Application Note

Establishment of iPS cells using Ceglu™, chemically defined scaffold

Background

The establishment of induced pluripotent stem cells (iPSCs) is one of the most important processes in regenerative medicine and drug discovery because their safety and quality affect later applications. Sendai virus (SeV) vectors, which have a low risk of genome damage and excellent transduction efficiency, are used to introduce the reprogramming factors into cells. However, their residual is a concern in terms of safety and quality and they must be eliminated at an early stage. Herein, we developed a new method for establishing iPSC using Ceglu™, a chemically defined scaffold, in order to eliminate the remaining SeV vectors in the cells.

KeyPoint

- ✓ **Reduction of viral vector usage**
- ✓ **Early disappearance of viral vectors**
- ✓ **Establishment of high-quality iPSCs**

Method

This method uses adherent cells as a raw material; Ceglu also can be applied with the conventional establishment method using floating cells.

1. Research grade frozen PBMCs are seeded onto a Ceglu multiwell plate (**Fig1**)
2. After 5 days cultivation, remove floating cells by medium exchange and confirm adherent cells on the plate
3. SeV vector (Tokiwa Bio, SRV™ iPSC-4 Vector) is added to the wells
4. Leave for 2 hours to infect cells with SeV vector
5. Medium change to remove SeV vector
6. Gradually switch to iPSCs medium during culture
7. Colonies were detached and passaged (P0) approximately 2 weeks after infection

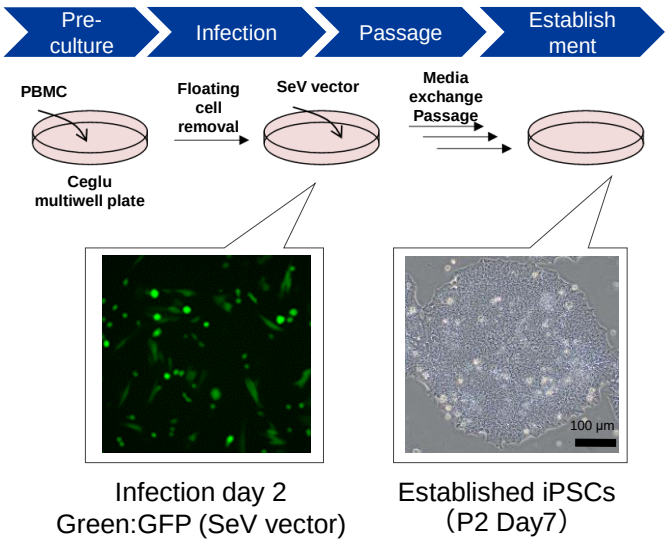


Fig1. iPSCs establishment flow and cell images

Result

- **Vector usage and iPSCs proliferation**

The relationship between vector usage and the proliferation of established iPSCs, as well as the reduction of vector usage, was evaluated (**Fig2**). As a result, it was confirmed that highly proliferative iPSCs can be established by this method of infecting SeV vector with cells adherent on Ceglu. It was also confirmed that iPSCs with proliferation could be obtained even when the amount of vector was reduced. These results suggest that a suitable scaffold environment for the establishment of iPSCs is provided by Ceglu, resulting in higher efficiency. Therefore, Ceglu supports high-quality iPSCs establishment in small-scale like 96-well plates and reduces experimental costs.

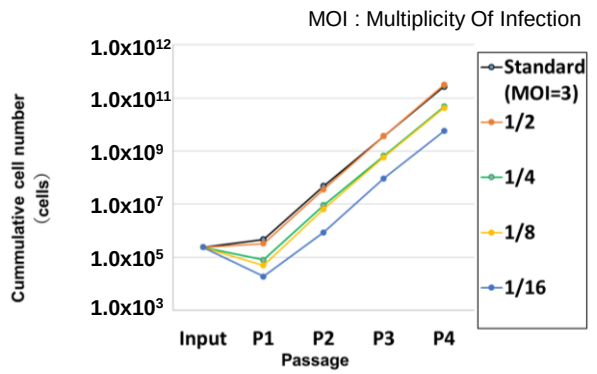


Fig2. SeV vector usage and iPSC proliferation

Under Development

Product specifications are subject to change without notice.

Result

Comparative data between iPSCs established from floating cells on protein-based scaffolds and adherent cells cultured on Ceglu are shown below

- **Remaining SeV vectors in cells**

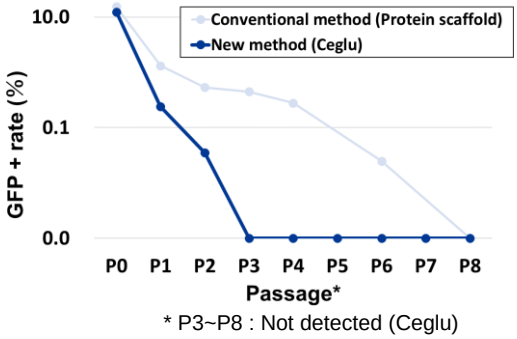
Vector residual of iPSCs established on Ceglu was evaluated using SeV vectors carrying the GFP gene (**Fig3**). We confirmed that iPSCs established on Ceglu show no fluorescence of vector-derived GFP after P3. This result suggests that the virus disappears earlier than with the conventional establishment method using floating cells.

- **Positive rate of undifferentiated markers**

The positive rate of the undifferentiated marker TRA-1-60 was evaluated for the obtained iPSCs over time (**Fig4**). iPSCs obtained by the Ceglu-based method showed a high TRA-1-60 positive rate from P1, confirming a tendency to obtain highly pure iPSCs at an early stage[1].

- **Embryoid bodies (EBs) forming**

iPSCs obtained by both methods were seeded onto 96-well plates (Corning® ULA plates) with ultra-low adhesion culture surface, and their ability to form embryoid bodies (EBs) was evaluated (**Fig5**). iPSCs established by Ceglu reproducibly produced spherical EBs. On the other hand, EBs formation were not observed in iPSCs established from floating cells. These results suggest that this method using Ceglu can be useful to improve the efficiency of differentiation applications through EBs, such as neural organoids[2,3].



* P3~P8 : Not detected (Ceglu)

Fig3. Comparison of Residual SeV vevtors

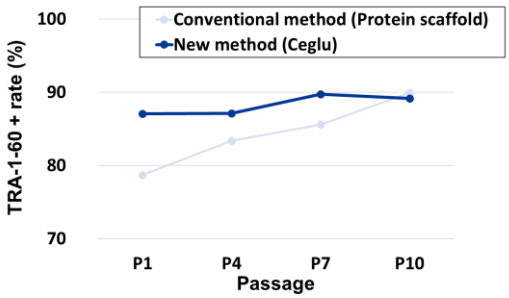


Fig4. Comparison of Undifferentiated Markers

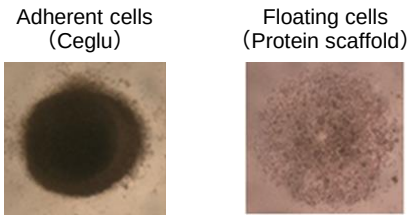


Fig5. Embryoid bodies formation*
(Experiments with seeding in ultra-low adhesion plates)
*n=6

Products

Product	Plate format	Catalog #
Ceglu™ multiwell plate	6-well	Coming soon
	96-well	Coming soon

Reference

1. Regen. Med. 2018 Oct 1;13(7):859-66.
2. PNAS. 2013 Dec 10;110(50):20284-9.
3. Scientific Rep. 2018 Jan 10;8(1):241.

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