Application Note

Differentiation of iPSCs into cardiomyocyte using Ceglu™ , a chemically defined scaffold

Background

The utilization of cardiomyocytes from human induced pluripotent stem cells (iPSCs) is anticipated to be extensively adopted in various applications, including cardiotoxicity assay systems in drug discovery, disease modeling, and cell therapy for cardiac diseases. In particular, when used for cell therapy, it is desirable to have highly differentiated cardmyocytes with high purity and no residual iPSCs. In this study, we examined the differentiation of iPSCs into cardiomyocytes on Ceglu™ multiwell plate under specific conditons (**Fig.1**)

Key Points

- ✓ **Cardiomyocyte differentiation efficiency at the same level as conventional methods**
- ✓ **Consistent scaffold environment from culture to differentiation**
- ✓ **No animal-origin components**

Methods

- Maintenance culture and cardiomyocyte differentiation were performed using the Gibco™ PSC cardiomyocyte differentiation kit manufactured by Thermo Fisher Scientific. Inc. according to protocol[1]
- iPSCs are acclimatized with Ceglu

1. Seed iPSCs (SCTi003-A cell line, STEMCELL Technologies) on Ceglu multiwell plate.
2. Change the medium to iPSCs maintenance medium (mTeSR™ Plus, STEMCELL Technologies) the day after seeding.
3. On the 4th day after seeding, exchange the medium to cardiomyocyte differentiation medium A (Day 0 of differentiation).
4. On day 2 of differentiation, change the medium to cardiomyocyte differentiation medium B.
5. On days 4, 7, 9, 11, and 14, change the culture medium to cardiomyocyte maintenance medium.
6. Measure cardiac muscle troponin (cTnT) on day 15.

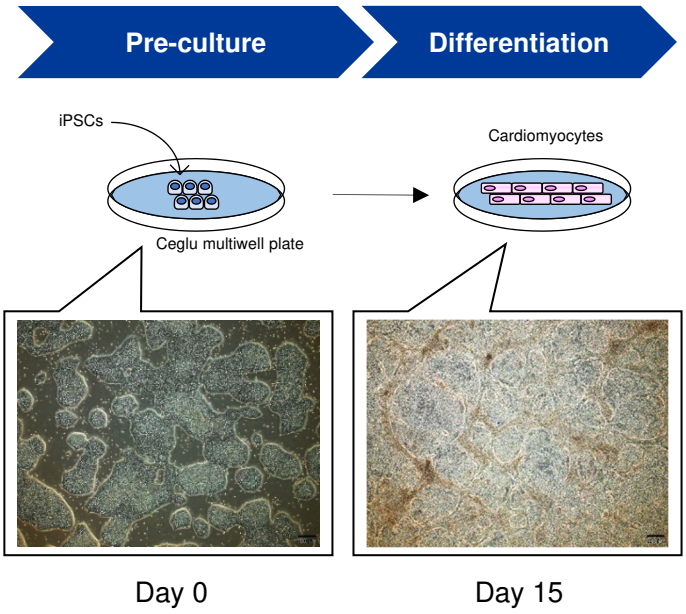

Fig.1 Workflow of cardiomyocytes using Ceglu and cell images after differentiation (Day 15)

Results

iPSCs into cardiomyocytes were differentiated on Ceglu and protein scaffolds, and cell morphology, cell number, and expression of cardiac muscle troponin (cTnT) were evaluated. As iPSCs differentiated into cardiomyocytes, the layered cells were connected and the formation of structures was confirmed (**Fig.2**) After Day 8, beating was observed in some cells, and by Day 15, beating was observed in cells of the entire well. The number of cells obtained was 1.94 x 10^6 cells per well (2.6 cardiomyocytes were produced from single iPSC). Similar cell morphology and beating were observed when compared to differentiation on protein scaffolds.

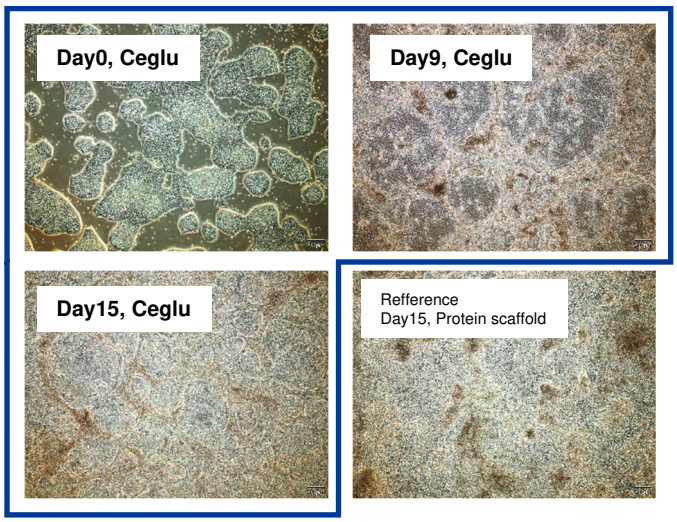

Fig.2 Differentiation of iPSCs into cardiomyocytes on Ceglu

The positive rate of cardiac muscle troponin (cTnT) in the obtained cardiomyocytes was also confirmed to be at the same level as that of protein scaffolds (protein scaffold: 43.4%, Ceglu: 40.7%) (**Fig.3).** These results suggest that cardiomyocyte can be differentiated by Ceglu at the same level as protein scaffolds. Further improvement in differentiation efficiency is expected by optimizing the conditions.

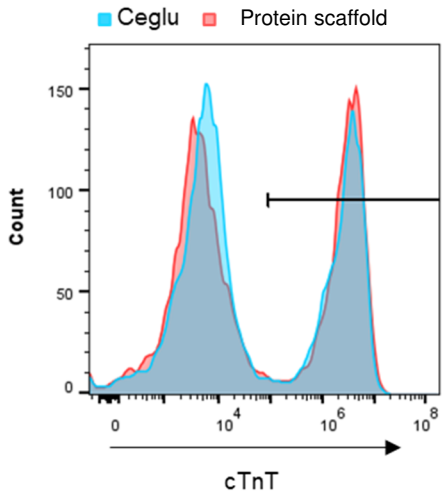

Fig.3 cTnT-positive rate of cardiomyocytes after differentiation

Products

Product	Plate type	Cat. No.
Ceglu™ multiwell plate	6-well	ASPL060001
Ceglu™ multiwell plate	96-well	ASPL970001
Ceglu™ dish	100 mm dish	ASPL100001

References

1. ThermoFisher SCIENTIFIC, Gibco™ PSC Cardiomyocyte Differentiation Kit, Prod. Info

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