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RESEARCH PAPER

Construction of Tumor Tissue Microarray on a Microfluidic Chip

WANG Wei-Xin[1], LIU Wei-Ping[1], WU Bin[1,2], LIANG Guang-Tie[1,2], LIU Da-Yu[1,2,*]

[1] Department of Laboratory Medicine, Guangzhou First People's Hospital, Affiliated Hospital of Guangzhou Medical University, Guangzhou 510180, China

[2] Clinical Molecular Medicine and Molecular Diagnosis Key Laboratory of Guangdong Province, Guangzhou 510180, China

Abstract: A microfluidic approach for tumor tissue microarray construction was developed in this work. The 64 cell arrays were prepared by integrating cell introduction, 3D hydrogel scaffold formation and perfusion culture on the microfluidic chip. By using breast cancer MCF-7 cell as a model, cell viabilities, densities, proliferation rates and intracellular pH values were monitored throughout cell culture, and immunohistochemical staining was performed using frozen tissue sections. Breast cancer cells cultured on chip aggregated into tumor spheroids, showing a tissue structure similar to glandular tube. E-cadherin and Vinculin staining indicated the formation of cell-cell and cell-mesenchyme connections. Cancer cells kept proliferating within 15 days after seeding, and the cell viability and cell proliferation rate detected on the 15th day were 85% and 10%, respectively. Intracellular pH assay showed acidification in the cells, which was ascribed to the accumulation of acidic metabolic products caused by shortage of oxygen inside the tumor tissue. The developed microfluidic approach for tumor tissue microarray construction is simple and efficient for tumor research.

Key Words: Microfluidic chip; Tumor tissue microarray; Microenvironment; Three-dimensional cell culture

1 Introduction

Tumor tissue microarray consists of separate tumor tissue cores assembled in array fashion to allow multiplex assays, such as gene expression analysis, antibodies/probes optimization, tissue bank construction, drug screening and disease diagnosis[1]. Tissue microarrays link the analyses of genetic structure, gene expressions and histomorphology, thus provide an advantage tool for cancer research. Despite its advantages, conventional tumor tissue microarray techniques also associates with limitations, including: (1) source limitation in resected tumor tissue, (2) bias in experimental results due to tumor heterogeneity, (3) inconvenience in tissue microarray preparation and application, and (4) infeasibility of performing live cell analysis. These limitations mentioned above hinder the expansion of tumor tissue microarray applications, especially in high throughput drug screening.

Microfluidic chip offers ideal platforms for cell manipulation and analysis due to its advantages in flexible design and scalable integration. A series of research works reported tumor tissue microarray construction on microfluidic chips[2–8], showing the advantages including high throughput, low consumption and automatic operation. The early generation of microfluidic tissue microarray majorly relied on 2D cell cultures, showing great discrepancies with the in-vivo tissues with regard to cell morphology, structure and function. To overcome this issue, 3D cell cultures were implemented on microfluidic chip for constructing tissue microarrays[9–14]. In comparison with their 2D counterparts, microfluidic tissue microarrays using 3D cell cultures are readily for mass production, and suitable for high throughput pharmacology or toxin toxicity experiment due to their biomimetic tissue complexity and homogeneity.

Sodium alginate was widely used in microfluidic cell

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* Corresponding author. Email: dyliu@foxmail.com
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approaches to serve as the scaffold for 3D cell cultures. As an example, Yu *et al*[12] reported a microfluidic system for 3D cultures of tumor cells in alginate beads. Sodium alginate droplets with suspended breast tumor cells were firstly dispersed in oil and then released into calcium bath for gelation, and the resultant alginate beads were trapped in microsiever arrays for continuous perfusion culture. The alginate environment permitted cell proliferation and the formation of multicellular spheroids, which mimicked the complexity of in vivo tumor microenvironment. The dose-dependent response of the tumor spheroids to anticancer drug, showed multicellular resistance compared to conventional monolayer culture. This system allows convenient tumor tissue microarray construction thus hold the potential for eventual high-throughput drug screening. However, the presented microfluidic approach is somehow inconvenient as it involves two chips and two-phase fluid handling.

