Automatic Filter Layer-by-Layer Coating System for Construction of iPS-Derived 3D Tissues by Cell Accumulation Technique

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Statement of Purpose: Human induced pluripotent stem cell (iPS) technology is expected to accelerate development of regenerative medicine. However, it is difficult to evaluate real tissue functions by monolayer culture. Therefore, construction of three dimensional (3D) tissue models using iPS cell is crucial for many applications, such as evaluation test in cosmetics field, graft use in the medical field, drug effect and toxicity test in the drug discovery field.

We reported cell accumulation technique (figure1 (a)) for constructing 3D-tissues models [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 extra cellular matrix (ECM) films [3]. Less than 10 nm sized ECM-films induced cell-cell interaction in three dimensions.

This technique needed centrifugation process to collect the coated cells in each immersion. It would damage cell membrane, and give lower yield to sensitive cells, especially iPS derived cells.

In this study, we develop a novel method of cell surface coating, named filter layer by layer (F-LbL). It uses micron-size pore filter for separating cells from ECM solution with lower cell damage than conventional centrifugation layer by layer method (C-LbL). We also developed automatic system (figure1 (b)) of F-LbL for industrialization. This system has feeding and breeding features of ECM solution, and a swinging chamber in which cells are covered with ECMs by mixing the cells and the solution. The automatic F-LbL coating system have great potential for R&D and industrialization of 3D tissue fabrication.

Methods: The cardiomyocytes derived from iPS cells (iPS-CM) and the normal human cardiac fibroblasts (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. In each steps, cells have to be separated by ECM solution and the two methods were applied for the separation; C-LbL and F-LbL. In the former, the cells were centrifuged by 240 times gravity for one minute. In the latter method, for the separation of the cells the chamber of which diameter is about 1.5 mm are rotated at 1000 rpm corresponding to about two times gravity for 15 seconds.

The FN-G coated cells including iPS-CM and NHCF, were seeded into 24 well cell culture insert to construct the iPS-3D-CM tissue. The ratio of NHCF to all cells was adjusted 25%. After 4 days of incubation, the tissues were stained with Hematoxylin-Eosin (HE) and the cross-sections were observed by the microscopy.

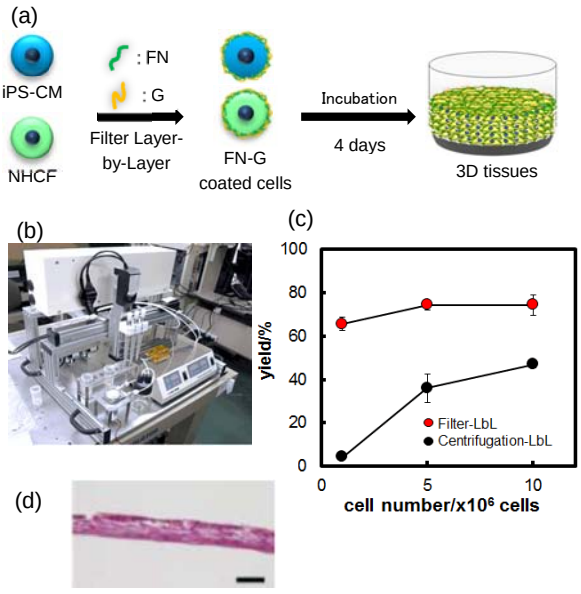

Figure 1. (a) Schematic illustration of construction of the iPSC-derived 3D tissue models by the cell-accumulation technique. (b) Image of automatic filter layer-by-layer coating system. (c) Yields of the cardiomyocytes in the two coating processes; filter layer-by-layer and centrifugation layer-by-layer. The F-LbL enable the better yield ratio than C-LbL. (d) Histological HE staining image of iPS-CM-3D tissues constructed with the automatic coating system. (Scale=50um)

Results: Figure1 (c) shows the yields of the living coated cardiomyocytes on the two types of coating process. F-LbL method enable us to collect coated cells more effectively than C-LbL method. Moreover, the yields of C-LbL were reduced as the number of cells decrease. Based on these results, we developed automatic F-LbL coating system shown in figure (b). Using the automatic system, the iPS-CM-3D tissues were successfully constructed as shown in figure1 (d).

Conclusions: We proposed new coating method, filter-layer-by-layer method, which has better yield constant than conventional centrifugation layer-by-layer method. Automatic coating system based on the filter layer-by-layer method was developed. This system would be greatly helpful for achieving stable and repeatable coating process especially for using damageable cells like iPS derived cardiomyocytes.

Reference:

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