# Culture and Differentiation of iPS cells Using Temperature-Responsive Substrates

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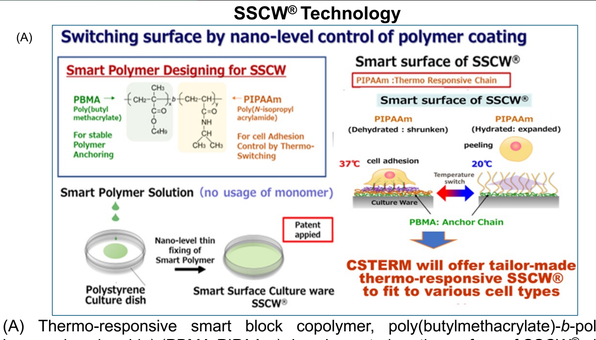
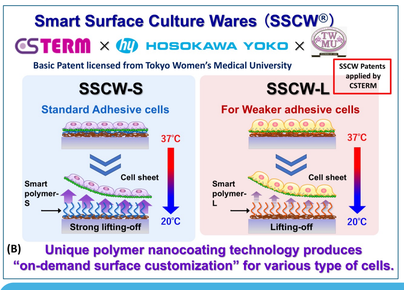
**This study, we focused on exploring the potential of clamp seeding for iPSCs using Thermo-responsive smart surface culture ware (SSCW®) and successfully implemented a clamp seeding technique with iMatrix-511.**

**Background:**
Thermo-responsive culture ware containers allow cell sheets to be easily detached by adjusting the temperature. However, this technique had not previously been applied to the culture of iPSCs.
**Objective:**
This study aimed to develop and evaluate thermo-responsive culture ware optimised for iPSC culture.
**Study Design:**
Thermo-responsive culture ware for iPS cells was custom-made at CSTERMi. We tested iPSC adhesion and detachment on these plates using StemFit AK03 medium with iMatrix-511. The iPSC line used was CFiS-S01.
**Results:**
iPSCs formed ES-like colonies and could be passaged for over five generations using temperature-dependent detachment. Analyses of methylation, flow cytometry, immunostaining, image analysis, karyotyping and genetic mutations (KaryoStat™), as well as pluripotency (PluriTest™), revealed no abnormalities.
**Conclusion:**
SSCW® is optimised for iPSC adhesion and low-damage, temperature-induced detachment, enabling a trypsin-free process. This technology supports the automated production of iPSCs, which is especially beneficial for difficult-to-handle cell types.

## SSCW® Technology



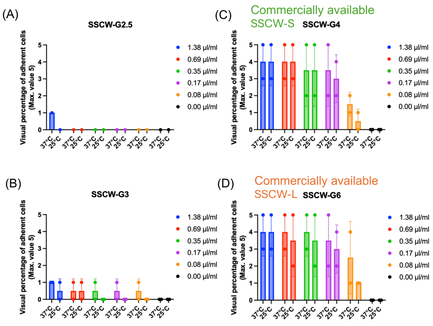

(A) Thermo-responsive smart block copolymer, poly(butylmethacrylate)-*b*-poly(*N*-isopropylacryl amide) (PBMA-PIPAAm), is spin-coated on the surface of SSCW® plates.




(B) In case of SSCW® plates used for this experiment including the initial screening tests, surface types of SSCW® show higher cell adhesiveness for G6.0(SSCW-L)>G4.0(SSCW-S)>G3.75>G3.5>G3.25>G3.0>G2.5 and also higher cell detachability for G2.5>G3.0>G3.25>G3.5>G3.75>G4.0(SSCW-S)>G6.0(SSCW-L).

**Tailored nano coating of smart polymer (PBMA-PIPAAm) realized optimal conditions for iPS cell adhesion and detachment in StemFit medium containing iMatrix-511.**

## Commercially SSCW® & Customized SSCW® (SSCW-G2.5, G3.0)




iMatrix-511 (0.5 mg/mL) was added at the following concentrations: 1.38, 0.69, 0.35, 0.17, and 0.08 μL/mL. Visual scoring of iPS cell adhesion on a 0–5 scale was used to evaluate the cell adhesion status. (A) SSCW® (surface type G2.5) and (B) SSCW® (surface type G3.0) exhibited poor adhesion and high detachment when the temperature of the plates was lowered from 37° C to 25° C. In contrast, (C) SSCW® (surface type G4.0) and (D) SSCW® (surface type G6.0), which are commercially available as SSCW-S and SSCW-L, respectively, showed smooth cell adhesion and low cell detachment using the aforementioned harvest method.

