Application Note

Differentiation of iPSCs into hematopoietic progenitor cells and monocyte using Ceglu™, a chemically defined scaffold

Background

Various immune cells derived from induced pluripotent stem cells (iPSCs) are being investigated for their potential application in cancer immunotherapy, with promising therapeutic efficacy. The differentiation of these immune cells proceeds through mesodermal lineage, vascular endothelial cells, and hematopoietic progenitor cells (HPCs). Traditionally, this process has relied on two-dimensional (2D) culture systems utilizing feeder cells such as mouse bone marrow stromal cells. More recently, feeder-free 2D culture methods using Matigel® have also been established[1,2]. However, for clinical translation, it is essential to ensure safety and reproducibility, which necessitates the elimination of animal-derived components.

In this study, we confirmed the feasibility of using Ceglu™, a chemically-defined scaffold material free of animal-derived components, for the differentiation of iPSCs into HPCs and monocytes (**Fig.1**)

KeyPoint

- ✓ **Differentiation efficiency at the same level as conventional methods**
- ✓ **No animal-origin components**
- ✓ **High-purity monocytes differentiation**

Methods

- **Differentiation into HPCs**

1. Acclimate iPSCs (in-house line or SCTi003-A) on a Ceglu multiwell plate for two passages (14 days).
2. Dissociate iPSCs using Gentle Cell Dissociation Reagent and detach with a cell scraper.
3. Adjust clumps to ~200 µm and seed ~100 clumps per well in a Ceglu 6-well plate.
4. Switch differentiation medium sequentially to induce mesoderm, vascular endothelial cells, and HPCs.
5. Harvest adherent and suspended cells at appropriate time points.
6. Immunostain HPC markers and analyze by FACS.

- **Differentiation into monocytes**

1. On day 7 after HPC step 3, switch to monocyte-inducing medium.
2. Harvest adherent and suspended cells at appropriate time points.
3. Immunostain monocyte markers and analyze by FACS.

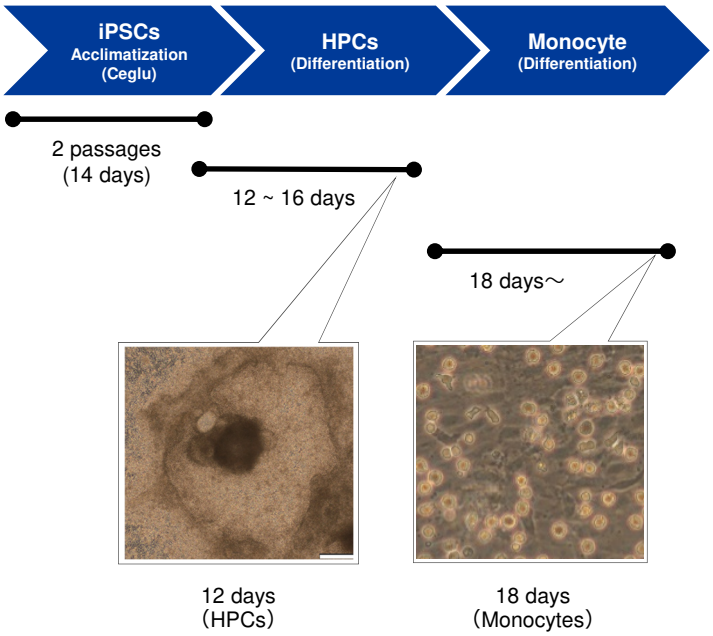

Fig.1 Workflow and cell morphology of HPCs and monocytes

Results

The following differentiation data into HPCs were obtained using both Ceglu and Matrigel® under equivalent experimental conditions.

● Differentiation into HPCs

Differentiation of HPCs was proceeded on Ceglu and Matrigel®, and cell morphology as well as cell surface marker expression were evaluated. On day 0 (two days after seeding), multiple colonies ranging from 50 to 500 μm in diameter were observed. Upon switching to hematopoietic differentiation medium, these colonies into cell aggregates and by day 7, a substantial number of suspended cells appeared around the aggregates (**Fig.3**). The suspended cells were collected and analyzed for cell surface marker expression. Co-expression of CD34 and CD43, markers indicative of HPCs, was confirmed by immunostaining and flow cytometry (**Fig.4**). Notably, the proportion of CD34+, CD43+ cells was comparable between the two conditions, with Ceglu and Matigel® yielding 54.0% double-positive cells.

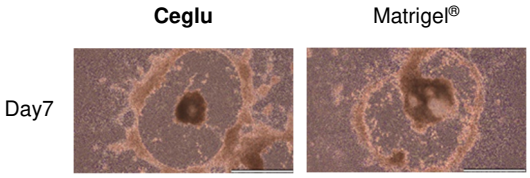

Fig.3 Cell morphology of HPCs (Day 7)

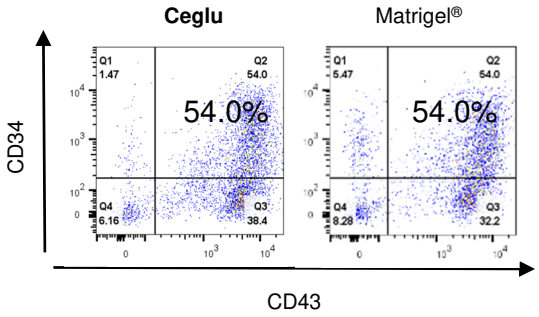

Fig.4 Expression of cell surface markers on HPCs

● Differentiation into monocytes

On day 7 of the HPCs differentiation, the culture medium was switched to a monocyte-inducing medium, and surface marker expression was subsequently evaluated. Similar to the HPCs differentiation, cell aggregates and surrounding suspended cells were observed during the monocyte differentiation process. Analysis of the suspended cells on day 18 revealed co-expression of CD45 and CD14. Notably, 84.3% of the cell population was double-positive for CD45 and CD14, showing successful and high-purity differentiation into monocytes using Ceglu (**Fig.5**).

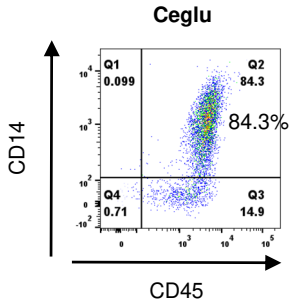

Fig.5 Expression of cell surface markers on monocytes

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. STEMCELL Technologies社 STEMdiff™ Hematopoietic Kit Protocol
2. *Cell Stem Cell.* 2013, 12.1, 114-126

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