Characterization of iPSC-derived Skeletal Muscle Generated with Rapid Differentiation Method

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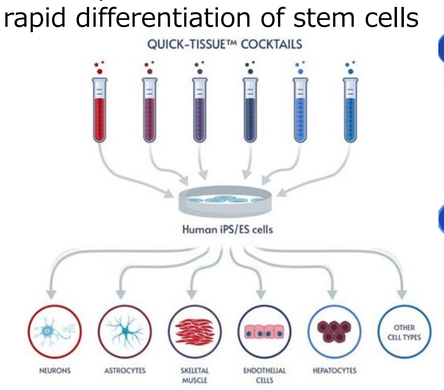
Introduction

Recently, iPSC-derived skeletal muscle cells (iSkMC) have been used in disease and drug discovery research. However, in order to obtain the desired cells from iPS cells (iPSC), a long differentiation process is generally required, which is one of the issues in disease and drug discovery research. In this study, we characterized iSkMC that were differentiated using Quick-Tissue™ Technology, which enables cell differentiation in a short period of time. Specifically, the expression of representative markers and the function of acetylcholine receptors were evaluated to verify the possibility of shortening the time required for disease and drug discovery research.

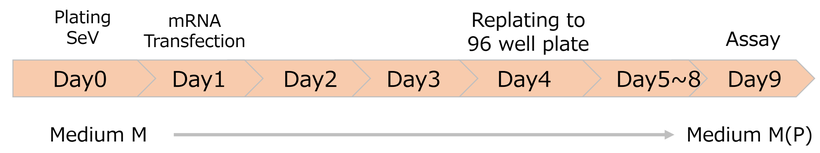
Quick-Tissue™ technology is a transcription factor-based method for rapid differentiation of stem cells