**G2.5 and G3 exhibited insufficient cell adhesion for iPSCs, even at 37° C. G4 (MP:SSCW-S) and G6 (MP:SSCW-L), on the other hand, showed excessive cell adhesion even at 25° C. Therefore, custom formulations G3.25, G3.5, and G3.75 were designed for iPSCs.**

## Characteristics of the Prepared Culture Dish

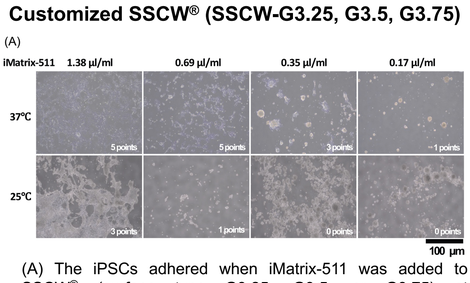
| Type | Surface | 37℃ | 20-25℃ |
|---|---|---|---|
| Cmmmercial SSCW | SSCW-G4.0(SSCW-S) | Adhesion | No cell detachment |
| | SSCW-G6.0(SSCW-L) | Adhesion | No cell detachment |
| Custom SSCW | SSCW-G2.5 | Non-adhesive | Non-adhesive |
| | SSCW-G3.0 | Non-adhesive | Non-adhesive |
| | SSCW-G3.25 | Adhesion | Cell detachment |
| | SSCW-G3.5 | Adhesion | Cell detachment |
| | SSCW-G3.75 | Adhesion | Cell detachment |

A temperature-responsive surface optimized for iPSCs now enables enzyme-free cell detachment simply by lowering the temperature. This advancement, which was made by modifying PBMA-PIPAAm to promote iPSC-specific adhesion and colony formation, improves clinical iPSC manufacturing by shortening processes, facilitating automation, minimizing contamination risks, and enhancing cell quality.

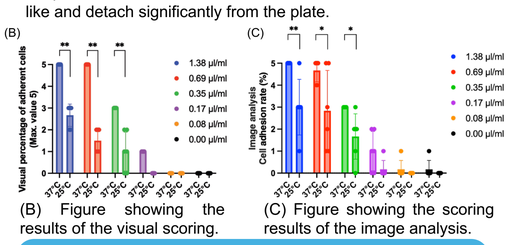
**Acknowledgments**
SSCW® plates (surface type - G2.5, G3.0, G3.25, G3.5, G3.75) used for this experiment were provided by CSTERMi. This research was supported by AMED under Grant Number JP22bm0104001, JP23bm1323001h0101, JP24bm1323001h0102 and JP25bm1323001h0103. We would also like to express our sincere gratitude for the generous donations made to the CiRA Foundation, which greatly contributed to this research.
**COI Disclosure Statment**
Yoshiki Nakashima has no conflicts of interest to declare.

CSTERMi *Cell Sheet Tissue Engineering Regenerative Medicine Initiatives, Japan*
*Representative Director: Teruo Okano Ph.D.* https://www.csterm.com

## Customized SSCW® (SSCW-G3.25, G3.5, G3.75)




(A) The iPSCs adhered when iMatrix-511 was added to SSCW® (surface type G3.25, G3.5, or G3.75) at concentrations of 1.38, 0.69, or 0.35 μL/mL, and the plate was kept at 37° C. After a 7-day seeding period, lowering the temperature to 25° C caused the iPSCs to become cell sheet-like and detach significantly from the plate.

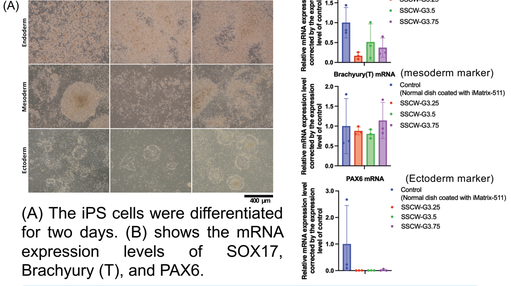



(B) Figure showing the results of the visual scoring.

(C) Figure showing the results of the image analysis.

