Application Note

Reproducible automated iPSCs 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**

Methods

Evaluation of Cell Culture Surfaces

1. 6-well plates were coated manually with protein scaffolds following standard protocols[1, 2].
2. Ceglu coating solution was applied to the 6-well plates using a coating machine.
3. 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.

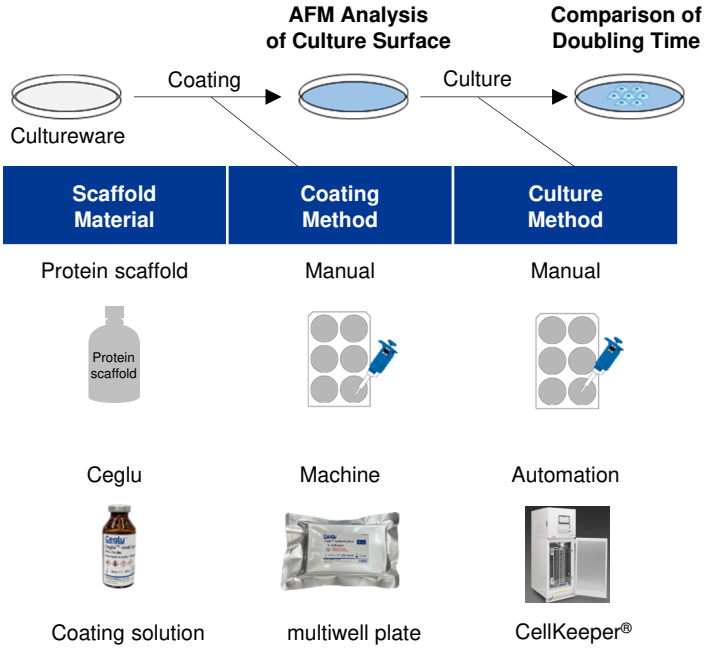


Fig. 1 Comparison of surface characteristics and reproducibility using protein scaffolds and Ceglu

Results

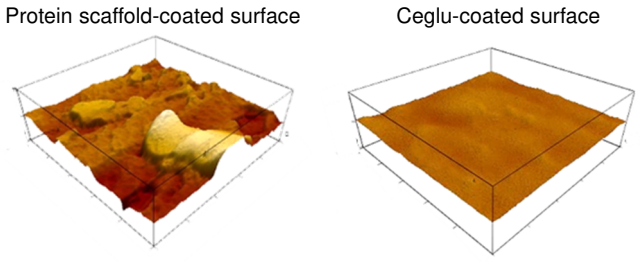
- **Uniformity of Cell Culture Surface**

Cell culture surfaces coated with protein scaffolds and Ceglu were evaluated by AFM in water (**Fig. 2**). Protein scaffold-coated surfaces exhibited an uneven appearance, with noticeable differences between regions where proteins were spontaneously adsorbed and those where they were not. In contrast, Ceglu-coated surfaces displayed 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.



*Comparison under the same measurement conditions

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

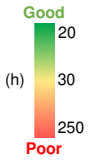


Fig. 3 Heatmap of iPSC doubling times (h) comparing the manual protein scaffold method (**Condition 1**) and the automated Ceglu method (**Condition 2**)

Products

Product	Plate type	Cat. No.
Ceglu™ coating solution	-	Contact us
Ceglu™ multiwell plate	6-well	ASPL060001
Ceglu™ multiwell plate	96-well	ASPL970001
Ceglu™ dish	100 mm dish	ASPL100001

References

1. User Protocol for Human induced Plutipotent 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

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