Differentiation
Typically 1-2 weeks

Robustness
Low dependency on iPSC lines

Differentiation Protocol

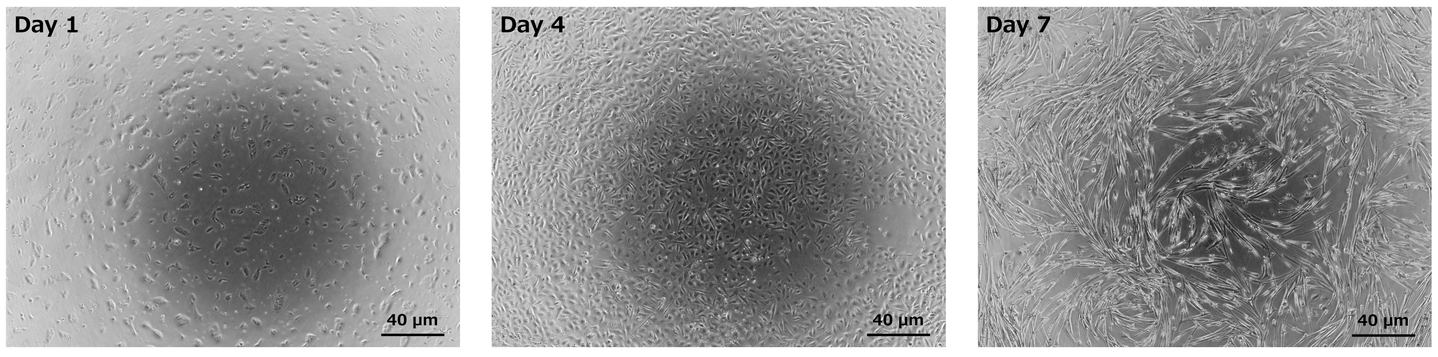


Materials

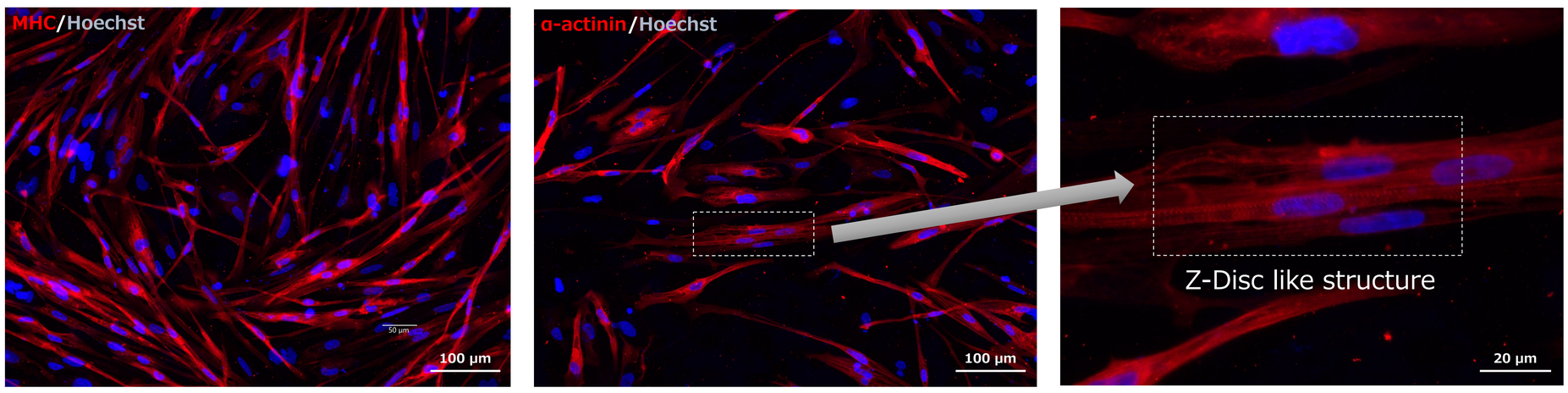
- ✓ iPSC : Riken, HPS1005 1383D2
- ✓ Differentiation reagent : Quick-Muscle™ Skeletal - SeV Kit (Elixirgen Scientific)

Results

1. Characterization of iPSC-derived Skeletal Muscle Cells



(a) Morphological changes (Phase contrast images)



(b) Marker expression (Left: MHC, Center and Right: α-actinin)

The iPSC-derived skeletal muscle cells were characterized for the morphology and protein expression of skeletal muscle markers including myosin heavy chain (MHC) and sarcomeric alpha-actinin (α-actinin).

(a) Spindle-shaped cells were observed on day 4 and myotube-like cells were observed on day7 after the start of differentiation induction.

(b) The immunocytochemistry (ICC) images show the expression of the marker proteins and multinucleation. In addition, a Z-Disc-like structure was observed.

Antibody :

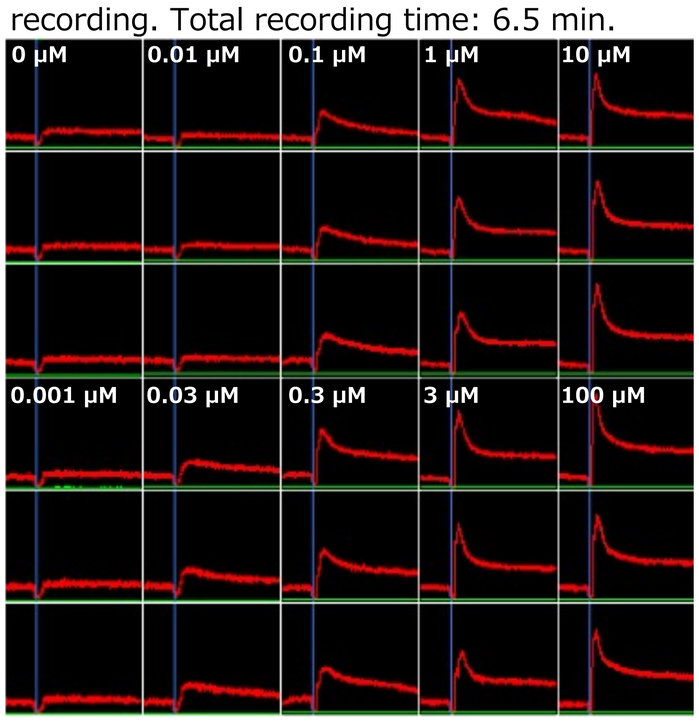
- MHC (MAB4470, R&D Systems)
- α-actinin (A7811, Sigma)

Skeletal muscle cells induced by Quick-Tissue™ technology showed tubular morphology and multinucleation after 7 days from iPSCs. In addition, expression of skeletal muscle cell markers and formation of the Z-disc-like structures were observed. These results indicate that the cells have characteristics of skeletal muscle cells.

