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RESEARCH PAPER

Assessing Plant Antioxidants by Cellular Antioxidant Activity Assay Based on Microfluidic Cell Chip with Arrayed Microchannels

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Abstract: An integrated microfluidic chip with arrayed micro channels consisted of eight repeat arrayed 6×6 cell culture chamber was designed and fabricated. The analytical microsystem combined with designed microchip, measuring device and environmental control unit was established for the cell culture and parallel cellular antioxidant activity (CAA) analysis of plant antioxidants. The microfluidic chip included a PDMS cover and a glass substrate which consisted of two hundreds and eighty-eight round cell culture micro chambers and forty-eight independent parallel array channels. Eight groups of different samples with six different concentrations could be investigated simultaneously with multimode reader in one test. HepG2 cells were successfully cultured on the microchip. Moreover, the viability percentage of the HepG2 cells exposed to these plant antioxidants solutions at different concentrations for 24 h was higher than 90%. With 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) as a fluorescence probe, 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP) as the initiator of intracellular reactive oxygen species (ROS), we tested the inhibitory effect of several plant antioxidants such as quercetin, rutin and kaempferol on free radicals. The CAA units were calculated by the data measured from cellular morphology and fluorescence intensity over time. It was shown that the CAA units of quercetin, rutin and kaempferol were (71.42 ± 0.19) μM, (74.31 ± 0.36) μM and (69.92 ± 0.09) μM ($x \pm s$, $n = 3$), while the calculated IC_{50} were (7.20 ± 0.06) μM, (52.06 ± 0.14) μM and 32.55±0.03 μM ($x \pm s$, $n = 3$), respectively.

Key Words: Microfluidic cell chip with arrayed microchannels; Cellular antioxidant activity; High-throughput and content screening

1 Introduction

In recent years, cell toxicity and efficacy evaluation has become the main outcome measure in the modern drug screening and development. In the study of the antioxidant activity analysis, Liu[1] established the cellular antioxidant activity (CAA) analysis method, and measured the inhibitory effect of antioxidants at cell level. Meanwhile, the activity of active substances in fruits and vegetables was determined. Subsequently, this method was used to analyze plant extracts by Karl[2] and Zhao[3]. However, these methods were still faced with the problem of low flux and complex testing process. Therefore, it was still a challenge to combine the CAA test method with high throughput and high content analysis technology for the fast and accurate, high automatic drug screening.

In order to evaluate the safety and efficacy of the compound in drug research process as soon as possible, a lot of techniques facilities and materials have appeared and been applied in this field. Compared with previous screening

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techniques, the striking microfluidic chip analysis showed some advantages, such as miniaturized sample pretreatment, chemical reaction, derivative, separation and detecting steps, shorter analytical time by the use of fluid and array multi passage, and a better cellular physiological and biochemical analysis of micro environment. Thus microfluidic chip became a potential drug testing platform [4].

In addition to the recombinant organ chip based on micro liquid drop of Frey[5] and the centrifugal cell chip of Espulgar[6], there were still many representative chips. Lin[7] designed a microfluidic chip, which was composed of eight symmetrical drug concentration gradient generator and cell culture area. Eight samples at eight different concentration levels could be selected simultaneously, and the hepatomicrosome was cultured on this chip. Integrated the chip electrophoresis with cell culture units, drug metabolites detection and toxicity evaluation could be performed simultaneously. Gao[8] designed a very simple chip based on concentration gradient generator and three-dimensional cell culture unit and established a drug sensitivity test platform. The concentration gradient relied on three micro channels with different lengths (5.7/7.5/12.5 mm) and three curved channel with different diameters (0.2/0.23/0.3 mm). With this chip, three samples at four different concentration levels were taken one time for lung cancer treatment. Therefore, the idea to combine the micro fluidic chip technology with the study of cell antioxidants was also instructive for the high throughput and high content test and screening of antioxidants.

