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Microvalve controlled multi-functional microfluidic chip for divisional cell co-culture

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ABSTRACT

Pneumatic micro-valve controlled microfluidic chip provides precise fluidic control for cell manipulation. In this paper, a multi-functional microfluidic chip was designed for three separate experiments: 1. Different cell lines were dispensed and cultured; 2. Three transfected SH-SY5Y cells were introduced and treated with methyl-phenyl-pyridinium (MPP^+) as drug delivery mode; 3. Specific protection and interaction were observed among cell co-culture after nerve damage. The outcomes revealed the potential and practicability of our entire multi-functional pneumatic chip system on different cell biology applications.

Introduction

Cell culture and manipulation act are the key elements for understanding basic biological mechanisms. In fact, multifarious technologies have emerged to provide better solutions for commercial cell culture demands [1,2]. Among these solutions, microfluidics technology has gained rapid development [3–6] for its advantages of reducing reagents consumption, providing customized microenvironment, improving spatiotemporal control and increasing portability [7,8]. Consequently, this miniature, integrated and high-throughput platform provide abundant opportunities for biological research [9–13]. The Polydimethylsiloxane (PDMS) chips that incorporate with pneumatic microvalves, have been well developed owing to the high elasticity of the material [14–16]. Hojin Kim et al. [17] combined a microfluidic-based dynamic microbead array system with pneumatically driven elastomeric valves to perform single-microbead arraying and rapid microarray reset. Pneumatic microvalves also provide great opportunities for cell manipulations on a chip. For example, cell mechanical function measurement [18] and metabolism [19] can be processed with the help of pneumatic microvalve array and its external controllers. However, most microfluidic chips fabricated in the laboratory are only designed for a unique rigid application, while commercial and pharmaceutical companies prefer to achieve more complex multi-functional tasks.

Here, we present a multi-functional microfluidic chip for parallel and independent cells culture/co-culture studies. The properties of the pneumatic microvalves were assessed by a series of gas driven test. The chip has been tested for three independent experiments. For further perspective, it can offer opportunities for different cell related studies.

Methods

Fabrication of microfluidic chip

Microfluidic chips were fabricated based on standard soft lithography technology [20,21]. Photolithography was drawn by Adobe Illustrator (AI) software and then printed on a plastic mask. The pneumatic control layer mold was made by negative photoresist (SU8-2050, Newton), then exposed and developed to form a 50 μm height rectangle section mold. The fluid layer mold was made by positive photoresist (AZ-50XT, AZ Electronic Materials) using the same fabrication steps as that of SU8-2050 with an additional reflow technique [22] to form an arc shape section mold. Mixed PDMS (Sylgard 184, Dow Corning) with a weight ratio of 10:1, 20:1, 5:1 (PDMS to curing agent) was poured and cured as the bottom layer, control layer, and fluid layer. Finally, the bonded chip was sealed by oxygen plasma treatment and post-bake at 80 °C for 6 h.

Decoration of the gas control system

A nitrogen cylinder with pressure regulator was utilized for the driving gas and connected with a self-made gas control system. The system was assembled by five main parts: 1) a 12 V/6 A DC power module (JW12-6-S, Zhong Tai Jing Wei), 2) a precision regulator (IR1000-01, SMC Corporation), 3) four electromagnetic valves and switches (3V1-06E, AirTAC), 4) gas tubes and wires, 5) the plastic case. When the gas was delivered to the control device, a precise pressure reducing valve was used to decrease the pressure to an adjustable range

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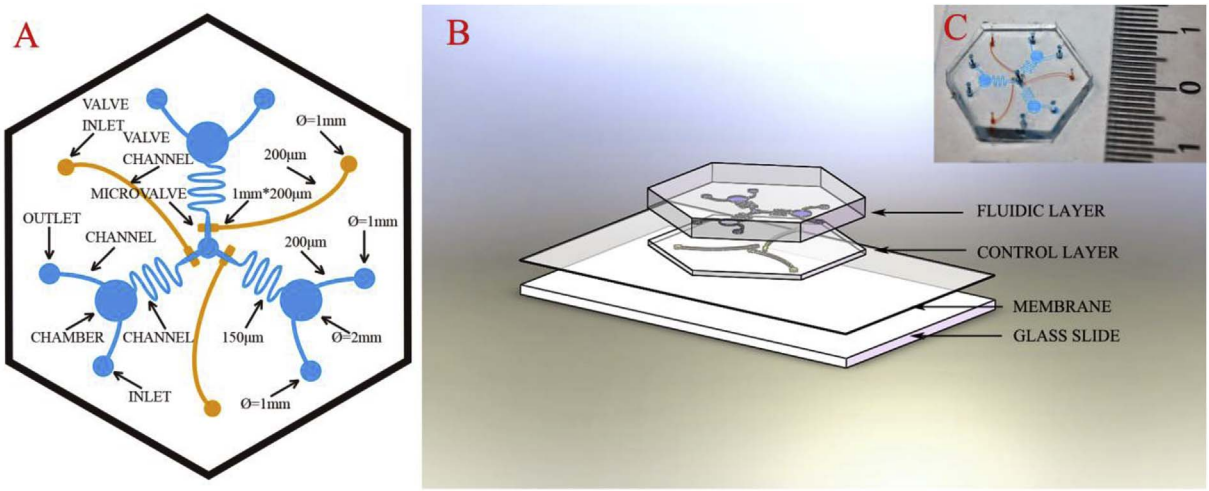

Fig. 1. Configuration of the microfluidic chip: A. Schematic representation of the size and function of different parts of the chip B.3D hierarchical structure of the microfluidic chip. From top to bottom are the fluidic layer, control layer, thin PDMS membrane and the glass slide. C. Photograph of the microfluidic chip. The channels of fluidic layer and control layer were loaded with blue and orange dye separately for better distinguishing of the two layers.