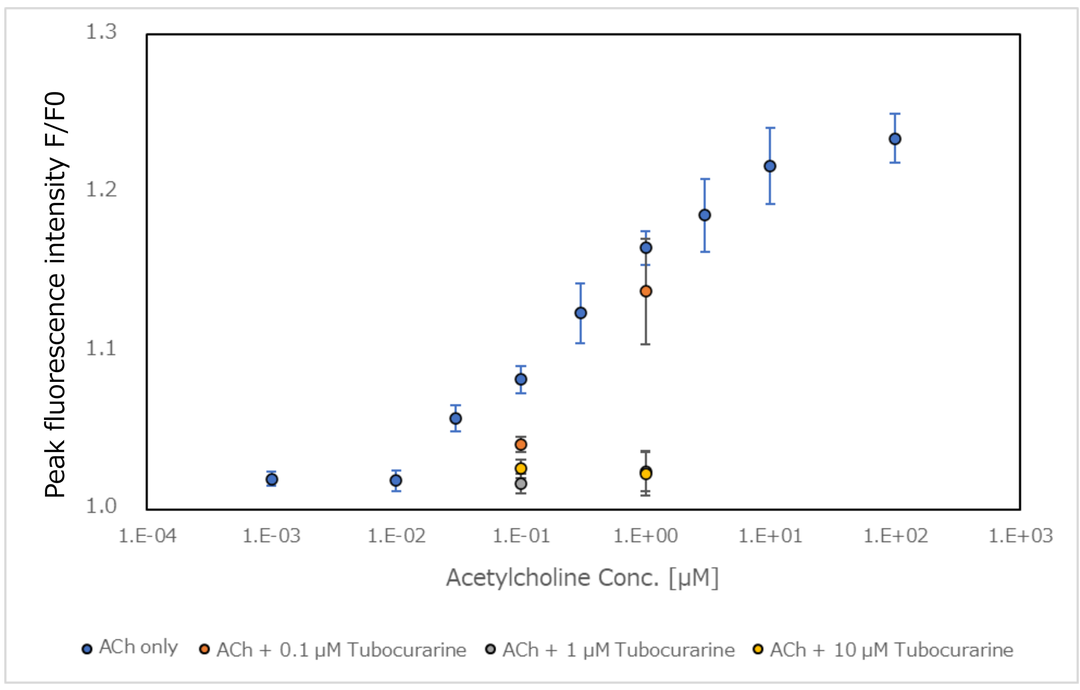
2. Evaluation of Acetylcholine (ACh) Receptor Functions

Methods:
Equipment: FDSS/µCELL (Hamamatsu Photonics)
Method: ACh was added 1.5 min after the onset of recording. Total recording time: 6.5 min.

Pretreatment
Ca^{2+} indicator: EarlyTox Cardiotoxicity Kit (Molecular Device)
Concentration: 50%. Incubation: 2 h at 37°C.



(a) Ca^{2+} Intensity after the addition of ACh



(b) Dependency of the Ca^{2+} signal intensity on the ACh concentration

The changes of the intracellular Ca^{2+} density after the addition of ACh were measured using Ca^{2+} indicators.

(a) Ca^{2+} fluorescence intensity before and after the addition of ACh. Immediately after the addition of ACh, the signal intensity increased and reached its peak in 10 seconds and then slowly returned to the steady state. The peak intensity depended on the ACh concentration.

(b) The peak fluorescence intensity positively correlated with the ACh concentration (blue circle). When the ACh receptor antagonist, Tubocurarine, was added in advance, the signal was clearly suppressed.

Ca^{2+} signals increased in a concentration-dependent manner and was inhibited by the antagonist. These results suggest that the acetylcholine receptors of iPS cell-derived skeletal muscle cells differentiated by Quick-Tissue™ technology function similarly to the in vivo counterparts.

Conclusions

In this study, we showed that the iPSC cell-derived skeletal muscle cells (iSkMCs) generated with the Quick Tissue™ technology exhibit cellular characteristics expected for human muscle cells including expression of skeletal muscle markers, the Z-disk like structure, and acetylcholine responses. Therefore, the Quick Tissue™ technology and the human iSkMCs provide simple and quick methods for skeletal muscle research in the fields of disease analysis and drug discovery.