Application Note

# Differentiation of iPSCs into neural progenitor cells and astrocytes using Ceglu™, a chemically defined scaffold

## Background

Astrocytes, a type of glial cell derived from neural progenitor cells play key roles in neural functions and are implicated in many neurodegenerative diseases, including Alzheimer's and Parkinson's disease. However, the quality of cells differentiated from induced pluripotent stem cells (iPSCs) can be influenced by factors such as cold storage and the complexity of the scaffold coating process.
In this study, we verified a simple and highly efficient method for differentiating iPSCs into astrocytes via neural progenitor cells (NPCs) using Ceglu™, a chemically defined scaffold (**Fig. 1**).

### Key Points

- ✓ **Consistent scaffold environment for differentiation into NPCs and astrocytes**
- ✓ **Highly efficient astrocyte differentiation**

## Method

- **Differentiation into neural progenitor cells**[1]

*Dual SMAD inhibition method*

1. Culture iPSCs (253G1 line) on Ceglu multiwell plate for two weeks prior to induction
2. Dissociate the iPSCs using Gentle Cell Dissociation Reagent.
3. Resuspend the dissociated cells as single cells in neural induction medium and seed onto Ceglu.
4. Culture for more than three weeks to induce differentiation into neural progenitor cells.

- **Differentiation into astrocytes**[2]

1. Thaw frozen neural progenitor cell stocks and resuspend in neural progenitor cell medium. Seed onto Ceglu at high, medium, and low densities*.
2. From the following day, replace the medium with astrocyte differentiation medium and culture for three weeks.
3. Continue culturing in astrocyte maturation medium for an additional three weeks.

* Cells were seeded at baseline density of 1.8 × $10^5$ cells/$cm^2$, with high and low densities set by adjusting the cell number ±30% relative to the medium density.

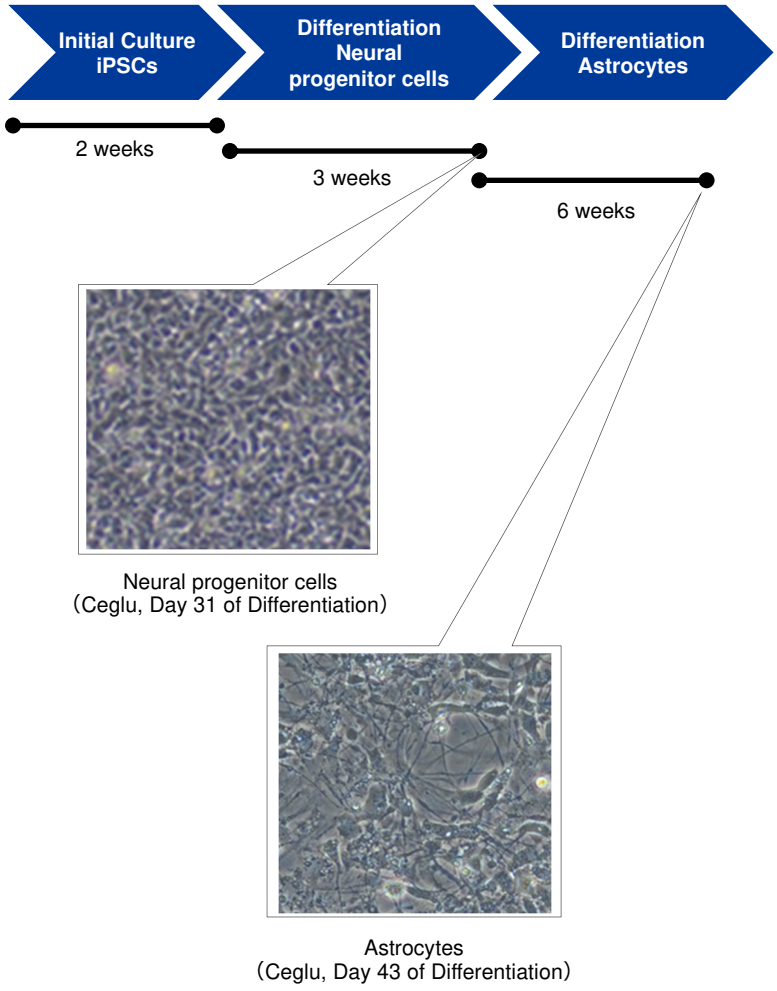


**Fig.1** Workflow and cell morphology of neural progenitor cells and astrocytes

## Result

The following results are shown together with comparative data from iPSC differentiation using protein scaffolds.

