

# Reproducible Automated iPSC Culture using Ceglu™, a chemically defined scaffold

## Background

The industrialization of regenerative medicine requires a reliable culture process that consistently reproduces stem cell culture conditions. However, manual coating with protein scaffolds can introduce variability issues, such as uneven coating and inter-operator variability. To address these issues, we combined Ceglu™ multiwell plates pre-coated with Ceglu, a chemically defined scaffold and an automated culture system.

In this study, we compared cell culture surfaces in multiwell plates manually coated with protein scaffolds versus those coated with Ceglu using a coating machine. We also evaluated culture reproducibility by measuring doubling time in both manual and automated systems (**Fig. 1**).

### Key Points

- ✓ Uniform cell culture surface
- ✓ High cell culture reproducibility

## Method

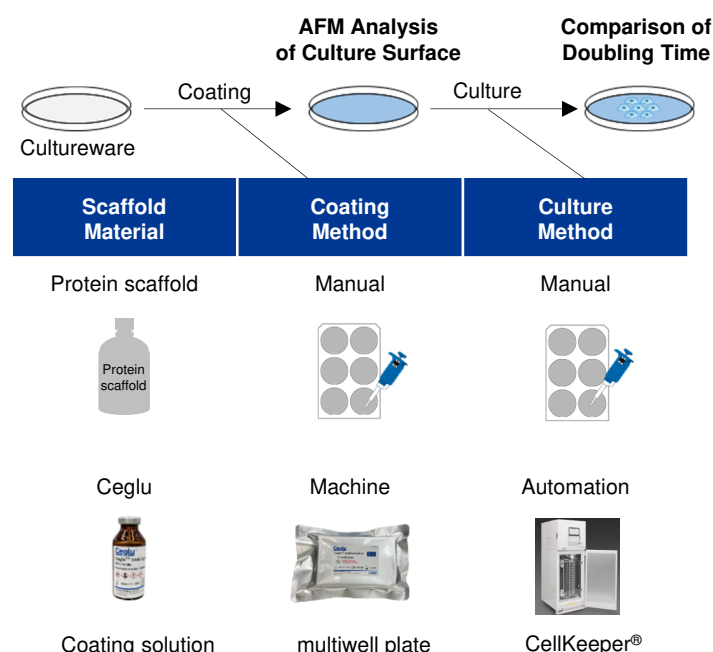
### Evaluation of Cell Culture Surfaces

- 6-well plates were coated manually with protein scaffolds following standard protocols<sup>1, 2</sup>.
- Ceglu coating solution was applied to the 6-well plates using a coating machine.
- Cell culture surfaces prepared in steps 1 and 2 were evaluated using atomic force microscopy (AFM).

### Comparison of Reproducibility

- Condition 1:** Three technicians manually coated 6-well plates with protein scaffolds and performed medium changes. Each technician cultured iPSCs in three 6-well plates (9 plates in total).
- Condition 2:** iPSCs were cultured in nine 6-well plates using Ceglu multiwell plates, with medium exchange by an automated machine (CellKeeper® by RORZE Lifescience Inc.)

After 5 days of culture under both conditions, cell counts were performed, doubling times for each well were calculated, and reproducibility was assessed.



**Fig. 1** Comparison of surface characteristics and reproducibility using protein scaffolds and Ceglu

## Result

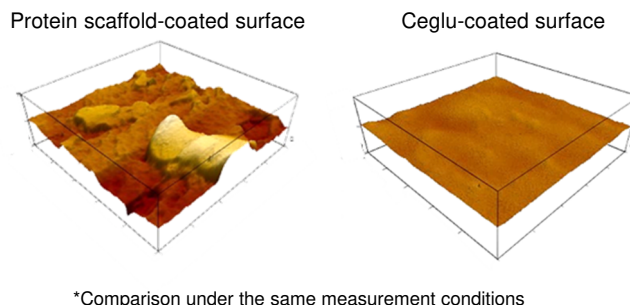
### ● Uniformity of Cell Culture Surface

Cell culture surfaces coated with protein scaffolds and Ceglu were evaluated by AFM in water (**Fig. 2**). Protein scaffold-coated surfaces appeared uneven, with differences between areas where protein was spontaneously adsorbed and areas where it was not. In contrast, the Ceglu-coated surfaces showed a more uniform topography.

### ● Comparison of Reproducibility

Culture reproducibility was assessed by comparing doubling times between manually coated protein scaffold plates and Ceglu multiwell plates with automated coating and culture (**Fig. 3**). The protein scaffold system (**Condition 1**) showed large variations in doubling time due to manual handling. In contrast, the Ceglu system (**Condition 2**), exhibited minimal variation, demonstrating high culture reproducibility.

These results demonstrate the potential of pre-coated Ceglu multiwell plates combined with automation for robust, reproducible stem cell culture. Further development of Ceglu applications will focus on exploring different plate formats and integration with additional automated systems.



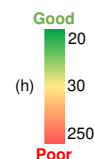
**Fig. 2** Atomic force microscopy (AFM) analysis of culture surface conditions.

#### Condition 1: Protein scaffold, manual method

	Protein scaffold, Manual method								
	Operator A			Operator B			Operator C		
	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9
Well 1	24.2	22.0	22.9	25.1	24.4	25.4	69.7	21.7	23.0
Well 2	23.1	22.5	23.2	26.1	22.9	30.2	229.9	22.7	23.2
Well 3	23.2	21.1	22.9	25.5	23.7	25.4	183.9	22.1	22.1
Well 4	24.4	21.1	24.0	24.2	25.0	29.5	82.5	22.1	21.9
Well 5	24.3	23.6	23.0	24.5	22.7	29.0	235.3	23.0	22.3
Well 6	24.9	36.3	22.9	24.7	21.0	29.2	104.3	21.2	23.0

#### Condition 2: Ceglu, automated method

	Ceglu, Automation method								
	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9
Well 1	21.0	22.1	20.5	20.5	21.1	20.5	21.1	20.8	20.5
Well 2	22.1	21.8	20.9	21.6	21.9	21.2	21.4	21.3	20.8
Well 3	21.9	22.3	23.6	21.4	22.0	20.9	21.4	22.8	21.3
Well 4	21.4	21.6	19.8	20.9	21.2	20.3	21.0	21.6	20.4
Well 5	22.3	21.5	21.0	21.9	22.0	20.6	20.8	22.2	20.4
Well 6	24.8	24.9	23.5	23.9	23.5	21.8	22.1	22.6	21.3



**Fig. 3** Heatmap of iPSC doubling times (h) comparing the manual protein scaffold method (**Condition 1**) and the automated Ceglu method (**Condition 2**)

## Products

Product	Catalog #
Ceglu™ coating solution	Coming soon
Ceglu™ multiwell plate (6-well)	Coming soon

## References

1. User Protocol for Human induced Pluripotent Stem Cells Version 4 (EBiSC®)
  2. CiRA\_Ff-iPSC\_protocol\_JP\_v140310 (CiRA-F™)
- \* EBiSC®: European Bank for induced pluripotent Stem Cells  
 \*\* CiRA-F: Center for iPS Cell Research and Application Foundation, Kyoto University

This materials is based on data presented at ISSCR 2023 (International Society for Stem Cell Research 2023) and JSRM 2022 (Japanese Society for Regenerative Medicine 2022).

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