Application Note

Generation of iPSCs using Ceglu™, a chemically defined scaffold

Background

The generation of induced pluripotent stem cells (iPSCs) is one of the most important processes in regenerative medicine and drug discovery as their safety and quality affect downstream applications. Sendai virus (SeV) vectors, which have a low risk of genome damage and high transduction efficiency, are used to introduce the reprogramming factors into cells. However, residual SeV may pose safety and quality concerns, requiring elimination at an early stage. Here, we report a new method for generating iPSCs using Ceglu™, a chemically defined scaffold, to eliminate residual vectors at early passages.

Key Points

- ✓ **Reduced viral vector dosage**
- ✓ **Early elimination of viral vectors**
- ✓ **Generation of high-quality iPSCs**

Methods

This method uses adherent cells as a starting material; Ceglu also can be used with the conventional reprogramming method using cell suspension.

1. Thaw and seed research grade frozen PBMCs onto a Ceglu multiwell plate (**Fig. 1**)
2. After 5 days in culture, remove floating cells by medium exchange and visually inspect adherent cells on the plate.
3. Add SeV vector (Tokiwa Bio, SRV™ iPSC-4 Vector) to the wells. *Use of adherent cells eliminates need for cell collection.
4. Incubate for 2 hours to infect cells with SeV vector.
5. Remove SeV vector and change medium.
6. Gradually switch to iPSC culture medium.
7. Approximately 2 weeks after infection, detach formed iPSC colonies and passage (P0).

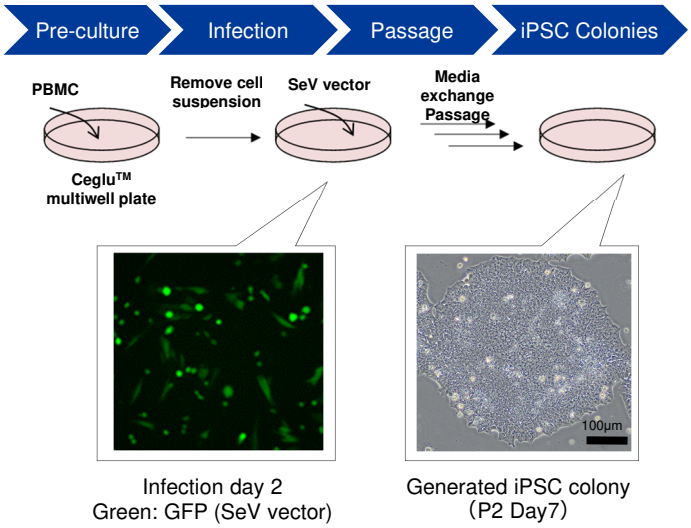

Fig. 1 Workflow of iPSC generation and cell images

Results

- **Viral vector dosage and iPSC proliferation**

The relationship between iPSC proliferation and the passage numbers, along with the reduction in viral vector dosage, is shown (**Fig. 2**). As expected, highly proliferative iPSCs were generated by infecting cells adherent on Ceglu with the SeV vector. Even at the lowest vector dosage tested, iPSCs exhibiting proliferative ability could be obtained. These results suggest that Ceglu provides a highly efficient scaffold environment conducive to the generation of iPSCs. Therefore, Ceglu enables generation of high-quality iPSCs, even in small-scale like 96-well plates, thereby contributing to the reduction of experimental costs.

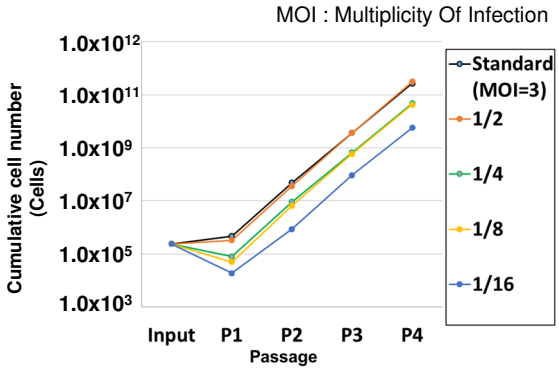

Fig. 2 SeV vector dosage and iPSC proliferation

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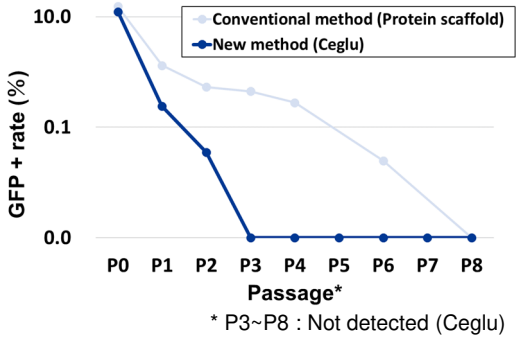
Results

Comparative data between iPSCs generated using conventional method with cell suspensions and protein-based scaffolds and the new method with adherent cells cultured on Ceglu are shown below.

● Residual SeV vectors in cells

Residual SeV in iPSCs generated on Ceglu was evaluated using SeV vectors carrying the GFP gene (**Fig. 3**). After P3, iPSCs generated on Ceglu, no fluorescence from vector-derived GFP was observed. These results suggest that SeV is eliminated earlier on Ceglu compared to the conventional method using suspended cells.



* P3~P8 : Not detected (Ceglu)

Fig. 3 Comparison of residual SeV vectors

● Expression of undifferentiated marker

The expression of the undifferentiated marker TRA-1-60 was evaluated in the obtained iPSCs over time (**Fig. 4**). The new Ceglu-based method generated a significantly higher percentage of TRA-1-60 positive iPSCs even at P1, confirming a tendency to yield highly pure iPSCs at an early stage[1].

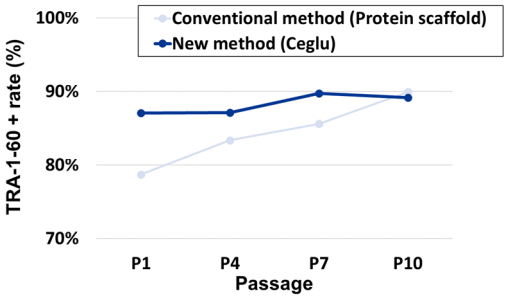
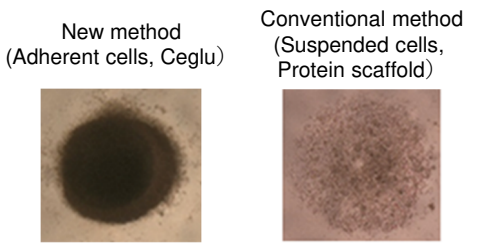


Fig. 4 Comparison of undifferentiated markers

● Embryoid body (EB) formation

iPSCs obtained by both methods were seeded onto 96-well plates (Corning® ULA plates) with an ultra-low attachment surface, and their ability to form embryoid bodies (EBs) was evaluated (**Fig. 5**). iPSCs generated using Ceglu reproducibly formed spherical EBs, whereas no EB formation was observed in iPSCs generated from suspended cells. These results suggest that the Ceglu-based method may improve the efficiency of differentiation applications involving EBs, such as neural organoids[2,3].



*n=6

Fig. 5 Embryoid body formation*
(Tests carried out by seeding in ultra-low attachment plates)

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. *Regen. Med.* 2018, 13, 7. 859-866
2. *PNAS.* 2013, 110, 50, 20284-20289
3. *Scientific Rep.* 2018, 8, 241

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