### ● Differentiation into Neural Progenitor Cells

Following differentiation, neural progenitor cells were evaluated by immunostaining (**Fig. 2**). Expression of neural progenitor markers NESTIN, PAX6 and SOX1 (data not shown) was observed in both Ceglu and protein scaffold systems, confirming the differentiation into neural progenitor cells.

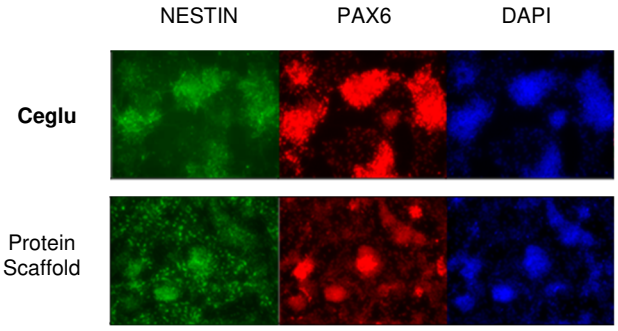


**Fig. 2** Immunostaining of neural progenitor cells

### ● Differentiation into Astrocytes

The resulting NPCs were induced to differentiate into astrocytes. Expression of GFAP, an astrocyte marker, was assessed by immunostaining. To evaluate the effect of seeding density (low, medium, or high) on differentiation efficiency, GFAP positivity was quantified by flow cytometry. After the 6-week differentiation protocol on Ceglu, astrocyte-specific cell morphology and GFAP expression were consistently observed across all seeding densities (**Fig. 3**). Additionally, a higher percentage of GFAP-positive cells was revealed on Ceglu compared to protein scaffold conditions at all densities (**Fig. 4**). The expression of neuronal marker DCX was negative across all conditions (data not shown), further supporting the astrocyte-specific differentiation. These results demonstrate that Ceglu supports both the efficient generation of neural progenitor cells and their subsequent differentiation into astrocytes, offering a simplified and consistent workflow.

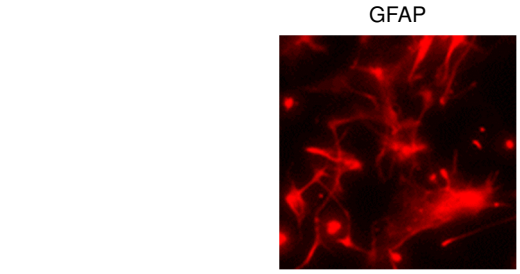


**Fig.3** Immunostaining of astrocytes on Ceglu

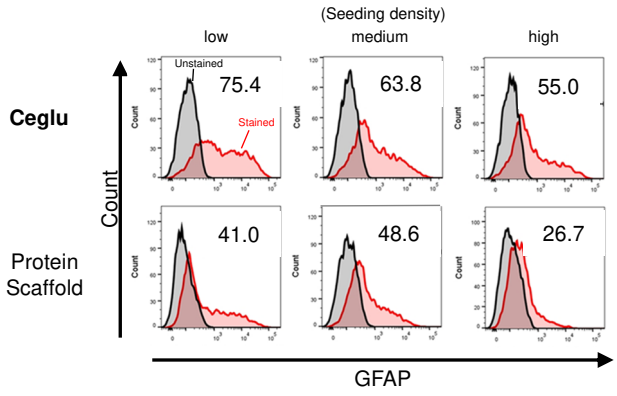


**Fig. 4** GFAP positivity of astrocytes

## Products

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

## Reference


1. STEMCELL Technologies, Generation and Culture of Neural Progenitor Cells Using the STEMdiff™ Neural System, 5.2 Monolayer Culture Protocol
2. STEMCELL Technologies, STEMdiff™ Astrocyte Differentiation Kit, STEMdiff™ Astrocyte Maturation Kit



SEKISUI AMERICA CORPORATION

6659 Top Gun Street
San Diego, CA 92121
Contact : support_life@sekisui.com


For more information
Please check our WEB