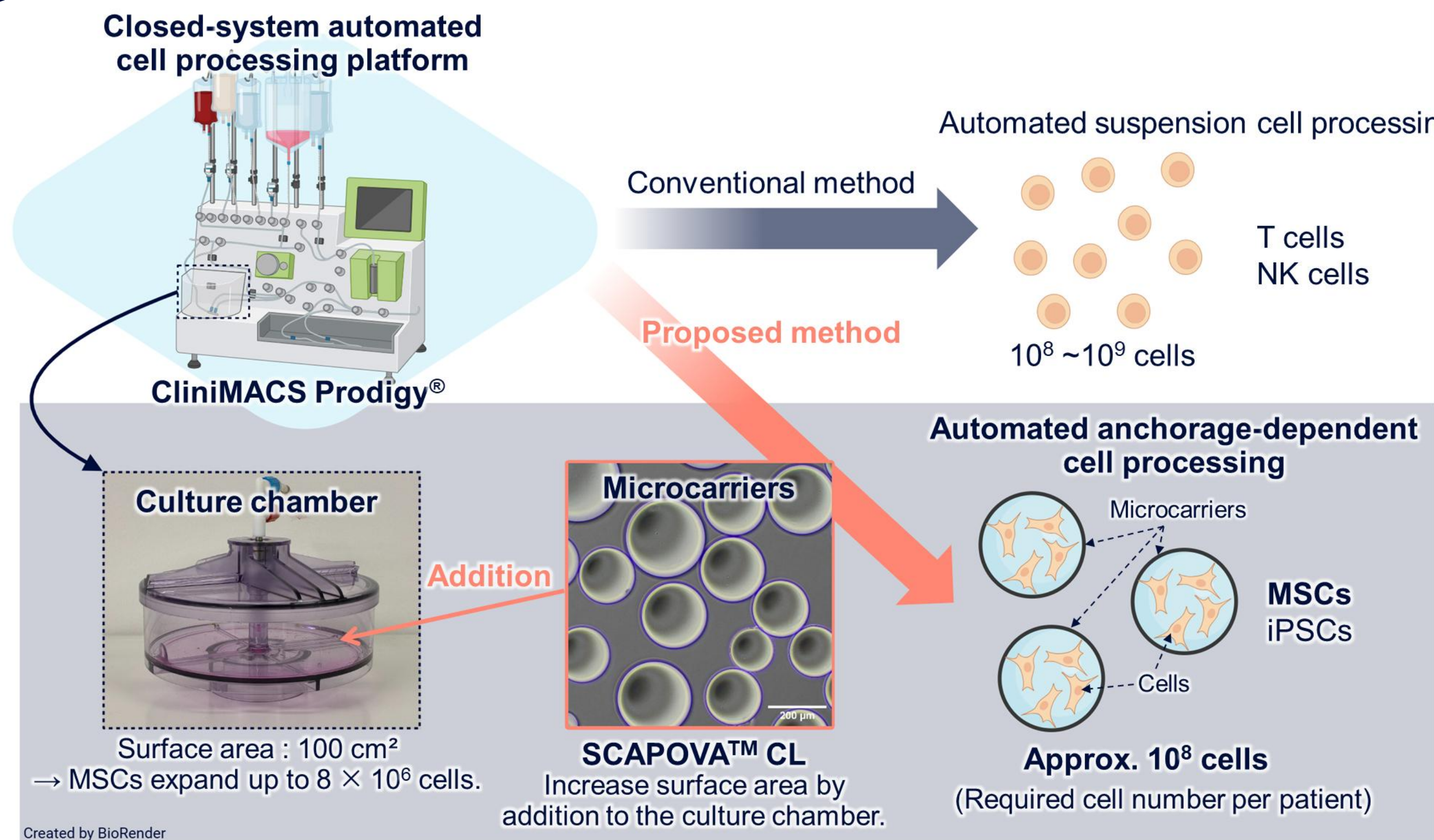


Development of an automated cell processing platform for mesenchymal stromal cells on SCAPOVA™ CL using the CliniMACS Prodigy®



R. Tanaka¹, M. Higa², M. Araki², T. Itagaki¹, T. Miwa¹, Y. Karino¹, A. Fujita¹, and G. Kobayashi¹
¹Kuraray co., Ltd., Japan, ²Miltenyi Biotec K.K., Japan

1 Introduction



Background

In recent years, research and development of cell-based therapies utilizing mesenchymal stromal cells (MSCs) has advanced⁽¹⁾. However, when cell culture is performed manually, challenges arise in ensuring safety, achieving scalable production, and controlling labor costs. Therefore, efficient and safe manufacturing methods for MSCs using automated culture systems are needed.

