Application Note

Differentiation of iPSCs into all three germ layers on Ceglu™, a chemically defined scaffold

Background

Differentiation of induced pluripotent stem cells (iPSCs) into the three germ layers (ectoderm, mesoderm, and endoderm) is an important process for assessing their differentiation potential and for downstream applications in generating differentiated cells. However, controlling this process experimentally can be complicated, often requiring the selection and preparation of appropriate scaffold materials based on the target germ layers and differentiated cells. In this study, Ceglu™ multiwell plates pre-coated with a chemically defined scaffold were used to induce iPSC differentiation into all three germ layers and various differentiated cell types including neural progenitor cells, cardiomyocytes, and hepatoblasts. We compared the current method using Ceglu, and conventional methods using protein-based scaffold materials by evaluating gene expression and cell morphology across the three germ layers and differentiated cells (**Fig. 1**).

Key Points

- ✓ **Consistent scaffold environment from maintenance culture to differentiation**
- ✓ **Differentiation efficiency equivalent to protein scaffolds**

Method	Maintenance culture	Three germ layers		Differentiation	
	Typical scaffold	Germ layer	Typical scaffold materials	Cell type	Typical scaffold materials
Conventional methods	Vitronectin iMatrix-511 Matrigel® etc.	Ectoderm	iMatrix-511 +POL*+PLL**	Neural progenitor cells	iMatrix-511 +POL*+PLL**
		Mesoderm	iMatrix-511	Cardiomyocytes	iMatrix-511
		Endoderm	Matrigel®	Hepatoblast	Matrigel®
Current method	**Ceglu**	Ectoderm	**Ceglu**	Neural progenitor cells	**Ceglu**
		Mesoderm		Cardiomyocytes	
		Endoderm		Hepatoblast	

*Poly-L-Ornithine **Poly-L-Lysine

Fig. 1 Comparison of iPSC differentiation into three germ layers using a conventional method (example) vs. Ceglu.

Methods

*iPSCs (253G1 line) were maintained and cultured on Ceglu for more than 10 passages and then differentiated into three germ layers[1]. Ceglu was used throughout the process from maintenance to differentiation induction.

Ectoderm and neural progenitor cells
Differentiation by Dual SMAD inhibition method[2].

Mesoderm and cardiomyocytes
Differentiation with various inhibitors[3].

Endoderm and hepatoblast
Differentiation with combination of hypoxic environment and supplements[4].

Results

- **Differentiation into three germ layers**

iPSCs maintained and cultured on Ceglu were used to induce differentiation into three germ layers. The expression of various differentiation markers was evaluated by comparing this method (Ceglu) with existing methods (iMatrix-511 and Matrigel®). In neural ectoderm differentiation, expression of differentiation markers such as SOX1, PAX6, and NES was confirmed (**Fig. 2**). In cardiac mesoderm differentiation, expression of T (Brachyury), a mesoderm marker was confirmed along with NKX2.5 and TNNT2, which are characteristic of cardiac mesoderm and cardiomyocytes (**Fig. 3**). In hepatic endoderm differentiation, expression of differentiation markers such as SOX17, CXCR4 and FOXA2 was confirmed (**Fig. 4**). With Ceglu, the same differentiation markers were expressed as in conventional methods, indicating that the scaffold material can be used for differentiation into all the three germ layers.

