Building Up Layered Tissues by Layer-by-Layer Cell Assembly

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Statement of Purpose: Tissues or organs consist of a complex organization of cell and extracellular matrix (ECM), especially a blood vessel and skin is a highly organized hierarchical layer composed of various types of cells and ECM layers. The *in vitro* construction of highly organized layered tissues composed of cells and ECMs has attracted much attention in biomedical fields. Some researchers have reported a novel technique to develop cellular multilayers, but all methods require complicated manipulation.

Recently, we reported the *in vitro* fabrication of three-dimensional (3D) cellular multilayers composed of various types of cells and fibronectin (FN)-gelatin (G) nanofilms by layer-by-layer (LbL) technique (Figure 1).[1)] FN and G are significant components of ECM for cell adhesion, thus we prepared 6 nm of FN-G films on the surface of the first layer of cells to allow the adhesion of second layer of cells. The 4-layered architecture of mouse L929 fibroblast cells was successfully constructed, and the layered architecture was well organized even after a few weeks of incubation. This methodology was useful to fabricate the xenogeneic cellular multilayers as a layered tissue model, such as a blood vessel composed of vascular endothelial cells and smooth muscle cells.

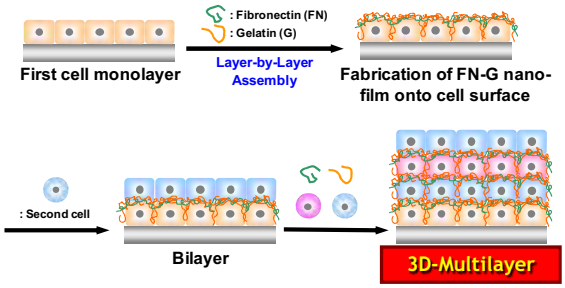

Figure 1. Fabrication of 3D-cellular multilayers by preparing FN-G nanofilms onto the cell surface.

Methods: Preparation of FN-G nanofilm on cell surface was performed by the following LbL assembly. Briefly, cover glass as a substrate was immersed in 50 mM Tris-HCl buffer (pH=7.4) of FN (0.2 mg/mL) for 15 min, and cells were seeded on the substrate at confluent condition and incubated in DMEM (10% FBS) for 6 hours at 37°C. The monolayered cells on the substrate were alternately immersed into 50 mM Tris-HCl buffer (pH=7.4) of FN (0.2 mg/mL) and G (0.2 mg/mL) for 1 min of each immersion at 37°C, and then rinsed with 50 mM Tris-HCl buffer (pH=7.4). After 9-step assembly of FN and G, cells as second layer was seeded at confluent condition and incubated for 6 hours at 37°C. Repeating these LbL steps, the multilayered architectures of cells were obtained.

Results: Figure 2 shows HE staining and immunostaining images of mouse C2C12 cell multilayers (left) or 5-layered blood vessel models composed of monolayer of endothelial cells (HUVEC) and 4-layered smooth muscle cells (UASMC) (right) after 3 days of incubation. These multilayered structures were stable even after 1 week of incubation and survival of cells in each layer were confirmed by WST-1 method. Functionalities of the layered architecture like skeletal muscle or blood vessel models are evaluating as compared to natural tissues.

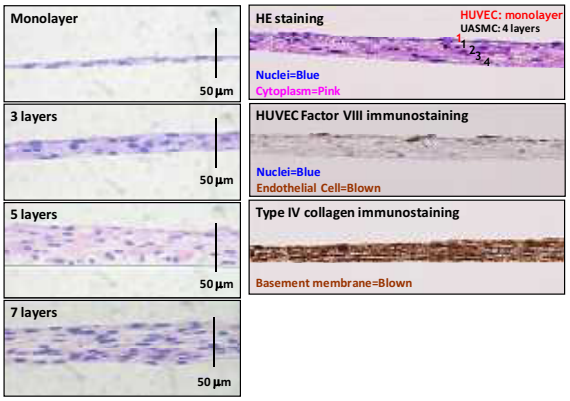

Figure 2. The HE staining images of multilayers of mouse C2C12 myoblasts prepared by the LbL assembly (left). The HE and immunostaining images of 5-layered blood vessel model composed of human endothelial cell monolayer and 4-layered smooth muscle cells (right).

Conclusions: In summary, we demonstrated that cellular multilayers were successfully fabricated *in vitro* by preparing FN-G nanofilms on the surface of each cell layer. We are developing now the "Tissue Chip" assembled the micrometer-sized various layered tissues on one substrate by inkjet technique. These novel technologies will be valuable to fabricate varied layered tissue models for tissue engineering or efficacy test of drugs.

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References:

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