(with 0.005–0.2 MPa as control range and 0–1 MPa as display range). Then four electromagnetic valves, which were controlled by their corresponding electronic switches, were connected to the pressure reducing valve with a cross pipe and controlled by electronic switches, respectively.

Cell culture

Human neuroblastoma cell line SH-SY5Y, uterine cervical cancer cell line Hela, colon carcinoma cell line HCT 116 and human glioblastoma multiform cell line U87 were obtained from Beijing Institute of technology. Stable expression EGFP-N2, EGFP-synWT and EGFP-synA30P nerve tumor SH-SY5Y cell line (SH-N_2, SH-α, and SH-P) were provided by laboratory colleague, whose former work includes: corresponding plasmids construction, transfection, and selection of stably expressed cell line. These cells were cultured with Dulbecco's Modified Eagle's Medium (DMEM, Gibco®) supplemented with 10% fetal bovine serum (FBS, Gibco®), and 1% penicillin and streptomycin (Gibco®). Besides, an additional 1% Essential Amino Acids Solution (NEAA, Gibco®) was also added in the medium of U87. All the cells were incubated in a 37 ˚C, 5% CO2 humid incubator. Before the microfluidic experiments, cells were detached with 0.25% trypsin-EDTA (Life Technology) and centrifuged at 1000 rpm for 5 min, then resuspended and prepared for seeding.

Cell seeding and drug stimulation in chip

The chips were pretreated by 1 M HCl (more explanations on Fig. S) and washed with phosphate buffered saline (PBS), then treated by 400 μg/ml fibronectin (Fn, Sorlarbio) to enhance cells adherence. The gas controlling system was assembled with the chip and adjusted to 0.06 MPa pressure. Therefore, each chamber was isolated for parallel operating. Three parallel experiments were carried out: 1. SH-SY5Y, HCT116 and Hela cells were dispensed into the corresponding chamber at a density of 5×10^5 cell/ml separately. Then the chip was put into a 37 ˚C, 5% CO2 incubator. Cells' behaviors were observed timely using inverted fluorescence microscope (OLYMPUS-IX71, OLYMPUS). 2. SH-N_2, SH-α, and SH-P cells were dispensed into their corresponding chamber at a density of 10^6 cell/ml separately and then put into an incubator for 24 h with sufficient culture medium. 600 μg/ml MPP^+ (Sigma) were subsequently continuous delivered from the central inlet to the chip with a speed of 60 μL/h. After 24 h treatment, cells were observed under the inverted fluorescence microscope. 3. SH-SY5Y cells (5×10^5 cell/ml) were mixed with the same volume of SH-SY5Y, U87, and Hela (5×10^5 cell/ml) separately and introduced into the separated chambers and incubated for 24 h. Then, 500 μg/ml MPP^+ were continuously delivered from the central inlet at 60 μL/h for 48 h. Cells were finally observed and counted.

Results and discussions

Multifunctional chip design

As illustrated in Fig. 1A, we labeled the chip's detail designation and measure. The microchip has three PDMS layers and a glass slide substrate (Fig. 1B). The fluid layer contains three separate parts that sharing one common inlet in the middle to ensure fluid exchange, three parallel independent round-shape chambers (with a diameter of 2 mm) with inlet and outlet channels (with a width of 200 μm) are designed for cell culture. The winding channels between the shared inlet and chambers act as a buffer zone to avoid cells flowing out from corresponding chambers. Fig. 1C shows the photography of the microfluidic chip.

The advantage of the chip design is to provide a new pattern for multiple cell operations. Fluid or samples can be introduced from either center or side inlet by controlling the microvalves. For common treatments, reagents can be introduced through the center inlet. Meanwhile, each chamber can also be handled independently. The microfluidic chip provides three operational areas, seven inlets/outlets, which can be transformed into many combinations that enable the possibility for more complex applications.

Valve control and multiple pathway manipulations

Pneumatic microvalves were controlled by a self-made device. Fig. 2A, B, C, D gave the typical microvalve photograph under 0 MPa, 0.02 MPa, 0.04 MPa and 0.06 MPa. The red dotted boxes indicated the sealing area. The fitting relationship between pressure and sealing area data acquired from four parallel experiments obeyed the trigonometric functions (Fig. 2E $F(x) = 1.191e^5 \times \sin(18.36\ x - 0.02075)$ Goodness of fit: Sum of Squared Residuals (SSE): 7.508e+007 Root Mean Square Error (RMSE): 2740).

The pneumatic valves can block the channels at approximately 0.06 MPa and the switching can be done in 1s. The sealing area will not increase obviously beyond 0.06 MPa. Therefore, the blocking pressure was maintained at 0.06 MPa during the experiments.