Fabrication of Three-dimensional Human Tissue Chips by Combination of Inkjet Cell Printing and Hierarchical Cell Manipulation

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Statement of Purpose: Cell-based drug evaluations have been performed on a plastic dish at monolayer (two-dimensional; 2D) cell conditions. Since nearly all tissues are integrated three-dimensional (3D) structures of multiple types of cells and extracellular matrices (ECMs), and since intercellular signalling is important for tissue functions, it is generally difficult to evaluate actual tissue functions by 2D-culture methods. Furthermore, high-throughput assays of human tissue responses using micro-arrays are valuable for the rapid assessment of drugs, chemicals and cosmetics. Accordingly, the development of 3D human tissue chips consisting of simplified tissue structures with multiple types of cells and ECMs is a key challenge for pharmaceutical evaluations.

We recently reported a simple and unique bottom-up approach, termed "hierarchical cell manipulation", to develop 3D cellular multilayers with the desired layer number and location by the fabrication of nanometer-sized LbL fibronectin (FN) -gelatin (G) (FN-G) films as a nano-ECM onto the cell surfaces [1-3]. Here, we demonstrate a Layer-by-Layer (LbL) assembly using the inkjet printing of single cells and proteins to enable the fabrication of 3D human micro-tissue arrays (**Figure 1**). We fabricated a liver tissue chip which integrated 440 simplified and multilayered 3D-micro liver tissues consisting of hepatocyte carcinoma (HepG2) and human umbilical vein endothelial cells (HUVEC) because the most common cell types in the liver are hepatocytes and endothelial cells, and then evaluated liver functions to understand the most suitable 3D-structures for stimulating HepG2 functions [4].

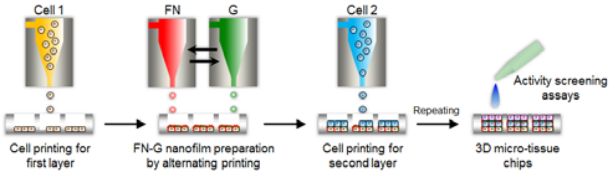

Figure 1. Schematic illustration of the development of 3D micro-tissue arrays by the LbL printing of single cells and proteins.

Methods: We prepared 440 micro-wells in a plastic plate, and the diameter and height of each well were 500 and 200 μm, respectively. One thousand drops of cells at 1 x 10^7 cells/mL concentration were printed onto the micro-wells, and the printed cells were incubated for 24 hours. Next, 1,000 drops of FN and G solutions were printed onto the surface of the printed cells to prepare the FN-G nanofilms, and then cells for the second layers were printed in the same manner. These processes were repeated to fabricate 3D-micro tissues. Three types of layers were constructed in 440 micro-well plates to investigate the effect of sandwich cell-cell interactions (**Figure 2**).

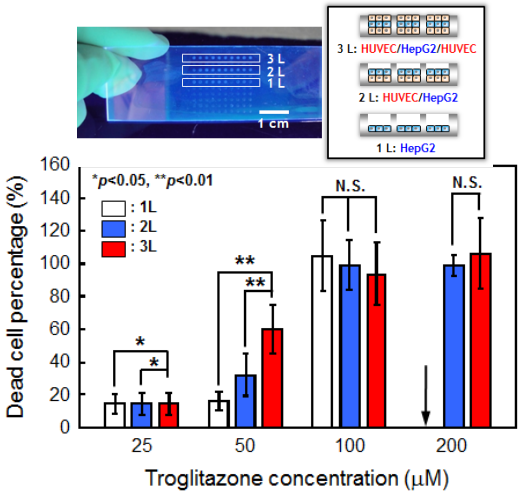

Figure 2. The Photograph of hepatic layer co-culture arrays prepared in 440 micro-well plates. e) The layer number and dose-dependent cytotoxicity of troglitazone in co-culture after 2 days of incubation (n=3, over 10 wells per image). The data were normalized to untreated cultures (100% activity). The arrow indicates undetectable samples due to the detachment of the dead cells. N.S. means no significant difference.

Results: To evaluate practical drug metabolism activity, we employed troglitazone (Rezulin®) which was withdrawn from 2,000 due to serious idiosyncratic hepatotoxicity. Thus, troglitazone is currently a good example to evaluate practical drug metabolism activity of CYP3A4 enzymes. Dose-dependent cell death was observed, and especially the 3L-multilayers clearly showed extensive cell death, even at a low concentration, as compared to the 1L and 2L. Quantitatively, the estimated IC_{50} values (concentration causing 50% cell death) of the 3L-multilayers reached 43 μM, whereas those of 1L- and 2L-multilayers were 66 and 60 μM, respectively, suggesting an approximately 1.5-fold higher CYP3A4 drug metabolism activity in relation to the higher CYP3A4 secretion and activity due to 3D-structures with HUVEC constructed with our method.

Conclusions: The rapid and automatic fabrication method of 3D human micro-tissue chips has great potential for tailor-made drug screenings and toxicological evaluations instead of animal experiments.

References:

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