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RESEARCH PAPER

Construction of Tumor Tissue Array on An Open- Access Microfluidic Chip

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Abstract: An open-access microfluidic chip which enabled automatic cell distribution and complex multi-step operations was developed. The microfluidic chip featured a key structure in which a nanoporous membrane was sandwiched by a cell culture chamber array layer and a corresponding media reservoir array layer. The microfluidic approach took advantage of the characteristics of nanoporous membrane. On one side, this membrane permitted the flow of air but not liquid, thus acting as a flow-stop valve to enable automatic cell distribution. On the other side, it allowed diffusion-based media exchange and thus, mimicked the endothelial layer. In synergy with a liquid transferring platform, the open-access microfluidic system enabled complex multi-step operations involving medium exchange, drug treatment, and cell viability testing. By using this microfluidic protocol, a 10 × 10 tissue arrays was constructed in 90 s, followed by schedule-dependent drug testing. Morphological and immunohistochemical assays results indicated that the resultant tumor tissue was faithful to that *in vivo*. Drug testing assays showed that the microfluidic tissue array promised multi-step cell assays under biomimetic microenvironment, thus providing an advantageous tool for cell research.

Key Words: Open-access microfluidic chip; Nanoporous membrane; Microenvironment; Tissue microarray; Anti-cancer drug testing

1 Introduction

Microfluidic chip has been accepted as a powerful tool for cell biology research[1-5]. Besides the common advantages of microfluidics, including high throughput, lower reagent consumptions and potential for integration, the most influential benefit of using microfluidics for cell assays is the ability to reconstitute cell microenvironment at microscale[6]. On one hand, microfluidic chip facilitates the transition from 2D to 3D cell culture, which is an important step in a trend towards better biomimetic tissue models[7–10]. On the other hand, microfluidic technology allows precise control over fluids in micrometer-sized channels, thus becoming a valuable tool to mimic the vascular vessel. The combination of 3D cell culture with microfluidic networks offers a great potential selection for in vivo-like tissue-based applications, such as the emerging organ-on-a-chip system.

The majority of microfluidic cell culture systems employ perfusion systems[11–14]. The perfusion of fluid within microchannels with comparable size to the capillary system mimics the fluidic behavior in vivo[15,16]. Although previously introduced perfusion microfluidics is very successful, there are still several challenges in culturing cells under flow conditions. First, it is difficult to precisely control and measure pH, oxygen, and nutrition levels at specific locations inside the microchannel in real time, which often requires careful validation experiments and feeding-schedule calibrations for every device. Secondly, the continuous perfusion cell culture systems often involve high reagent consumption and dead volume. Thirdly, the majority of

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perfusion systems did not offer flexibility to conduct complex multistep cell assays[17–20].

To solve the limitations mentioned above, open-access chip design for cell culture has been proposed[21–23]. In this design, the cells are cultured in open-access reservoirs, in which different reagents and stimulus can be added simultaneously or sequentially, to provide a stable microenvironment. Compared with the enclosed format microfluidic chip, the open-access microfluidic system enables complex multi-step operations[24–26]. Nevertheless, as these chips are free of microfluidic networks, cell array formation through cell distribution is difficult. Therefore, the analysis throughput of open-access microfluidic chips is usually limited. To solve these issues, Zhu *et al*[27] developed a semi-open microfluidic platform featuring an automated pipetting platform, in which a tapered capillary was used for droplet generation and liquid handling. This design makes the system capable of generating high-density cell arrays and programmed operations involving medium exchange, drug treatment, and cell viability testing[28].

Inspired by the concept of open-access microfluidics, the current work reports an open-access microfluidic chip for tissue array construction and conduct complex multistep cell assays. The microfluidic chip contains a nanoporous membrane for separation of the top layer with media reservoirs from the bottom layer with cell culture chamber arrays. The nanoporous membrane sandwiched between the top layer and the bottom layer permits the flow of air but not the passage of liquid, thus endowing the microchip with distinctive features: the nanoporous membrane acts as a flow-stop valve enabling automatic cell distribution; with a pore size similar to endothelial gaps, the membrane serves as a diffusion barrier that mimics the endothelial layer and thus, it is helpful to reconstitute a biomimetic microenvironment. The developed microfluidic protocol features several advantages. The first one is ease of tissue-array formation through automatic liquid distribution; Secondly, the reconstituted tumor tissue permitted the drug-testing assays to be performed under a biomimetic environment; Thirdly, it offfered feasibility and convenience of complex multistep liquid operations. In this study, breast cancer cells tissue microarray was constructed, and its construction of biomimetic tumor tissue was evaluated. The results of anti-tumor drug test showed that cell experiments involving complex operation procedures were allowed to be carried out under simulated microenvironment. The open-access microfluidic array chip can be a useful tool for cell research.

2 Experimental

2.1 Instruments and reagents

IX71 inverted fluorescence microscope (Olympus, Japan), IX81 laser confocal microscope (Olympus, Japan), and T2-AS yringe pumps (Longer Pump, China) were used in this experiment.

Polydimethylsiloxane (PDMS) was purchased from Dow Corning (USA). Polycarbonate membrane (200 nm) was obtained from Whatman (UK). SU-8 3035 Photoresist and developer was obtained from MicroChem (USA). PBS, DMEM, 1640, 0.25% Trypsin-EDTA, 4',6-diamidino-2-phenylindole (DAPI), Phalloidin, Calcein AM/EthD-1 were purchased from Thermo Fisher (USA). Anti-vinculin monoclonal antibody was obtained from Pierce (USA). Anti E-cadherin monoclonal antibody was obtained from BD (USA). Anti β-catenin monoclonal antibody was obtained from ABCAM (UK). FITC-conjugated second antibody and Cy3-conjugated second antibody were obtained from Boster (China). Fetal calf serum (FCS) was purchased from Sigma (USA). Doxorubicin hydrochloride and paclitaxel was urchased from Wanle (China). Sodium alginate, Triton X-100 and paraformaldehyde were purchased from Sangon (China). All reagents are analytical reagent. The 4-inch silicon slice was obtained from Lijing (China). MCF-7 breast cancer cell lines were provided by the oncology center of Sun Yat-Sen University, China.

2.2 Structure and fabrication of microfluidic chip

The open-access microchip contained three layers (Fig.1A): a bottom PDMS layer with 10 × 10 via-hole arrays fabricated using the standard soft lithography protocol[29], a top PDMS layer with tandem microchamber arrays aligned to the via-hole arrays, and a middle layer of nanoporous polycarbonate (PC, 100 nm pore size, Whatman, UK). The PC membrane was bonded to the PDMS layers following a protocol reported by Aran *et al*[30].

2.3 Cell culture

Human breast cancer cells (MCF-7) were cultured in DMEM medium and 10% FCS. After growing to 70%–80% confluence in cell-culture flasks, the cells were harvested and digested for 3 min using 0.25% trypsin-EDTA, followed by addition of fresh DMEM culture medium to terminate digestion, and centrifugation for 3 min at 1000 rpm. The cell pellet was resuspended in DMEM culture containing 2% sodium alginate.

2.4 Cell seeding and culture in microfluidic chip

The chip and polytetrafluoroethylene capillary were baked at 80 ℃ before use. The microsyringe was soaked in 75% ethanol for 6 h, and rinsed with sterilized distilled water. The chip, capillary and syringe were exposed to UV light. All the operations were performed on the clean bench.