**Cell adhesion and detachment of temperature-responsive iPSCs were achieved under iMatrix-511 concentration conditions of 1.38, 0.69, and 0.35 μL/mL.**

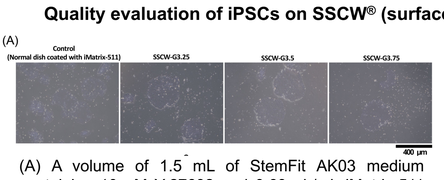
## Differentiation potential on SSCW®




(A) The iPS cells were differentiated for two days. (B) shows the mRNA expression levels of SOX17, Brachyury (T), and PAX6.

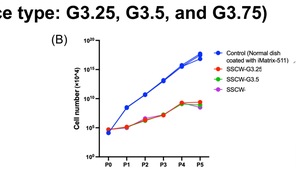
**iPSCs tend to detach easily when induced to differentiate on a custom SSCW®.**

## Quality evaluation of iPSCs on SSCW® (surface type: G3.25, G3.5, and G3.75)



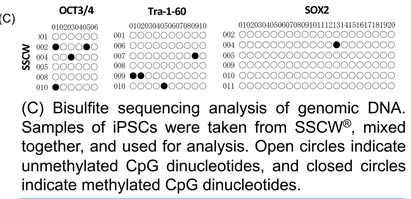

(A) A volume of 1.5 mL of StemFit AK03 medium containing 10 μM Y-27632 and 0.69 μL/mL iMatrix-511 (0.5 mg/mL) was added to SSCW®. iPSCs were seeded onto a control dish coated with iMatrix-511 or an SSCW® surface (types G3.25, G3.5, or G3.75) at a concentration of 1.3 × 10⁴ cells/well. Optical microscopy images were taken on day 7 after seeding the cells.

**The iPSC colony morphologies on SSCW3.25-3.5-3.75 were similar to those of ES cells.**



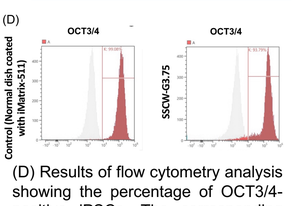

(B) For SSCW®, the cells were thermally detached and seeded at a concentration of one-tenth of the detached cells for each passaging. Cells from passages 0–5 were counted.

**Cells cultured on SSCW® can undergo five thermo-responsive passages.**



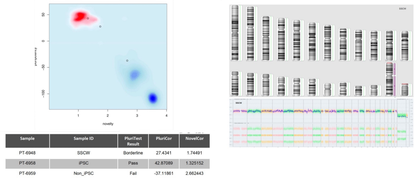

(C) Bisulfite sequencing analysis of genomic DNA. Samples of iPSCs were taken from SSCW®, mixed together, and used for analysis. Open circles indicate unmethylated CpG dinucleotides, and closed circles indicate methylated CpG dinucleotides.

**Analysis of DNA methylation in the promoter regions of the undifferentiated markers OCT3/4, Tra-1-60, and SOX2 revealed demethylation in all regions in iPSCs cultured on SSCW®.**



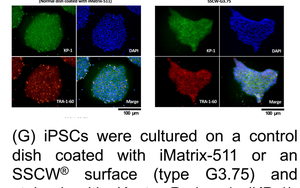

(D) Results of flow cytometry analysis showing the percentage of OCT3/4-positive iPSCs. The corresponding isotype is shown in gray.

**The expression of OCT3/4 exceeded 93% in iPSCs cultured on SSCW®.**




(E) Samples of iPSCs were taken from SSCW®, combined, and analyzed. The PluriTest score for the iPSCs cultured on SSCW® was 27.4.

(F) KaryoStat assays revealed no genetic or karyotypic mutations in iPSCs cultured on SSCW®.




(G) iPSCs were cultured on a control dish coated with iMatrix-511 or an SSCW® surface (type G3.75) and stained with Kyoto Probe 1 (KP-1), TRA-1-60, and Hoechst on day 5 after seeding at a density of 1.3 × 10⁴ cells per 35-mm dish. Fluorescence microscopy was then performed.

**The pluripotency of iPSCs cultured on SSCW® was confirmed.**

**No karyotypic or genetic changes were observed in the iPSCs cultured on SSCW®.**

**iPSCs cultured on SSCW® retain the characteristics of pluripotent iPSCs.**