High-throughput microwell plate for cell research

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INTRODUCTION

The capabilities of the micro-platform have been successfully augmented to reveal the underlying biological mechanisms masked by traditional laboratory systems. Microbiological changes such as the membrane potential, ion channel opening or closing, cell migration and proliferation, and cell to cell communication can now be studied in a microenvironment resembling the in vivo condition [1-4]. Quantitative analyses of nucleic acids and proteins in single cells [5, 6] and subcellular localization of biological action are now possible using the technologies such as sensors and micro-electromechanical systems (MEMS) [7-9]. But the cost of commercial chips and technical difficulties in designing and manufacturing cell culture chips in laboratories make these exciting technological advances unattainable for large scale applications.

We introduce a simple microwell plate for easy cell culture on chips without requiring the use of tubes and pump. The plate is originally designed for culturing mammalian cells but can be adapted for other culture systems.

METHODS AND RESULTS

Microwell plate design

The microwell plate consists of two levels: the bottom PDMS plate with a thickness of 1 mm, and a PMMA structure with a thickness of 2 mm (Fig.1). The outer format of the plate is 20 mm × 20 mm, with a thickness of about 3 mm. The whole plate has 4 equal divisions, each containing 4 groups of 6×6 microwells on the bottom PDMS layer (144 microwells in total). The microwell plate can be put in cell culture dish directly to enhance the convenience in cell culture and observation.

Microwell plate fabrication

The master mold for the microwell plate was...
created using standard photolithography techniques in an SU-8 negative photoresist on a bare silicon wafer (manufactured by Wenhao Chip Technology Co. Ltd, China). PDMS prepolymer (Sylgard 184, Dow Corning) was mixed with a curing reagent at a 10:1 mass ratio. The resulting mixture was poured into the mold, and degassed in a vacuum chamber for 30 min at room temperature to remove air bubbles. The PDMS was cured by heating at 60 °C for 2 h in an oven and then carefully detached from the master mold. The top PMMA structure was a laser-cut PMMA board (manufactured by Wenhao Chip Technology Co. Ltd, China). The PDMS structure and PMMA structure were fabricated with the mixed Sylgard 184 and heated for 2 h at 60 °C. PDMS consists of repeating (-OSi(CH3)3-) units with a hydrophobic surface suited for chemical research applications due to its relatively inert nature and low surface energy, but it is necessary to render the surface of PDMS hydrophilic for cell culture to improve cell adhesion. Ultraviolet (UV)-mediated polymerization was used for modifying PDMS surfaces to render the PDMS hydrophilic by attacking the siloxane backbone of the PDMS and forming an oxygen-rich SiOx silica-like layer and Si-OH surface structures (10, 11). The microwell plate was exposed to a 250 W ultraviolet lamp at a distance of 20 cm for 3 h (12, 13). The microwell surface was then coated with poly-L-lysine (0.01 %, Sigma-Aldrich) for 60 min in an incubator and washed 3 times with phosphate buffer saline (PBS). The microwell plate can also be coated with collagen and fibronectin (14, 15). The problem of bubbles in the microwells during coating and washing, due to the liquid surface tension, can be resolved with degassing (Fig.2).

**Cell culture on microwell plate**

The PC-12 cell line, derived from transplantable rat pheochromocytoma, was obtained from the Mammalian Culture Collection in Jinan University, China. PC-12 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, USA) supplemented with 5% (V/V if not specified otherwise) fetal bovine serum (FBS), 10% horse serum (HS), 5% penicillin (100 mg/ml), and 5% streptomycin (100 mg/ml) in T25 flasks until 70%-80% confluence, and then trypsinized and centrifuged. The cells were resuspended at the density of 1×10⁶ cells/ml in DMEM, seeded in the microwell plate at 2-5 cells per well (Fig.3a), and incubated at 37 °C in 5% CO₂ in an incubator, with the media changed every 24 h. The cell attachment and adherence was observed 12 h later (Fig.3b). After 72 h of incubation, the medium was replaced with a medium supplemented with cobalt chloride (COCl₂, 400 μmol/L) to establish a model of chemical hypoxia-induced injury, and cell apoptosis was observed 24 and 48 h later (Fig.4).

**DISCUSSION**

The advances in high-throughput cell culture chips and other micro-devices greatly facilitated drug discovery and cell culture research (16-18). But most microfluidic system chips require the use of macro-scale tubing and interconnect to cause unwanted dead volumes and increased reagent and sample consumption (19). Another problem associated with the interconnects and tubes is the presence of bubbles, especially for those made by PDMS, which has a hydrophobic surface to disturb local fluid flow and potentially cell growth (20). Local fluid flow may remove autocrine and paracrine signals released from cells (21), and can affect cell adhesion, proliferation, migration, and differentiation (22). In addition, mammalian cells are especially sensitive to small changes in their microenvironment including pH, osmolarity and other extracellular factors, which are unstable in microfluidic systems.
The microwell plate we designed was successfully tested for use in culturing PC-12 cells. These plates can be useful in all types of cell culture studies including high-resolution microscopy and automated imaging. This microdevice is easy to manufacture and can be used in routine laboratory settings without the requirement of inconvenient pipes or additional pumps. The microwell plate can be directly put into an incubator to allow the maintenance of favorable cell growth environment with stable temperature, humidity, air pressure, oxygen and CO₂ concentration. The microwell plate can be also useful for researches with rare cell samples or expensive reagents.

REFERENCES