Existing Technology

The CliniMACS Prodigy® automated cell processing platform developed by Miltenyi Biotec enables aseptic operations within a closed system and the automation of each process step.

Challenges

The bottom surface area available within the culture chamber is limited (100 cm²), making it difficult to obtain a sufficient number of MSCs for cell therapy. Based on calculations, at most 8×10^6 MSCs can be expanded on 100 cm²⁽²⁾, whereas approximately 10^8 cells are actually required⁽³⁾.

Approach

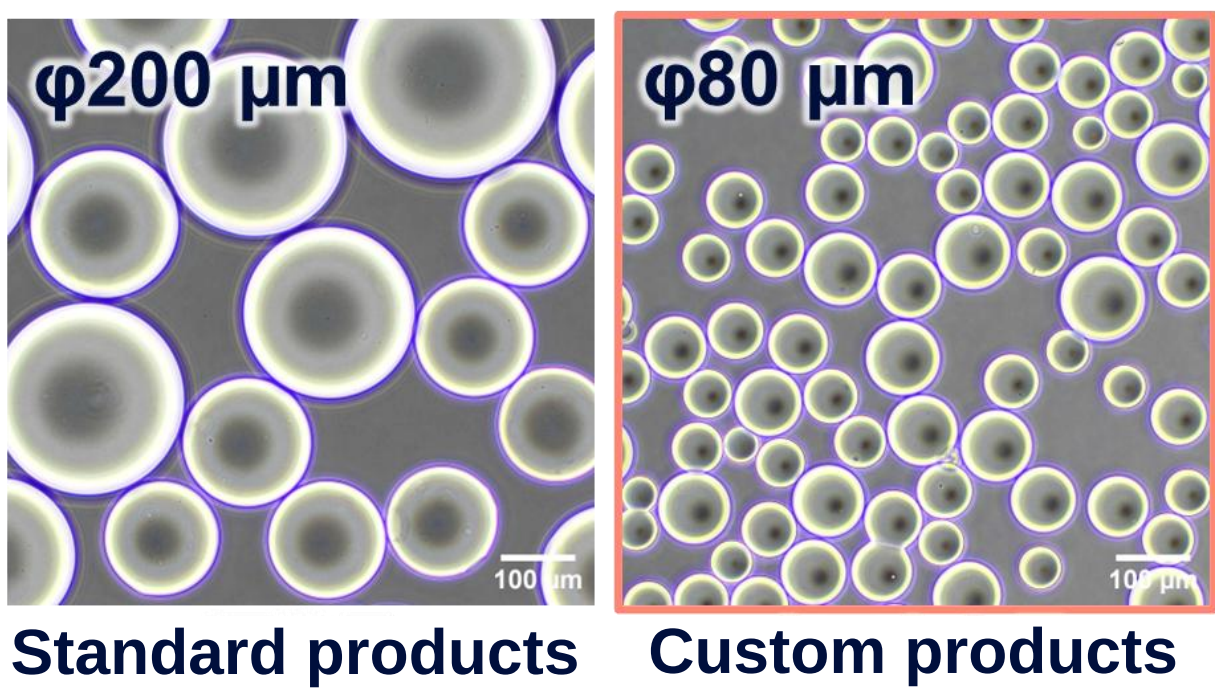
When combined with the SCAPOVA™ CL microcarrier developed by Kuraray, the available surface area for cell attachment is increased, enabling the culture of the number of cells required for treatment on a per-patient basis (above figure).

Research objective

To develop an automated culture system capable of producing the number of MSCs required for cell therapy in a single patient, this study aimed to define culture conditions that enable MSC expansion on SCAPOVA™ CL within the CliniMACS Prodigy®.