In this study, a microfluidic approach was developed for tumor tissue microarray construction by using a multilayer chip with embedded porous film. By serially fluid introduction, cell seeding, alginate hydrogel formation, cell culture and cell imaging were implemented on the same chip. For the on-chip cell culture, a porous polycarbonate (PC) membrane was used to mimic the endothelium layer that linked tumor tissue array with perfusion flow: the hydrogel supported tumor spheroids on the membrane mimicked tumor parenchyma, the perfusion microchannels beneath the membrane mimicked the capillary network, and the alginate hydrogel mimicked the tumor stroma to support and nourishe the cancer cells. The in-vivo mass exchange and transport were also mimicked by the combination of active perfusion through the microchannel and passive diffusion in the hydrogel.By using MCF-7 breast cancer cell line as a model, the microfluidic tumor tissue microarrays were constructed and tested.

2 Experimental

2.1 Instruments, reagents and materials

Inverted confocal fluorescence microscope (IX81) and AU2700 biochemical analyzer (Olympus, Japan), flow cytometry (GUAVA easyCyte HT, Merck Millipore), 4-channel syringe pump (TS-2A, Longer Pump, China) were used in the experiments.

Polycarbonate porous membrane (pore size 0.2 μm) and grade 114 filter paper were purchased from Whatman, UK. Polydimethylsiloxane (PDMS) precursor and initiator were from Dow Corning, USA. SU-8 3025 photoresist was from Microchem, USA. DMEM cell culture media and trypsin solution were from Thermo Fisher, USA. Low melting point agarose was from Takara, Japan.

The materials used in the work were as follows: Anti-vinculin (Pierce，USA), anti-E-cadherin (BD, USA); FITC and Cy3 conjugated secondary antibodies (Boster, China); DAPI and 2,7-Bis(2-Carboxyethyl)-5,6-Carboxyfluorescein (BCECF-AM) (Life technologies, USA); Propidium iodide (PI, Sangon Biotech, China), Calcein-AM (Dojindo, Japan). All the fluorescent dyes were prepared in PBS. All the reagents were of analytical grade, and the solutions were prepared in sterile double distilled water.

2.2 Chip fabrication

The microfluidic chip was fabricated according to the standard soft lithography protocol[15]. SU-8 photoresist was firstly coated on a glass wafer, and then the cover film was put on the wafer. The SU-8 mould was obtained after UV exposure and development, which was used to fabricate the PDMS substrate by replica molding PDMS on the glass templates. As shown in Fig.1, the microchip contained three layers of PDMS and one layer of PC membrane. The top PDMS layer contained 8 parallel cell introducing channels, crossing over the 8 × 8 microhole array in the second PDMS layer. The third layer was a porous PC membrane, and the bottom layer contained parallel perfusion micochannels. The chip was bonded in a clean room using a plasma assisted method. Firstly, the PDMS layers and the PC membrane were treated with O_2 plasma. The PC membrane was further soaked in 5% APTES/ethanol solution for 1 min[16], and then sandwiched between the second and the bottom PDMS layer. After applying a pressure for 5 min, these three layers were bonded together. The bonding of PC membrane and the microhole array layer formed cell culture well array, with a volume of 3 μL in each well. Finally, the top PDMS layer was bonded to the second layer to form an entire chip.

2.3 Cell culture

MCF-7 cells were cultured in flasks with DMEM medium and harvested in the logarithmic growth phase. After trypsin treatment, the cells were washed twice with PBS and then

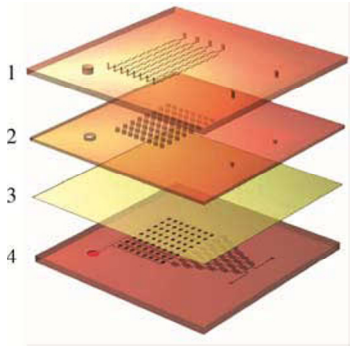

Fig.1 Diagram of microfluidic chip for tumor tissue microarray construction

The microchip was assembled by 4 layers, containing 8 cell introducing channels, 8 × 8 microhole arrays, porous PC membrane and perfusion channel networks, respectively