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24-well transwell format-based microfluidic platform and tilting tower system for parallel interconnection and cultivation

Chaewon Jin[1#], Yeo Jin Hwang[1#], Hongsoo Choi[2,3], Kyeong-Min Lee[1*] and Jin-Young Kim[1,3*]
[1] Division of Biomedical Technology, DGIST, Republic of Korea
[2] Department of Robotics and Mechatronics Engineering, DGIST, Republic of Korea
[3] DGIST-ETH Microrobotic Research Center, DGIST, Republic of Korea

Summary. This paper presents the simple and robust 24-well transwell format-based microfluidic platform and tilting tower system (TTS) for interconnection and cultivation of multi-layered cells in a high-throughput manner. The *in-vitro* network of multiple chambers can be realized by physically separating cells but fluidically interconnecting them in the microfluidic platform. Utilizing the transwell structure, it allows easy loading, culturing, unloading from developed device for measurement. The TTS enables long-term cultivation of the in-vitro network under perfusion condition in an incubator without any external tubing and pumps.

Introduction. The discovery of cell culture inserts with porous filter membranes has expanded the scope for using biomimetic environments [1]. Transwell systems still offer a distinct advantage in that they are relatively easy to setup, as well as offering a range of endpoints to study [2]. Although this commercialized trasnwell system creates a three-dimensional (3D) environment by attaching cells above and below the membrane, it still lacks in mimicking *in vivo*-like environments such as interconnection of different cell-type tissues and physiological flow conditions. In this paper, we present a simple and robust 24-well-based microfluidic platform which can interconnect multiple transwell-based tissues (human colon cancer, glomerular filtration barrier of kidney cell) and culture under the perfusion condition.

Experiments and results. The 24-well transwell format-based microfluidic device has 4 separate microfluidic channels and each channel contains 4 of transwell insert compartments (Figure 1). The 24-well transwell inserts were physically separated but fluidically interconnected so the network of multiple chambers were established *in-vitro*. A transwell insert can be easily inserted and removed from the transwell compartment. It also allows imaging of cells above and below the membrane. Medium was perfused through the microfluidic channel between two open reservoirs without any pumps and external tubing by the gravity driven-flow in the in-house made tiling tower system (TTS) in a conventional incubator (Figure 2). HCT116 (human colon cancer) cells were successfully cultured in the developed microfluidic platform under the perfusion for 4 days and were compared with the static condition (Figure 3).
According to bright-field images and Live/Dead images, HCT116 cells showed the highest cell density and live signal when cultured in perfusion condition. Also, viability measured by adenosine triphosphate (ATP) was 1.6 times higher in perfusion condition than static condition. Furthermore, Among the body simulation models using transwell, the representative cells are kidney cells. Therefore, for confirming the filter role of kidney cells through the transwell structure, a flow condition test was conducted. Using human kidney cells (podocyte, the glomerular mesangial cell and the glomerular endothelial cell), the results of culturing according to the presence of flow showed that the cell morphology changed as have some direction when flow condition cultivation. Also, it was confirmed that human kidney cells were well cultured in the developed device (Figure 4).

Conclusions. The 24-well transwell format-based microfluidic platform and TTS have potentials for simple, robust, highly user-friendly drug screening tools by interconnecting multiple transwell insert compartments, having compatibility with conventional 24-well transwell insert and cooperating with laboratory automation equipment.

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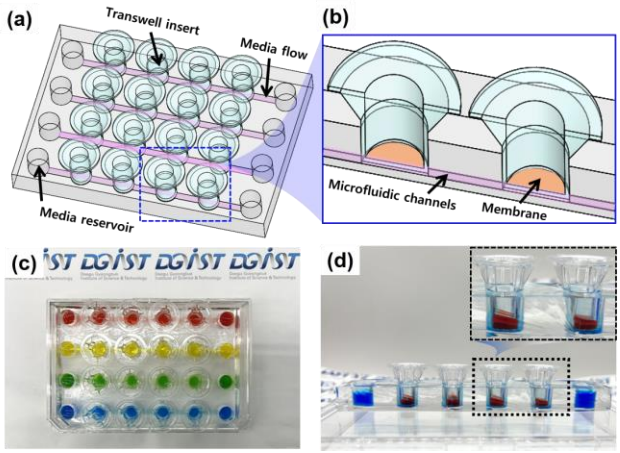


Figure 1. (a) Schematics of the 24-well Transwell format Body-on-a Chip (24W-BoC), (b)cross-section of Trnaswell insert compartment, (a) photograph of the fabricated 24W-BoC and (d) side view and enlarged photograph of insert