2 Materials and methods

2.1 SCAPOVA™ CL



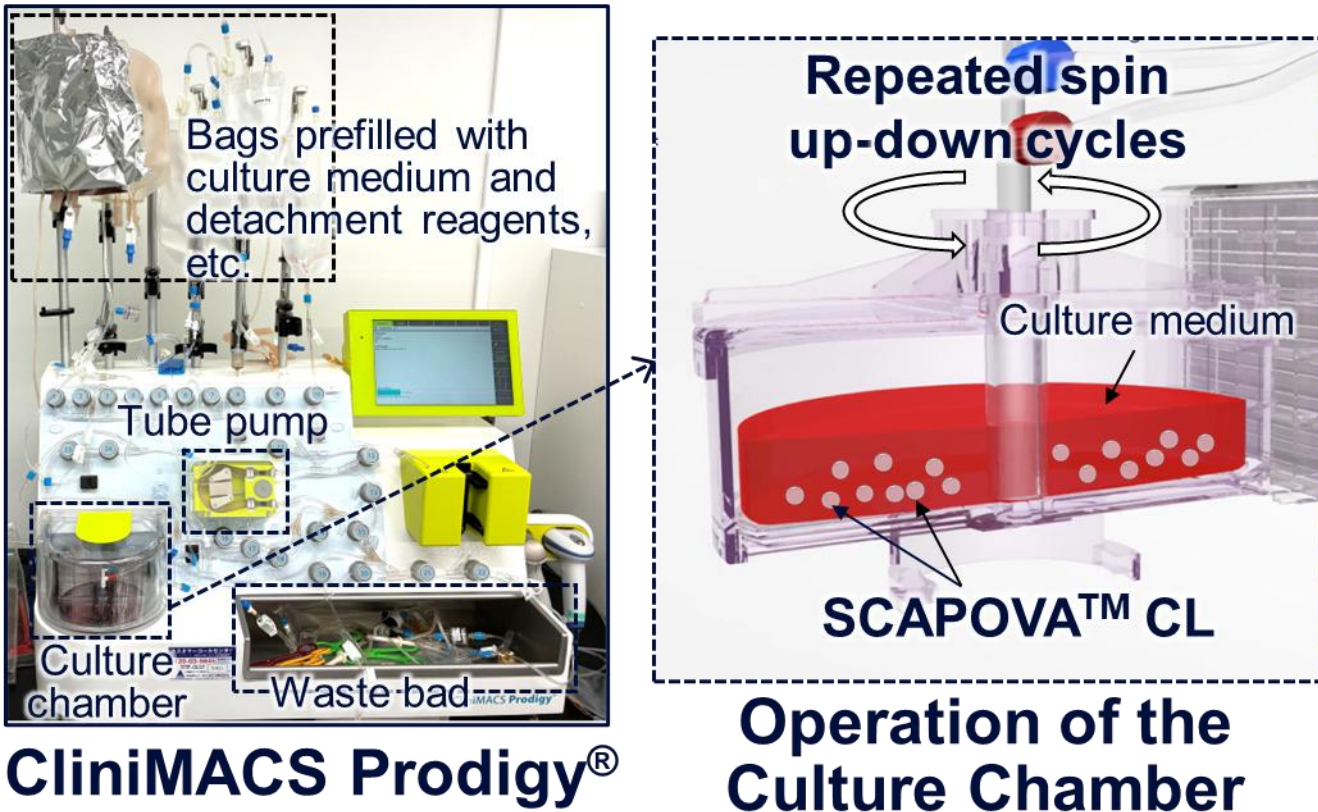
Polyvinyl alcohol(PVA)–based microcarriers. Collagen (CL) grafted onto the surface⁽⁴⁾

Features

- ✓ Quality control established with reference to GMP standards.
- ✓ Mechanically robust, with a low risk of foreign-matter contamination.
- ✓ Exhibits high proliferative capacity / supports robust MSC expansion.

Elasticity and particle size are customizable. In this study, to enhance flow through narrow channels (minimum channel diameter: 300 μm), we fabricated a CliniMACS Prodigy®-specific custom product with a smaller particle diameter (80 μm) (left figure).

2.2 CliniMACS Prodigy®



Closed-system culture platform for automated cell processing

- ✓ A closed, integrated automated system reduces manual operations and improves operational efficiency.
- ✓ The entire workflow—from cell washing, separation, activation, gene transfer (or transduction), harvest, formulation, to fill-finish—is completed on a single platform.
- ✓ At a 0.1–0.5 L culture scale, the system can manufacture the number of cells required to treat a single patient.