In this study, an integrated microfluidic cell chip with eight repeat 6 × 6 cell culture micro chambers was proposed and designed. The cells could be simultaneously cultured and grew well on this chip, as well the cell cytotoxicity assay was achieved. By combining the micro fluidic cell chip with CAA assay method, a chip analytical platform of CAA assay was established. The chip was used for the cell cytotoxicity assay of plant antioxidants quercetin, rutin and kaempferol, and the results showed that the chip could evaluate the activity of candidate material in vitro effectively.

2 Experimental

2.1 Apparatus and materials

HH.CP-7W CO2 incubator was purchased from Shanghai Boxun Industry & Commerce Co., Ltd, China. IX-7 inverted Fluorescence microscopy was obtained from Olympus Corporation (Japan). Synergy™ HT microplate reader was purchased from Bio-Tek Co., Ltd. (USA). Flow injection pump was purchased from Harward Company (USA).

DMEM medium supplemented with 10% fetal bovine serum and PBS powder were purchased from HyClone Company (USA). Poly-L-Lysine, ABAP, DCFH-DA and Carboxyfluorescein diacetate succinimidyl ester (CFSE) were purchased from Sigma Company (USA). Compounds quercetin, rutin, and kaempferol (purity: 98%) was purchased from Chengdu Push Herbchem Co., Ltd. (China). Polydimethylsiloxane (PDMS) (Sylgard 184) was purchased from Dow Corning (Germany). Human hepatoma cell HepG2 used in the experiment was provided by the Digestive Department of Southwest Hospital, Chongqing, China. The cells were incubated for some days, during which time cells initiated logarithmic growth and nitial cell concentration is 10^6 cells per milliliter.

2.2 Design and fabrication of microfluidic cell chip

A cell chip composed of a 6 × 6 array of cell culture micro chambers was designed. As shown in Fig.1A, the design of microfluidic chip presented here was composed of two parts: a glass substrate with micro channel and a cover formed by PDMS. The grooved glass substrate was fabricated by means of photolithography and wet etching. Mask graphics was

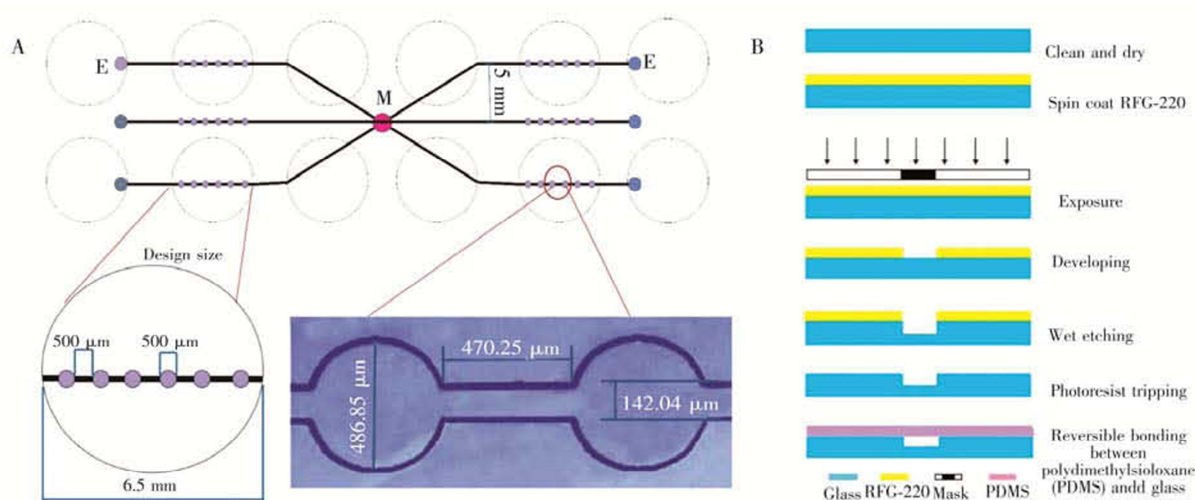

Fig.1 Design (A) and fabrication process (B) of the cell chip unit

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