary.
- Optimized voltage proved to be essential to the injection and separation of samples.
- The method is low-cost, time-saving, and eco-friendly.

GRAPHICAL ABSTRACT

The MCE-LIF system is composed of an intelligent electric driving instrument (IEED), a home-made cross microfluidic chip, a LIF detector (excitation wavelength/emission wavelengths: 473 nm/525 nm) and a data acquisition card. Based on the fluorescence quenching of fluorescinisothiocyate (FITC) by GSH, the internal pipeline of the microfluidic chips, the injection and separation condition were modified and optimized. Our study provided a basic and efficient method and technical support for the detection of GSH content in single hepatocytes.



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ABSTRACT

A rapid and accurate detection of glutathione (GSH) content in single cells is important to the early diagnosis and prevention of diseases. A microfluidic system allows the manipulation of trace amounts of reagents and single cells in a simple and cheap glass chip coupled with laser induced fluorescence (LIF) detection. 2,3-Naphthalenedicarboxaldehyde (NDA) was used as the derivatization reagent to label GSH in cells. Microchannel surface derivatization and optimization of injection and separation were investigated in detail, and then the GSH in single mice hepatocyte was separated and detected under optimum conditions with a linear range of 5×10^{-4} M $\sim 5 \times 10^{-3}$ M and a detection limit of 4.47×10^{-5} M. This study provides a simple and effective method for rapid GSH detection in single cells using few reagents.

Introduction

In recent years, the exploration of microfluidic system has drawn extensive interest. These systems, also known as miniaturized total analysis systems (μTAS) or Lab-On-a-Chip (LoC), have made great strides in the past two decades [1]. In comparison with

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conventional techniques, microfluidic systems have distinctive advantages, such as less sample and reagent consumption, shorter analysis time, high integration and automatization [2,3]. The integrated microfluidic systems can accomplish many biochemistry analyses, for example, sample preparation, separation, transportation, reaction and detection in a single chip [4–6].

Glutathione (GSH) is an intracellular thiol-containing tripeptide which is a normally used biomarker of oxidative stress (OS) and an important antioxidant in the cells of organisms [7,8]. The basic function of GSH in organisms is protecting cells from reactive oxygen species (ROS), including free radicals, hydrogen peroxide, and other peroxides [9,10]; furthermore, GSH is essential in the detoxification process in organisms. In consequence, variation of the content of GSH is related to a variety of diseases, including Parkinson's disease, diabetes, different kinds of cancers and senility [7,11–15].

Liver, which has the function of detoxification, is an important organ of most mammals. In conventional cell tests, the averaged data are usually obtained from a large number of cells, however, at the early stage of many diseases, lesions appear only in a small number of the cells, therefore the detection of intracellular constituents of single cells has vital importance to the early diagnosis and treatment of many diseases [16,17]. In view of that, in this study we established an effective and fast microfluidic-based method to detect the intracellular constituents in single cells. In order to provide the experimental basis, we firstly optimized the microchannel surface and the voltages of sample cell injection and separation. Afterwards, the mice hepatocytes were injected, driven, separated and detected in the optimized chips, and we obtained the electropherograms of GSH in the single cells.

Materials and methods

Materials

GSH (content > 98%) and D-Hanks solution were purchased from Sinopharm Chemical Reagent Co., Ltd. 2,3-Naphthalenedicarboxaldehyde (NDA) was obtained from Nippon Kasei Chemical Co., Ltd. Methanol, HCl, acetonitrile, acrylamide solution, ammonium persulfate solution, *N,N,N,N′*-tetramethylethylenediamine (TEMED), and methacryloxy propyl trimethoxyl silane (MAPTS) were purchased from Sigma. A stock solution of GSH was prepared at a concentration of 0.01 M in 25 mL 0.02 M Na_2EDTA solution. Phosphate buffered saline (PBS) was made of 0.155 M NaCl, 7.6×10^{-3} M Na_2HPO_4 and 2.4×10^{-3} M NaH_2PO_4 (pH 7.4). 20 M sodium tetraborate buffer (pH 9.2) was used as buffer solution, and 0.1 M NaOH solution was used to wash the microfluidic chip. All reagents used were of analytical reagent grade and purchased from standard reagent suppliers, and ultrapure water (18.25 MΩ cm) was used throughout.