Typically used for suspension cells; here, SCAPOVA™ CL were added to culture anchorage-dependent cells.

2.3 Culture methods

In the CliniMACS Prodigy®, agitation culture is performed by intermittently repeating spin-up (rotation) and spin-down (pause) cycles of the culture chamber at fixed intervals. In this study, we compared (i) a condition using the standard SCAPOVA™ CL together with the standard agitation program (Active Shaker 1) with (ii) a condition using a custom SCAPOVA™ CL and a modified agitation method designed to reduce cell damage.

Agitation protocol		SCAPOVA™ CL
(i) Standard cond.	Spin-up (3 s) and spin-down (27 s) repeated cyclically.	Standard products (φ200 μm)
(ii) Modified cond.	At 3-hours intervals, spin-up and spin-down repeated cyclically.	Custom products (φ80 μm)

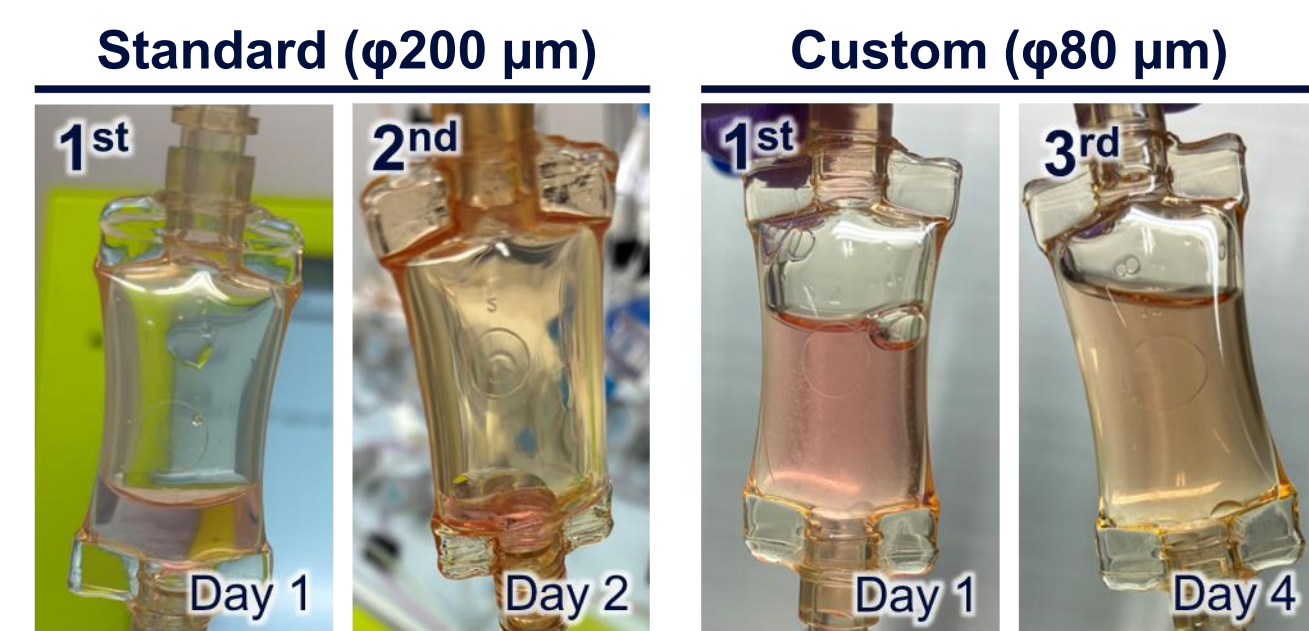
The cell types, culture medium, seeding cell number, and the dose of SCAPOVA™ CL microcarriers are listed in the table below.

Materials		Culture conditions	
Cells	Human Adipose-Derived Stem Cells (Lonza, PT-5006, P4)	Number of cells seeded	7.5×10 ⁶ cells
Medium	ADSC-1 (Kohjin Bio) +1% P/S	Microcarrier addition amount	Total surface area:1500 cm ²
Dissociation reagent	TrypLE™ Express (Gibco)	Medium volume	150 mL (D0) → 225 mL (D1)
Tubing set	TS520 (Miltenyi Biotec)	Environmental conditions	37°C, 5%CO ₂

After 4 days of automated culture under the standard and modified conditions, cells were detached from SCAPOVA™ CL using TrypLE and recovered into a separation bag. Proliferative capacity and harvested-cell quality were assessed.