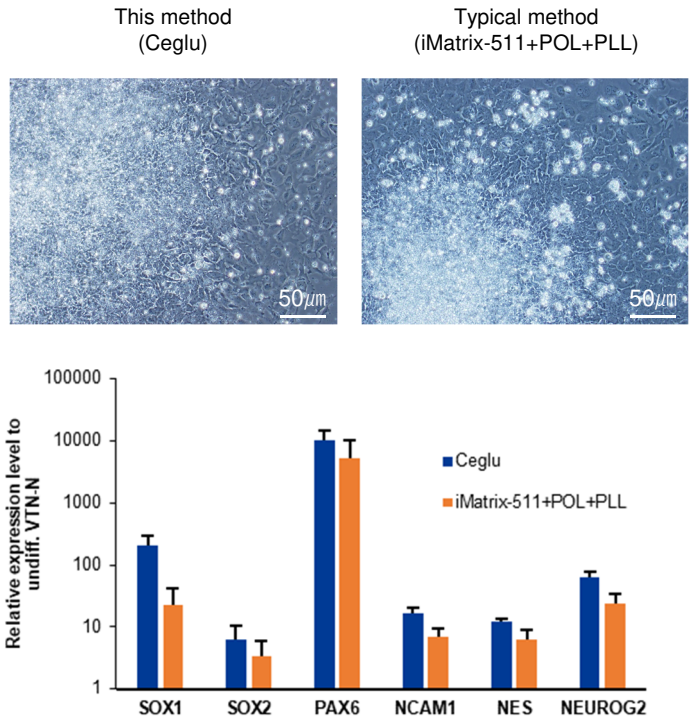

Fig. 2 Cell morphology and differentiation marker expression of neuroectoderm

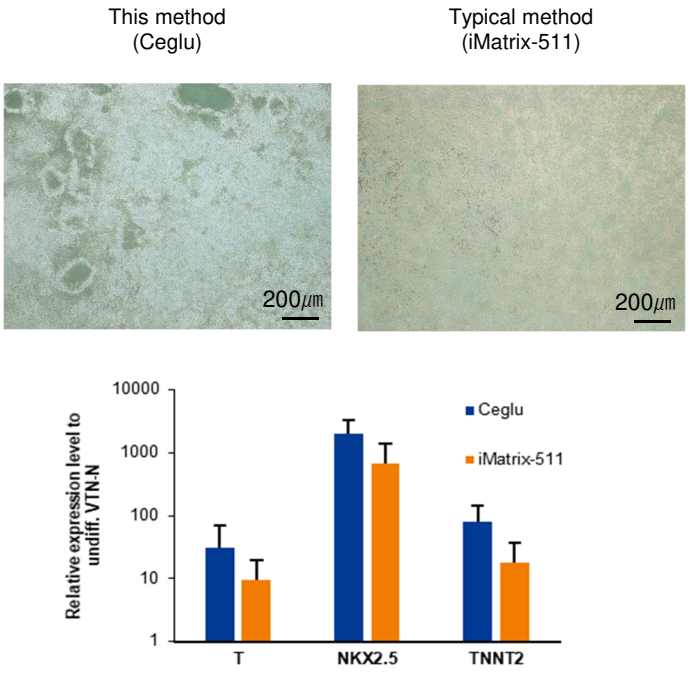

Fig. 3 Cell morphology and differentiation marker expression of cardiac mesoderm

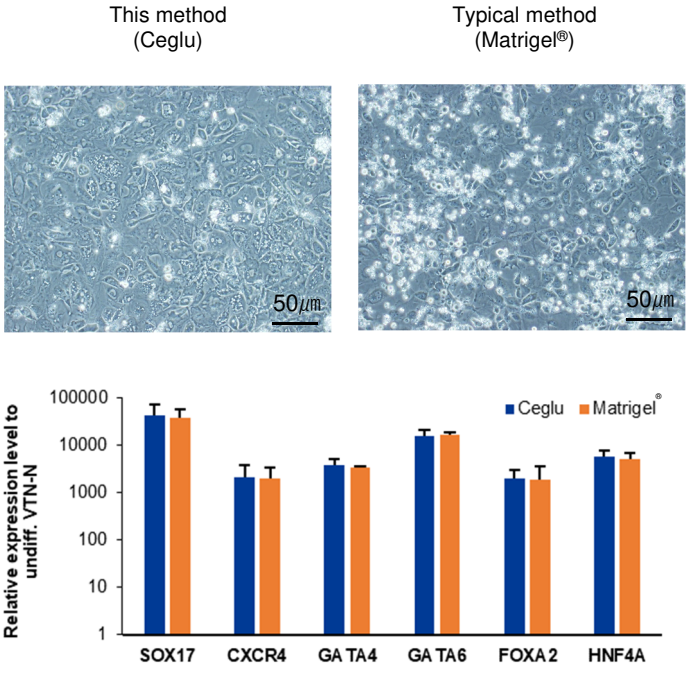

Fig. 4 Cell morphology and differentiation marker expression of hepatic endoderm

Results

- **Differentiation into cells (neural progenitors, cardiomyocytes, and hepatoblasts)**

Using cells differentiated into three germ layers on Ceglu, we induced differentiation into neural progenitor cells, cardiomyocytes, and hepatoblasts, evaluated cell morphology and differentiation marker expression. In neural progenitor differentiation, rosette-like colonies were formed, and expression of β-III tubulin was confirmed (**Fig. 5**). In cardiomyocyte differentiation, beating cells were observed and cardiac troponin (cTnT) expression was confirmed (**Fig. 6**). In hepatoblast differentiation, characteristic polygonal cell morphology and tight intercellular adhesions were observed, along with FOXA2 expression (**Fig. 7**).This study showed that Ceglu is a scaffold material that can be used consistently from maintenance culture to differentiation.
These results suggest that iPSCs cultured on Ceglu can be induced into all three germ layers and their subsequent differentiated cells.