A cross-linked microfluidic chip (Fig. 1) electrophoresis system together with laser induced fluorescence detection system (MCE-LIF system) was used in the experiment (Fig. 2). A pH-meter (type pHs-3C), an electronic balance (type of FA2004B), and an optical microscope were used in the experiment.

Experimental principle

NDA can react with GSH rapidly, and the derivative emits strong yellow fluorescence under alkaline conditions. The fluorescence of the derivative is related to the content of GSH and NDA (Scheme 1). We used excess NDA reacted with a concentration gradient of GSH to draw a standard curve in order to detect the GSH content in hepatocytes.

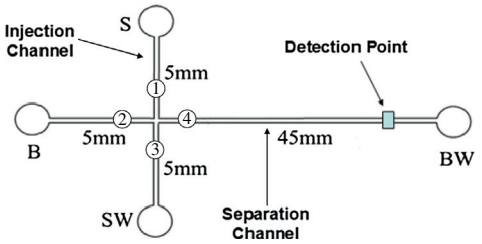

Fig. 1. Home-made cross microfluidic chip and the position of the detecting points. As the chip was not fastened to the LIF worktable, the detection points could be set at different positions, in order to optimize the voltages of the four electrodes. (S: Sample Reservoir; SW: Sample Waste Reservoir; B: Buffer Reservoir; BW: Buffer Waste Reservoir).

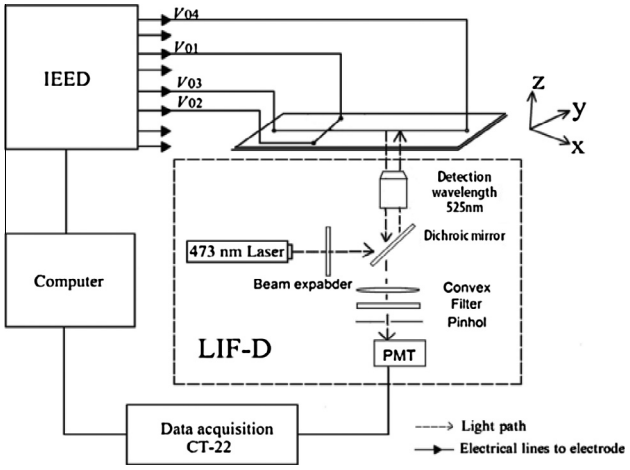

Fig. 2. The composition of the MCE-LIF system. The MCE-LIF system is composed of an intelligent eight-path electric driving instrument (IEED), a home-made cross microfluidic chip (Fig. 1), an LIF detector (excitation wavelength/emission wavelengths: 473 nm/525 nm) and a data acquisition card.

Sample treatment

The healthy male mice (3–6 months old, C57BL/6J) were obtained from School of Life Science of Shandong University. The mice were killed and dissected, the liver was taken out and the bloodiness was wiped off in a clean culture dish. Then the liver was washed by D-Hanks solution 2–3 times, and it was cut into pieces in some D-Hanks solution, and then the fragmented liver was filtrated by cell strainer. Secondly, the hepatocytes were washed by D-Hanks for 3 times and centrifuged for 5–10 min at 1000 rpm. At last, the hepatocytes were resuspended in 1.0 mL PBS and centrifuged again, and then the hepatocytes suspension was diluted to 1×10^5 cells mL^{-1}, afterwards, the hepatocytes suspension (500 μL) was mixed with 1×10^{-2} M NDA solution and reacted for 30 min.

Optimizing experimental conditions

Microchannel surface derivatization

Firstly, the microchannel was dried, and then rinsed with 0.1 M NaOH for 4 h. A mixed reagent of methanol/HCl (1:1) was passed through the channel for 15 min; then the liquid was drawn out from the microchannel and the chip placed in a vacuum drier (80 °C) for 20 min. Finally, we filled the microchannel with 4% MAPTS (20 μL MAPTS + 1 μL glacial acetic acid + 500 μL acetonitrile) and then let the chip soak at room temperature for two hours [18,19], so that the surface activity of the chip was restrained.