3 Results and discussion

3.1 Sampling function



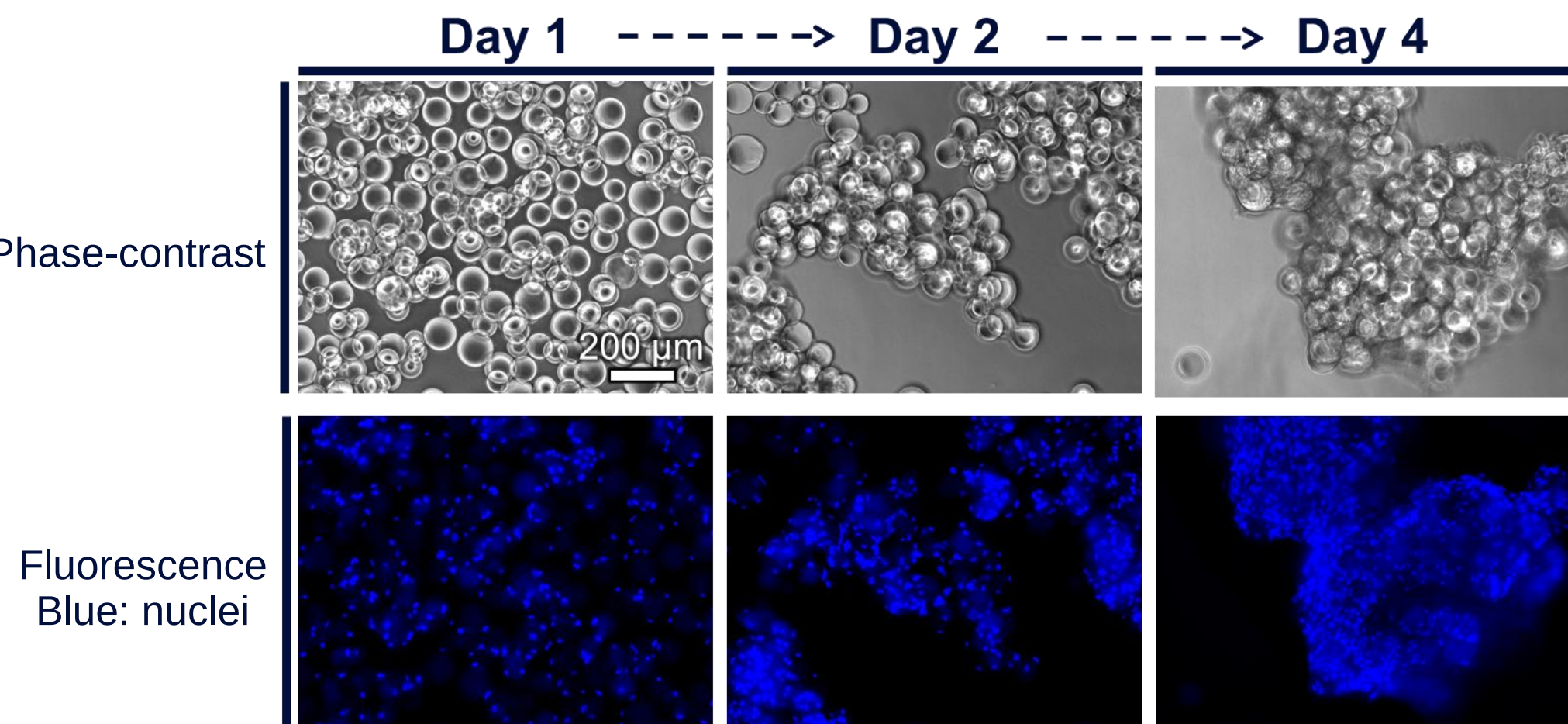
The sampling function enables in-process quality control (QC) during automated culture while maintaining the closed system.

- ✓ φ200 μm: clogging → no sampling
- ✓ φ80 μm: sampling feasible

→ In-process monitoring of culture medium composition and cell state during automated culture is enabled.