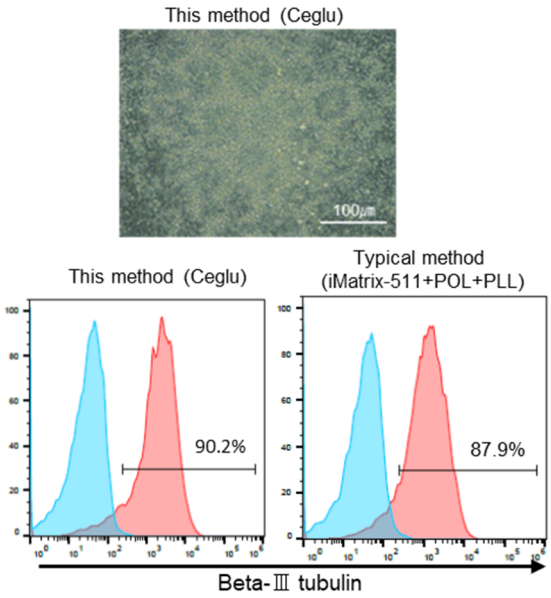

Fig. 5 Neural progenitor cells, cell morphology and differentiation marker expression

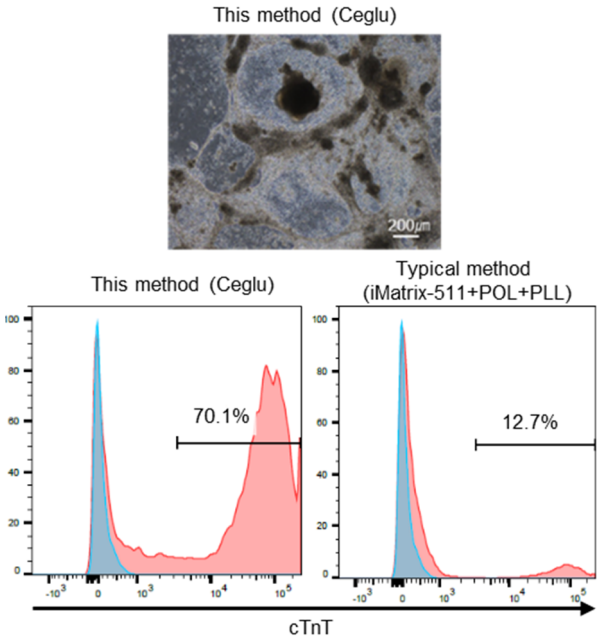

Fig. 6 Cardiomyocytes, cell morphology and differentiation marker expression

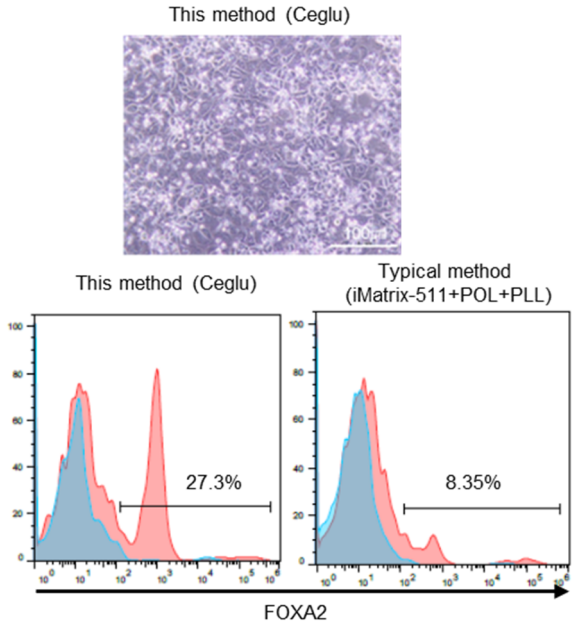

Fig. 7 Hepatoblats, cell morphology and differentiation marker expression

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. *Scientific Rep.* 2022, 15, 12, 2516
2. *Nat. Biotechnol.* 2009, 27, 3, 275-280
3. *Nat. Protoc.* 2013, 8, 1, 162-175
4. *Hepatology* 2010, 51, 1, 297-305

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