Construction of iPSC-Derived 3D-Cardiac Muscle Tissues Using Cell-Accumulation Technique for Pharmaceutical Applications.

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Statement of Purpose: General drug discovery systems 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. Therefore, the use of induced pluripotent stem cell (iPSC), which can provide many types of normal and diseased human cell sources, has enormous potential for pharmaceutical assays. However, since nearly all tissues are integrated three-dimensional (3D) structures of multiple types of cells and extracellular matrices (ECMs), and since intercellular signaling is important for tissue functions, it is difficult to evaluate actual tissue functions by 2D-culture method.

In this study, we aim to develop 3D-cardiac tissue models composed of normal and diseased iPS cells 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, such as fibronectin (FN)-gelatin (G) or type IV collagen (Col IV)-laminin (LN), induced cell-cell interaction in three dimensions. By this method, iPSC-derived 3D-cardiac muscle tissues were successfully fabricated. The obtained tissues showed the synchronized contractions. The iPSC-derived 3D-cardiac muscle tissues have great potential for pharmaceutical applications instead of animal experiments.

Methods: Neonatal rat cardiac myoblasts (rCMs) were emplayed to obtain basic information. The isolated 5 x 10^6 cells/mL cells were alternatively incubated with 0.2 mg/mL Col IV (M_w = 5.4 × 10^6 Da) and LN (M_w = 8.5 × 10^6 Da) in 50 mM Tris-HCl (pH = 7.4) for 1 min at 30 rpm. After repeating the nine steps of immersion, the $(Col\ IV/LN)_4$ Col IV films with about 10 nm thickness were prepared on the cell surface. The Col IV-LN coated cells were seeded into a 24 micro well cell culture insert (1 x 10^5 cells/layers) and cultured to construct multilayered tissues. The beating properties of the tissues at three different places of each sample were observed and counted for 9 days by confocal laser scanning microscopy (CLSM).

Results: Histological evalution showed successful fabrication of multilayered rCM tissues (Figure 1b). Within 24 hours of cell seeding, the tissues began to synchronously contract and continued to beat for about 10 days (Figure 1c). Interestingly, the beating rate of the tissues increased and continued with increasing the layer number, indicating importance of the 3D-structures for higher functions. When the tissues were exposed to the media with adrenergic drug, isoproterenol, that can accelerate the beating rate, the values increased from 53/min to 86/min. This result suggests that this model can be used as a drug testing

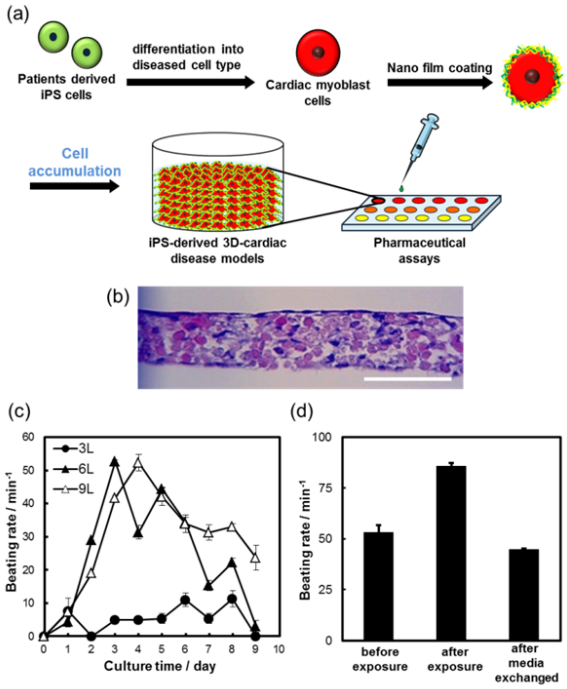

Figure 1. (a) Schematic illustration of the fabrication of 3D-myocardiac tissue models derived from iPSCs by the cell-accumulation technique. (b) Cross-sectional image obtained from the HE staining of 9L-tissue constructed with rCMs coated with Col IV-LN films after culture periods of 4 days. Scale bar represents 50 μm. (c) The temporal changes of beating rate of 3, 6, and 9L-rCM tissues. (d) Positive chronotropic responses of 10L-rCM tissue to isoproterenol (1μM).

models. Moreover, as shown in Figure d, we successfully fabricated seven layered (7L) iPSC-derived 3D-cardiac tissues same as rCM tissues. The iPSC-derived tissues revealed good beating properties and further experiments are now in progress.

Conclusions: We demonstrated the construction of 3-9L rCM tissues. They showed successful stimulation of beating rete in response to adrenergic drug. Furthermore, we succeeded to fabricate 7L-iPSC-derived 3D-cardiac muscle tissues. This approach would be useful for tailor-made drug screenings and toxicological evaluations. In the conference, we will present functional data of 3D-cardiac tissues including iPSC-derived tissues. These reconstructed 3D-cardiac tissues will be also useful for tissue engineering application, such as ischemic diseases.

Reference:

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