Construction of iPS-Derived 3D-Cardiac Myoblast Tissues Containing Blood Capillary Network by Cell Accumulation Technique

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Statement of Purpose: Ordinary drug discovery system are time-consuming and costly because the pharmaceutical assays using cell monolayer and animal models have many issues due to different drug responses as compared to human body. It is difficult to evaluate actual tissue functions by 2D-culture or animal models. Therefore, the use of human induced pluripotent stem cell (iPS), which can provide many types of normal and diseased human cell sources, has enormous potential for pharmaceutical assays by constructed three-dimensional (3D) tissue model with it. However, for the 3D-tissue living, it is necessary for capillary within the tissue to provide medium to inside. Especially, it's important for the cardiac myoblast (CM) tissues because of beating. But, it is still very challenging to introduce capillary into the iPS-3D-CM tissues.

In this study, we aim to develop iPS-3D-CM tissue models containing blood capillary by the cell-accumulation technique (Figure 1a). We reported a bottom-up approach, termed "accumulation technique" [1] which was improved method of our previous technique, hierarchical cell manipulation [2], to develop multilayered thick tissues (>100 μm) by cell surface coating with nanometer-sized ECM-films [3]. Less than 10 nm sized ECM-films induced cell-cell interaction in three dimensions. By this method, iPS-3D-CM tissues were successfully fabricated. Moreover, co-culture with iPS-CM, Normal Human Cardiac Fibroblast (NHCF), and Normal Human Cardiac Microvascular Endotherial Cell (NHCMEC) achieved to introduce the blood capillary into the tissues. The iPS-3D-CM tissues have great potential for pharmaceutical applications and tissue engineering.

Methods: The iPS-CM and NHCF were alternatively incubated with 0.2 mg/mL Fibronectin (FN) (M_w = 4.6 ×10^5) and Gelatin (G) (M_w = 1.0 ×10^5) in 50 mM Tris-HCl (pH = 7.4) for 1 min respectively. After repeating the nine steps of immersion, the $(FN/G)_4FN$ films with about 10 nm thickness were prepared on the cell surfaces. 5.0 x10^5 FN-G coated cells, including iPS-CM and NHCF, were seeded into 24 well cell culture insert to construction the iPS-3D-CM tissue. In case of introducing the capillary into the tissue, 1.0 x10^6 FN-G coated cells, including iPS-CM and NHCF, and 1.0 x10^5 NHCMEC were seeded. The ratio of NHCF to iPS-CM and NHCF was adjusted 0%, 25%, 50%, and 75%. After 4 days of incubation, the tissues were stained with Hematoxylin-Eosin (HE) or immunostained of human CD31. The ratio of capillary was evaluated by metamolph software.

Results: Thickness and cell density of the obrained the iPS-3D-CM tissues increased with increasing the NHCF ratio.The 25% and 50% NHCF introduced tissues showed synchronous beating, while 0% and

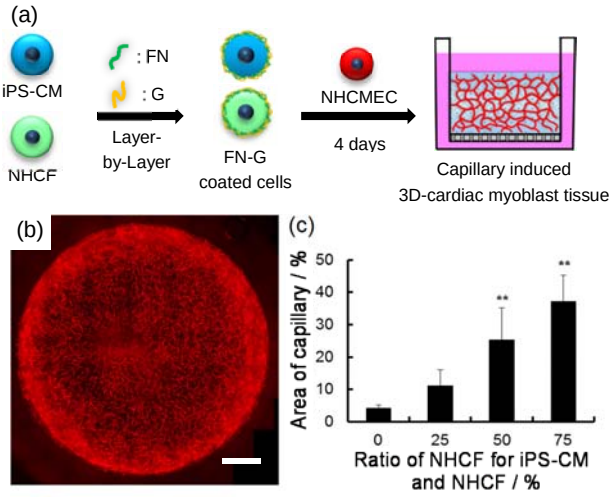

Figure 1. (a) Schematic illustration of construction of the iPSC-derived 3D-cardiac myoblast tissue models containing capillary network by the cell-accumulation technique. (b) Map image of the capillary network induced iPSC-CM tissue containing 50% NHCF. Scale bar represents 1 mm. (c) Quantification of the area of capillary in the 0%, 25%, 50%, and 75% NHCF introduced tissues respectively. **P < 0.05 when compared with 0%.

75% ones showed heterogeneous beating. These results suggested importance of NHCF for higher functions of the obtained iPS-3D-CM tissues.

Blood capillary network was successfully introduced into the tissues by co-culture with NHCF and NHCMEC. Figure 1b shows the confocal laser scanning microscopy (CLSM) image of 50% NHCF containing iPS-3D-CM tissues immunostained with an anti-CD31 antibody. The quantification data of the area of blood capillary indicates that introducing of NHCF promotes network formation in the tissues. As described in this image, homogeneous blood capillary network was constructed in the tissues (Figure 1c).

Conclusions: We demonstrated the construction of iPS-3D-CM tissues and introducing blood capillary by cell-accumulation technique. 25% or 50% NHCF introduced tissue showed homogeneous synchronous beating than 0% and 75% tissues. Moreover, addition of NHCF promotes blood capillary network formation. These reconstructed 3D-cardiac tissues will be useful for tissue engineering and pharmaceutical application.

Reference:

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