3.2 Sampled SCAPOVA™ and MSCs

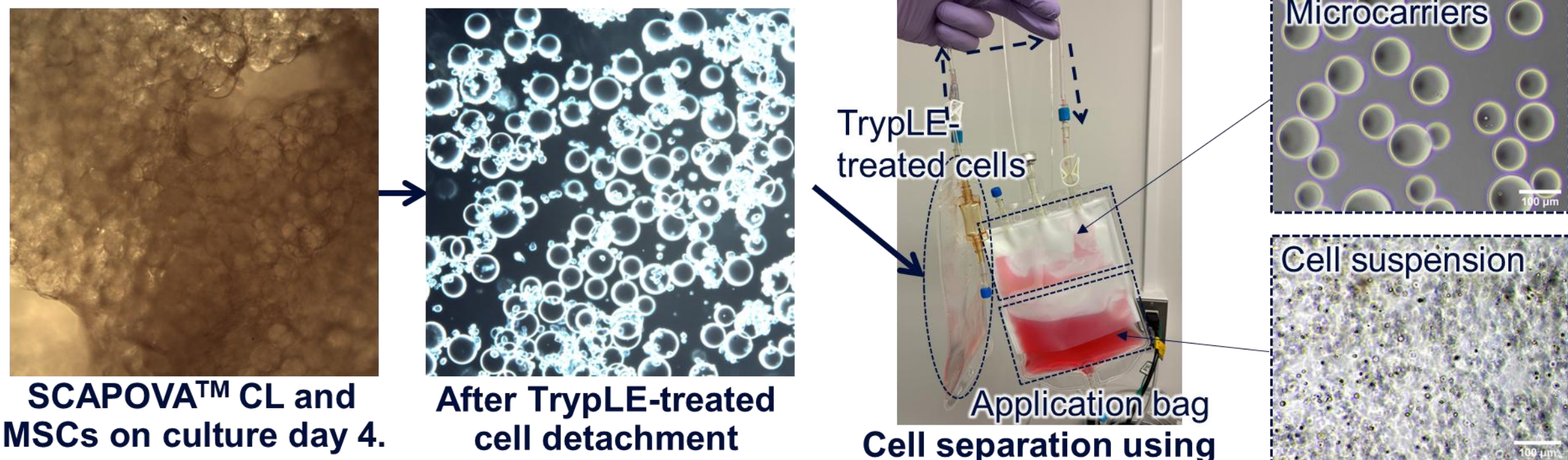
SCAPOVA® CL (φ80 μm) recovered by sampling were examined; cell nuclei were stained with Hoechst.



✓ MSC proliferation on SCAPOVA™ CL was confirmed.

3.3 Cell detachment and separation process

On culture day 4, cell harvest was initiated. After washing with PBS within the CliniMACS Prodigy®, cells were detached using TrypLE. The resulting suspension was passed through the supplied filter bag to separate the cells from the SCAPOVA™ CL.

