Multichannel lens-free CMOS sensors for real-time monitoring of cell growth

Ko-Tung Chang

Department of Biological Science and Technology,
National Pingtung University of Science and Technology
Tel：08-7703202 ext 6362
E-mail：kotungc@mail.npust.edu.tw

Yao-Nan Wang

Department of Vehicle Engineering,
National Pingtung University of Science and Technology
Tel：08-7703202 ext 7456
E-mail：yanwang@mail.npust.edu.tw

1. Introduction

A low-cost platform is proposed for the growth and real-time monitoring of biological cells. The main components of the platform include a PMMA cell culturemicrochip and a multichannel lens-free CMOS (complementary metal-oxide-semiconductor) / LED imaging system. The PMMA microchip comprises a three-layer structure and is fabricated using a low-cost CO_2 laser ablation technique. The CMOS / LED monitoring system is controlled using a self-written LabVIEW program. The platform has overall dimensions of just 130 × 104 × 115 mm^3 and can therefore be placed within a commercial incubator. The feasibility of the proposed system is demonstrated using HepG2 cancer cell samples with concentrations of 5000, 10 000, 20 000, and 40 000 cells/mL. In addition, cell cytotoxicity tests are performed using 8, 16, and 32 mM cyclophosphamide. For all of the tests, the cell growth is observed over a period of 48 h. The cell growth rate is found to vary in the range of 44-52% under normal conditions and from 17.4-34.5% under cyclophosphamide-treated conditions. In general, the results confirm the long-term cell growth and real-time monitoring ability of the proposed system. Moreover, the magnification provided by the lens-free CMOS / LED observation system is around 40× that provided by a traditional microscope. Consequently, the proposed system has significant potential for long-term cell proliferation and cytotoxicity evaluation investigations.

2. Design Concept

The present design proposes a simple low-cost platform for the growth and real-time monitoring of biological cells comprising a CO_2 laser-ablated PMMA microchip and a CMOS / LED observation system. The feasibility of the proposed platform is demonstrated by performing cultivation tests using HepG2 cancer cells and cytotoxicity tests using cyclophosphamide. In both tests, the images acquired from the CMOS observation system are compared with those obtained using a conventional optics-based microscope. In general, this design confirms that the proposed platform provides a promising solution for various cell culture and real-time monitoring applications.

3. Technique Development

Cell culture microchip fabrication

Figure 1A presents a schematic illustration of the proposed 3D PMMA-based cell culture microchip. As shown in Fig.1B, the microchip comprises three PMMA substrates with thicknesses of 1.6, 3.2, and 6 mm, respectively, stacked on top of a glass plate (Deckglaser, Germany) with dimensions of 24 mm × 60 mm × 150 mm. The upper PMMA substrate (substrate #1 in Fig. 1B) contains a circular hole with a radius of 6 mm for installation of the cell culture medium tube and two rectangular holes at either end connected to the atmosphere in order to balance the pressure. The middle substrate (substrate #2) contains a circular mixing chamber with a radius of 5 mm. The radius of the mixing chamber is slightly smaller than that of the circular hole in the upper substrate. Hence, its perimeter serves as a support for the culture medium tube once installed in the microchip. The middle substrate is also patterned with two rectangular collection chambers with the same dimensions as the rectangular holes in substrate #1. Finally, the lower substrate (substrate #3) contains a circular medium chamber with a radius of 3 mm and a rectangular cell culture chamber with dimensions of 8 mm × 6 mm. The PMMA substrates were designed using commercial AutoCAD (2010) software and ablated using a defocused CO_2 laser beam system. (Note that a detailed description of the laser ablation system is available in a previous study by the present group1.) The PMMA substrates and glass microplate were carefully aligned and lightly secured in place using 3M double-sided adhesive tape (#9460, 3M, USA). A permanent bond was then achieved by means of a hot-press bonding technique, in which the substrates were maintained under a light contact pressure of 15 kg/cm^2 as the temperature was gradually increased to 60°C over a period of 5 min using a hot press plate (CV0–45L, Collaborative Enterprise,Taiwan). Following the bonding process, the pressure was released and the microchip was allowed to cool naturally to room temperature.

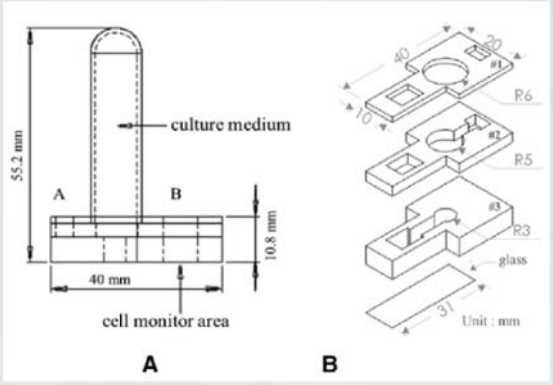

Figure 1. (A) Schematic illustration of 3D PMMA cell culture microchip (vertical direction). (B) Configuration of individual substrates in PMMA microchip.

