Layer-by-Layer Assembly of Cells and Proteins toward Vascularized Human Tissue Models

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Introduction

Development of artificial three-dimensional (3D) tissues possessing a similar structure and function as natural tissue is a key challenge for implantable tissues in tissue engineering, and for model tissues in pharmaceutical assay. We have developed a simple and unique bottom-up approach, hierarchical cell manipulation, using nanometer-sized Layer-by-Layer (LbL) films consisting of fibronectin and gelatin (FN-G) as a nano-extracellular matrix (**Figure 1**).[1)] The FN-G nanofilms were prepared directly on the cell surface, and we discovered that at least 6 nm thick FN-G films acted as a stable adhesive surface for adhesion of the second cell layer.[2,3)] Various 3D-layered constructs consisting of single or multiple types of cells were successfully fabricated, and the higher cellular activities induced from the 3D-structures as compared to monolayer structure were observed.[4)] Furthermore, the multilayered constructs like a blood vessel wall indicated almost the same drug response as *in vivo* natural blood vessel, suggesting the possibility to use as an *in vitro* blood vessel model to analyze drug response.[5-7)] Recently, 3D-fibrotic tissue constructs in pancreatic cancer were fabricated to evaluate nanoparticle delivery.[8)] This method is useful to fabricate cell multilayers one by one, but faces technical limitation for rapid construction of multilayered structures.

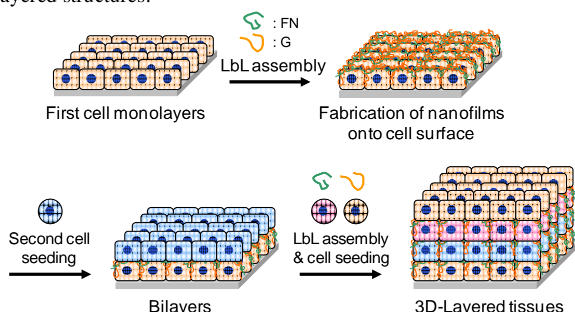

Figure 1. Schematic illustration of hierarchical cell manipulation by LbL assembly.

Cell Accumulation Technique

As described above, our hierarchical cell manipulation technique is simple and versatile enough to develop the multilayered constructs while controlling the cellular type and location. However, the fabrication of two layered (2L) tissues is limited due to the time required for stable cell adhesion. Therefore, a simple and rapid approach is strongly desired for the *in vitro* construction of thick multilayered tissue models. We currently developed a simple and rapid bottom-up approach, called the cell-accumulation technique, by a single cell coating using FN-G nanofilms (**Figure 2a**).[9)] Since the FN-G nanofilms prepared on individual cell surfaces provide an interactive property with the $\alpha_5\beta_1$ integrin receptor of the cell membrane, the cell-cell adhesion of all seeded cells in three-dimensions can be induced at the same time (cell accumulation). This rapid approach easily provided approximately over 10 to 20-layers (over 100 μm) after only one day of incubation. This rapid approach can apply various types of cells such as normal human dermal fibroblast (NHDF), human hepatocellular carcinoma (HepG2), human umbilical vein smooth muscle cells (UASMC), and mouse C2C12 myoblast. The obtained thickness were approximately same as 100 ~110 μm, whereas the layer numbers were significantly depending on cell types, such as NHDF was 23-layeres (23L) and HepG2 was 13-layers (13L) (**Figure 2b,c**).

We tried to construct thick multilayered tissues with endothelial tube networks by embedding HUVECs in 3D-tissues composed of NHDFs.[10,11)] The formation of capillary networks of HUVECs in the scaffolds or tissues by 3D-culture has been previously reported, but development of 3D-vascularized thick tissues possessing high density blood-capillary networks is still an outstanding issue for not only tissue engineering, but also for angiogenesis models for pharmaceutical assays. We performed a sandwich culture of HUVECs between 4L-NHDF for 1 week, and the HUVECs in the obtained tissues were then fluorescently labeled with an anti-CD31 antibody. After 7 days of incubation, highly-developed capillary networks and a tubular morphology of the HUVECs were clearly observed by CLSM analyses. Surprisingly, a dense and homogeneous vascularized morphology in the multilayered tissues of 1 cm width and 50 μm height was confirmed. The occupied area percentage and distance of this capillary network of HUVECs was calculated to be approximately 63 ± 12% and 50~150 μm by image analysis. Moreover, when human lymph endothelial cells (LEC) were employed together with HUVEC, individual tube networks of HUVEC and LEC were successfully obtained (**Figure 2d**). To the best of our knowledge, such widespread and dense both blood and lymph-capillary networks in 3D-multilayered tissues were reported for the first time here.

Conclusions

Several examples of our hierarchical cell manipulations using nanofilms have been described aimed at *in vitro* creation of 3D-tissue models. A bottom-up approach is valuable to develop 3D-cellular architectures using multiple types of cells under controlling the location and type of cells. Our techniques would be one of the solutions for the development of 3D-tissue models.

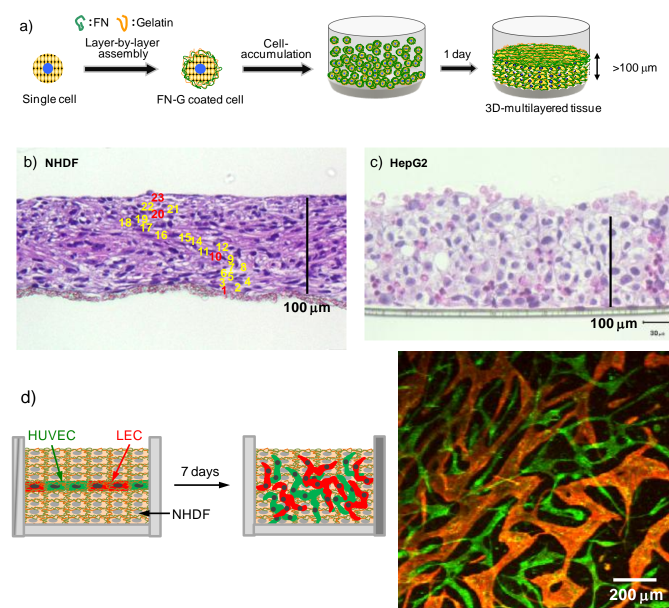

Figure 2. Rapid fabrication of thick layered tissues by cell accumulation technique (a). 23L-NHDF (b) and 13L-HepG2 (c) tissues fabricated by this technique after 1 days of incubation. Schematic illustration of sandwich co-culture of HUVEC and LEC to fabricate vascular and lymph networks (left) and confocal laser scanning microscope image of the obtained networks stained with CD31 for HUVEC (green) and LYVE-1 for LEC (red) antibodies, respectively (d).

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References

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