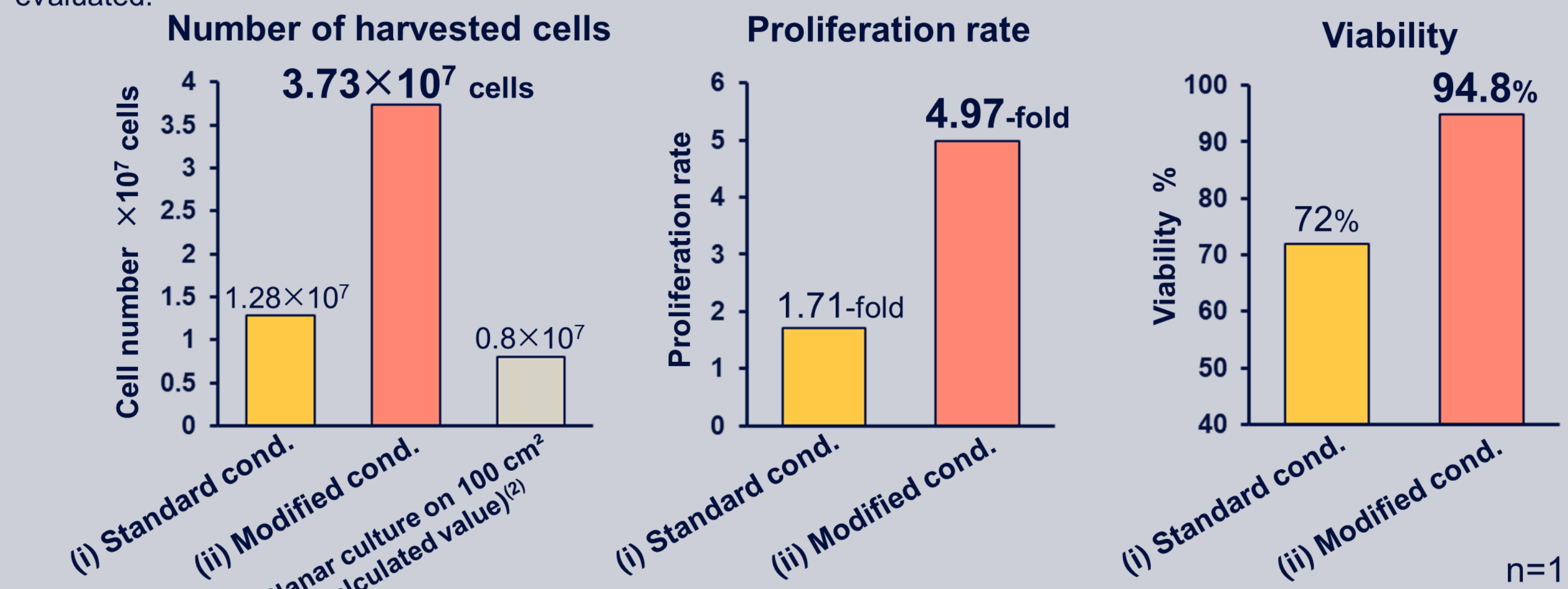


- ✓ Detachment of MSCs within the CliniMACS Prodigy® was confirmed.
- ✓ MSCs were successfully separated from SCAPOVA™ CL using the separation bag supplied with the tubing set.

3.4 Cell yield after automated culture

The cell number, proliferation rate, and viability of cells harvested on culture day 4 were evaluated.

- (i) Standard cond.: agitation at 30-s intervals; φ200 μm.
- (ii) Modified cond.: agitation at 3-h intervals; φ80 μm.



- ✓ Modified: expansion with maintained viability.
- ✓ Standard: poor expansion and viability.

Automated culture of MSCs was successfully achieved using the CliniMACS Prodigy® in combination with SCAPOVA™ CL.

→ As the standard agitation settings are optimized for suspension cells, are they unsuitable for SCAPOVA® CL? During agitation, SCAPOVA™ CL seldom entered suspension and instead were dragged across the chamber floor. Prior fluid analyses⁽⁵⁾ indicate outward bottom flow and upward wall flow during rotation, but this appears insufficient to suspend SCAPOVA™ CL. As a result, the modified, near-fixed-bed condition likely limited microcarrier dragging, reducing cell damage and improving expansion and viability.

3.5 Quality assessment of cells after automated culture

Post-culture cells were evaluated based on the definition of hMSCs proposed by the ISCT⁽⁶⁾.

Flow cytometric analysis		Tri-Lineage Differentiation		
MSC Markers	Percent positive cells	Alizarin Red	Oil Red	Alcian Blue
CD73	99.8%			
CD90	99.9%			
CD105	91.7%			
CD45, CD34, CD11b, CD19 HLA-DR	1.8%	Osteogenic	Adipogenic	Chondrogenic

n=1 ✓ It was confirmed that cells after automated culture retained MSC characteristics.

4 Conclusion

- ✓ Using small-diameter SCAPOVA™ CL (φ80 μm), sampling during automated culture was successfully achieved.
- ✓ Cell attachment and proliferation on the surface of SCAPOVA™ CL were confirmed.
- ✓ Under optimized agitation conditions, 3.7×10^7 MSCs were recovered after automated culture.

Outlook

- Further optimization of culture parameters is needed to achieve a process yielding $\geq 1 \times 10^8$ cells.
- Process designs that concurrently preserve proliferative capacity, cellular phenotype, differentiation capacity, and functional potency are expected to enable the establishment of a highly reproducible closed-system automated culture system.

References:

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