Real-time lens-free CMOS monitoring system

Figure 2 presents a schematic illustration of the proposed lens-free CMOS-based real-time cell growth and monitoring platform. As shown, the monitoring system comprises four CMOS image sensors arranged in a rectangular array on a lower PMMA substrate and four LED white light sources (5 mm, 1 W, Ho Hua electronic component, Taiwan) mounted on an upper PMMA substrate immediately above the corresponding image sensors. The platform was constructed using low-cost commercially-available CMOS image sensors (Logitech, C525), each with a field of view of approximately 2.0 mm × 3.5 mm and a resolution of 2.1 million pixels. The platform has overall dimensions of just 130 × 104 × 115 mm^3 and can therefore be placed in a traditional cell incubator (e.g., MCO-18AC, Sanyo, Japan) for cell cultivation purposes. In performing the cell cultivation experiments, the LED light sources and CMOS sensors were automatically controlled using a self-written LabVIEW program in such a way as to avoid interference with the cell growth. Specifically, the light sources and sensors were briefly turned on at predetermined 30 min intervals during the cultivation process and were then turned off as soon as the image acquisition process was completed.

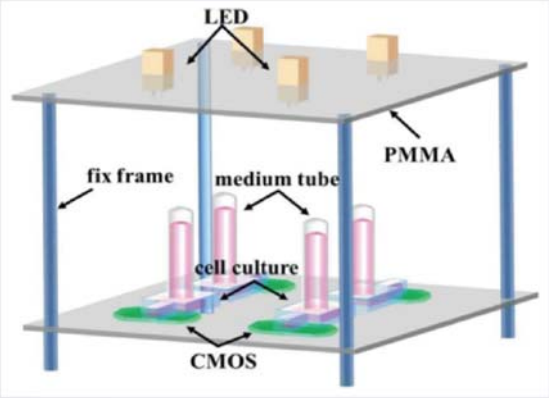

Figure 2. Schematic illustration of proposed cell growth and monitoring platform.

4.Technological Competitiveness

The proposed platform has several important advantages compared to traditional cell culture systems, including a low-cost, a small physical size, and a low weight. According to its performance, it provides an ideal solution for a wide range of cell proliferation and cytotoxicity tests in the biomedicine field.

5.R&D results

This project has presented a low-cost platform comprising a PMMA cell culture microchip and a CMOS-based imaging system for the cultivation and real-timemonitoring and analysis of biological cells. The feasibility of the proposed platform has been demonstrated by performing the long-term (48 h) cultivation of HepG2 cancer cell samples with initial concentrations of 5000, 10 000, 20 000, and 40 000 cells/mL. In addition, cytotoxicity tests have been performed by doping HepG2 samples with 8, 16, and 32 mM cyclophosphamide. The results have shown that for the original cell samples (i.e., no cyclophosphamide addition), the cell DT is relatively insensitive to the initial cell concentration. Moreover, the average DT is around 46 h. However, for the samples treated with cyclophosphamide, the cell growth rate is significantly reduced; leading to a notable increase in the DT. The effect of the cyclophosphamide in retarding the cell growth rate increases with an increasing dopant concentration. Thus, for the sample with a cyclophosphamide concentration of 32 mM, the average DT is around 160 h. It has been shown that the images obtained using the proposed lens-less CMOS real-time observation system are in good qualitative in agreement with those obtained using a conventional microscope under 40× magnification.

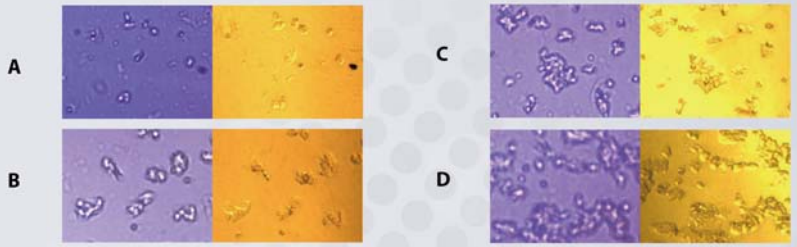

Figure 3. Comparison of images by CMOS-based imaging system (left) and traditional microscope with 40x magnification (right) following 48 h cultivation period given initial HepG2 sample concentration of : (A) 5000 cells/mL, (B) 10,000 cells/mL, (C) 20,000 cells/mL, (D) 40,000 cells/mL,

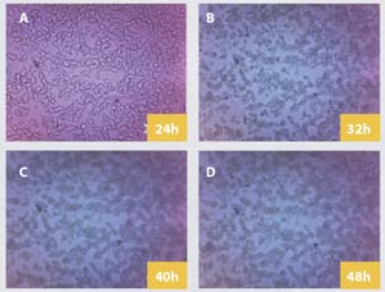

Figure 4. CMOS-based images of HepG2 cell growth in cyclophosphamide-treated condition (32 mM): (A) 24 h, (B) 32 h, (C) 40 h, (D) 48h.

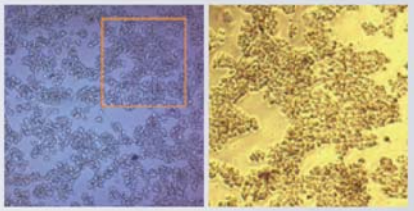

Figure 5. Comparision of images acquired by CMOS-based imaging system (left) and traditional microscope with 40x magnification (right) following 24 h cultivation period given by cyclophosphamide (32 mM).

Acknowledges

The financial support provided to this study by the Ministry of Science and Technology of Taiwan under Grant Nos. NSC 102- 2320-B-020-001 and NSC 99-2314-B-020-001-MY3 is gratefully acknowledged.

Reference

1. Hong, T. F., Ju, W. J., Wu, M. C., Tai, C. H., Tsai, C. H., Fu, L. M., *Microfluid. Nanofluidics* 2010, *9*, 1125–1133.
2. Ko-Tung Chang; Yu-Jen Chang; Chia-Ling Chen, Yao-Nan Wang (2015, Feb).Multichannel lens-free CMOS sensors for real-time monitoring of cell growth. *Electrophoresis*, 36(3):413-9.