Fabrication of Tissue Chip by Layer-by-Layer Inkjet Printing

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Statement of Purpose: Recently, development of cell chips have been attracted much attention for high-throughput screening of pharmaceutical assays. However, it is difficult to obtain tissue responses from the cell chip because tissues and organs consist of various cells and extracellular matrices (ECM) and three-dimensional (3D) structure is important for tissue functionality. In previous study, we reported *in vitro* fabrication of 3D cellular multilayers composed of various types of cells and ECM nanofilms by layer-by-layer (LbL) technique.[1)] Herein, we applied this cellular LbL system to inkjet printing method. This LbL printing can be applied for the integration of micrometer-sized 3D-layered structures as "tissue chip". The chip will be useful as a tissue chip for drug screening (Figure 1).

Methods: LbL printing of fibronectin (FN) and gelatin (G):The LbL printing was fabricated by alternate printing of 50 mM Tris-HCl buffer solution (pH=7.4) of rhodamine-labeled FN (Rh-FN) and 50 mM Tris-HCl buffer solution (pH=7.4) of FITC-labeled G (FITC-G) on the slide glass with piezo-type inkjet printing machine. Both of the concentration of Rh-FN and FITC-G were 0.2 mg mL^{-1}.

Cell printing and fabrication of 3D cellular multilayer: 50 mM Tris-HCl buffer solution (pH=7.4) of FN (0.2 mg mL^{-1}) and mouse C2C12 cell suspension ($1x10^7$ cell mL^{-1}) were directly printed on well substrates. The cells were incubated overnight at 37 ºC, 5% CO_2. 50 mM Tris-HCl buffer solution (pH=7.4) of FN (0.2 mg mL^{-1}) and 50 mM Tris-HCl buffer solution (pH=7.4) of G (0.2 mg mL^{-1}) were alternately printed onto the cell surface of the monolayered cells to fabricate LbL films. After the nine-step assembly of FN and G, C2C12 myoblast cell suspension ($1x10^7$ cell mL^{-1}), as the second layer, was printed. The FN-G assembly and cell printing were repeated four times to fabricate the micrometer-sized four-layered cellular architectures.

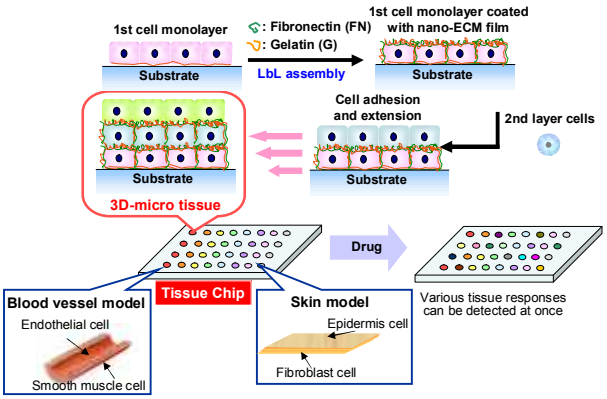

Figure .1 Schematic illustration of fabrication of 3D-cellular multilayers by inkjet printing.

Results: LbL spots of Rh-FN and FITC-G at the same position were clearly observed by fluorescence microscopy. The fluorescence intensity of the Rh-FN/ FITC-G spots increased with the LbL assembly step number. The results demonstrated the preparation of LbL FN-G spots by inkjet printing. To the best of our knowledge, this is the first report of LbL spots by inkjet printing technique.

We investigated viability and number of the printed cells. The viability of printed cells was over 99%, and printing cell number increased with increasing the drop number or the concentration of cell suspension. Furthermore, 3D-micrometer sized four-layered cellular architectures composed of mouse C2C12 myoblast cells were successfully fabricated by repeating FN, G and cell printing (Figure 2a, b). The four-layered cell assemblies were stably organized and easily peeled from the substrate. Hematoxylin and eosin (HE) - stained images of peeled constructs clearly indicated the four-layered structures of C2C12 myoblasts (Figure 2c). The thickness of four-layered cellular architecture was approximately 30 µm (Figure 2c). Furthermore 100 cell chips were successfully fabricated by using a 440 well (Figure 2d, e).

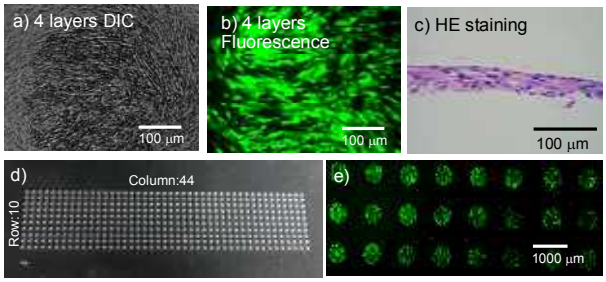

Figure 2. Focused differential interference contrast microscopic image a), fluorescent microscopic image b) and HE stained image c) of four-layered composed of mouse C2C12 myoblasts labeled with cell tracker green in 25 wells. d) Photograph of 440 wells. e) Fluorescent image of monolayered GFP-C2C12 myoblasts in 440 wells.

Conclusions: We demonstrated successful construction of micrometer-sized cellular multilayers with more than three layers in the well substrate by inkjet printing. The LbL printing of proteins and cells would be a useful technique for fabrication of tissue chip.

References: 1) M. Matsusaki et al., Angew. Chem. Int. Ed. 2007; 46: 4689-4692.