Fabrication of Perfusable 3D-Blood Capillary Models for In Vitro Drug Permeability Assays

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Statement of Purpose: Blood capillary is one of the important tissues that not only circulate nutrients and oxygen but also deliver drugs in the blood stream. To evaluate drug toxity and permeation to peripheral tissues from blood capillary, blood capillary models are required in drug screening. Up to date, in vivo animal experiments and in vitro cell experiments using vascular endothelial cells were employed for these evaluations. However, animal models have difficulties in the evaluations of species differences and cell monolayer models lack the responses from 3D tissue structures. Therefore, the constructions of perfusable blood capillary models are important for drug screening.

In this study, we fabricated the novel perfusable blood capillary models by improving our previous method. We reported a bottom-up approach, termed "cell accumulation technique" [1], which improved our previous method (hierarchical cell manipulation [2]), to develop multilayered thick tissue (>100 μm) by cell coating with nanometer-sized ECM films, fibronectin and gelatin (FN-G) films [3, 4]. The vascularized tissues were successfully fabricated by a sandwich culture of endothelial cells between fibroblast multilayers, as well as lymph-capillary models. However, since the constructed vasculatures did not reach to top and bottom surfaces, it was difficult to add chemical reagents from outside to inside the networks.

To develop the perfusable vascular networks, we added one more layer of endothelial cells at the top and bottom surfaces (Figure 1a). During the network formation, the added endothelial cells changed their morphologies to make opening pores at the both surfaces and the capillary networks reached to the outermost surfaces. When fluorescent molecules were added to the top surfaces, they reached to the outside at the bottom immediately. The improved method will be powerful technique to provide various perfusable 3D tissues in constructed micro-well plates for drug assessment.

Methods: The 7 x 10^4 Human umbilical vein endothelial cells (HUVEC) were seeded into cell culture insert and cultured for 12 h to adhere sufficiently. Secondary, the 2 x 10^6 cells/mL normal human dermal fibroblasts (NHDF) were alternatively incubated with 0.04 mg/mL FN ($M_w = 4.6 \times 10^5$) and G ($M_w = 1.0 \times 10^5$) in 50 mM Tris-HCl (pH = 7.4) for 1 min at 2,500 rpm to fabricate $(FN/G)_4FN$ films with about 7 nm thickness. The FN-G coated NHDF were seeded into a cell culture insert and cultured for 12 h to construct four layers. Next, $(FN/G)_4FN$ films were prepared on the tissue surfaces and 7 x 10^4 HUVECs were seeded on the surfaces. In the same manner, 1L-4L-1L-4L-1L tissue composed of 1-layered HUVEC and 4-layered NHDF was fabricated. After 2 days, perfusable blood capillary networks were formed and the histlogical evaluation and influx of FITC-labeled dextran 250k into tubular structure was observed by confocal laser scanning microscopy (CLSM).

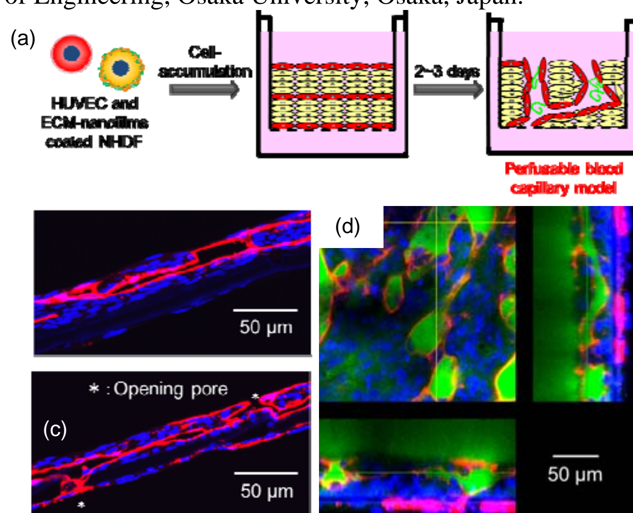

Figure 1. (a) Schematic illustration of the fabrication process of perfusable 3D-blood capillary model and their application for drug permeability assay and cell invasion model. (b) Histological evaluation of blood capillary networks. Asterisk denotes opening pores of blood capillary-like networks. (c) Confocal laser scanning microscopy image of HUVEC tubular structure with FD250k solution. Nuclei were labeled with DAPI (blue). HUVECs were immunostained with anti-CD31 antibody (red), and green color indicated FD250k, respectively.

Results: Histological evaluations with immunological staining clearly showed the successfully preparation of perfusable 3D-blood capillary networks (Figure 1b). When FITC-labeld dextran 250k solution were added to the top surfaces of the 3D tissues, the FITC-labeled dextran showed rapid diffusion inside the blood capillary networks within 10 minutes by confocal laser scanning microscopy (CLSM) (Figure 1c). These results indicated the constructed blood capillary networks had perfusable properties.

Conclusions: We demonstrated fabrication of in vitro perfusable 3D-blood capillary models and assessed the diameter of blood capillary. Moreover, we confirmed that the blood capillary model possessed the ability to perfuse solution. This perfusable 3D-blood capillary model would be useful for the drug permeability assay and cell, such as red blood cells and tumor cells, invasion model.

Reference:

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Abstract #15
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