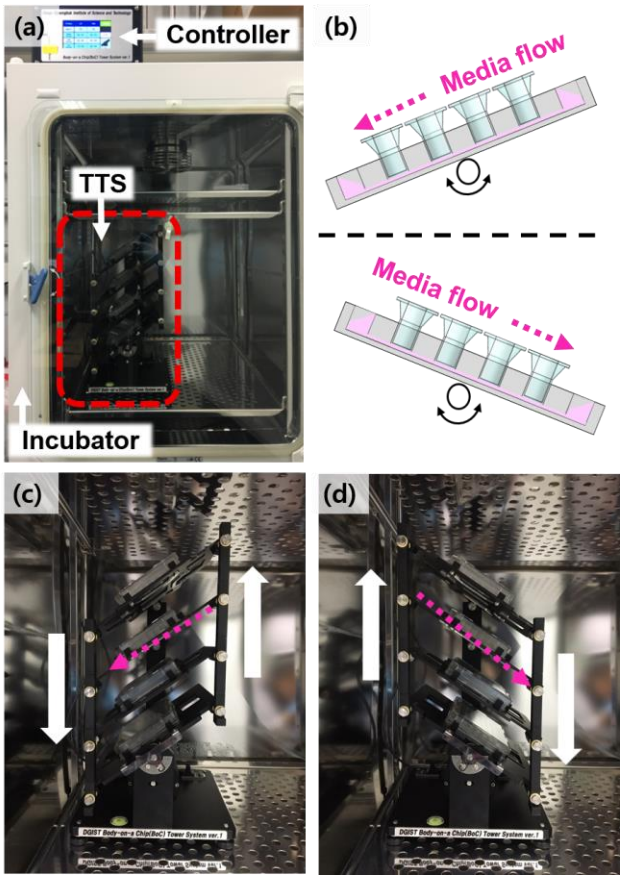


Figure 2. (a) Tilting tower set-up in an incubator, (b) gravity driven flow by tilting and media flow in microfluidic channels, and tilting to the (c) left and (d) right during cultivation in the 24-well format-based microfluidic platform for 4 days

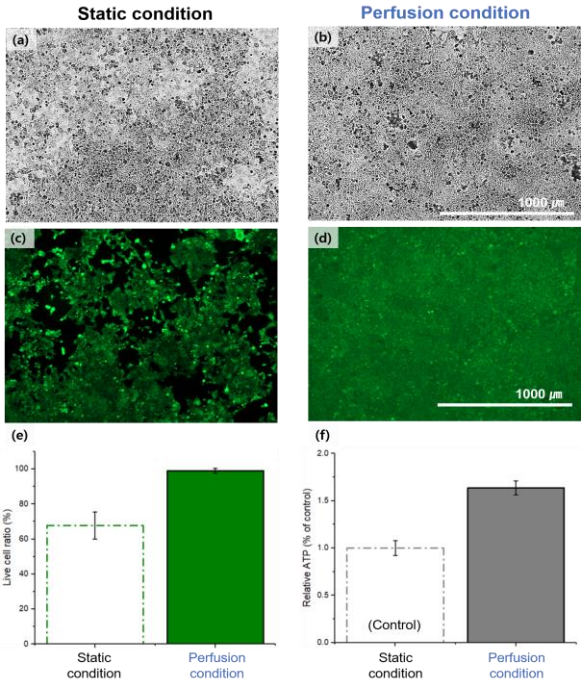


Figure 3. HCT116 (colon cancer cell) Bright-field image of the (a)static condition and (b) perfusion condition, Live/Dead image of (c) static condition and (d) perfusion condition (Green: Live, Red: Dead), (e) live cell ratio and (f) viability of HCT116 cells cultured under static and perfusion condition in the 24W-BoC on Day 4

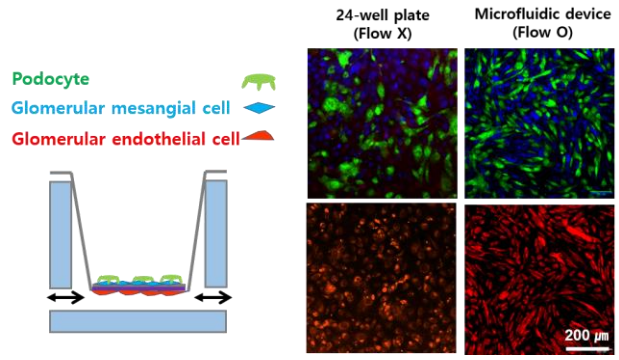


Figure 4. Kidney cell morphology change according to flow condition using human kidney cells

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