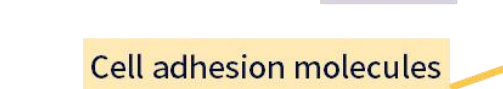


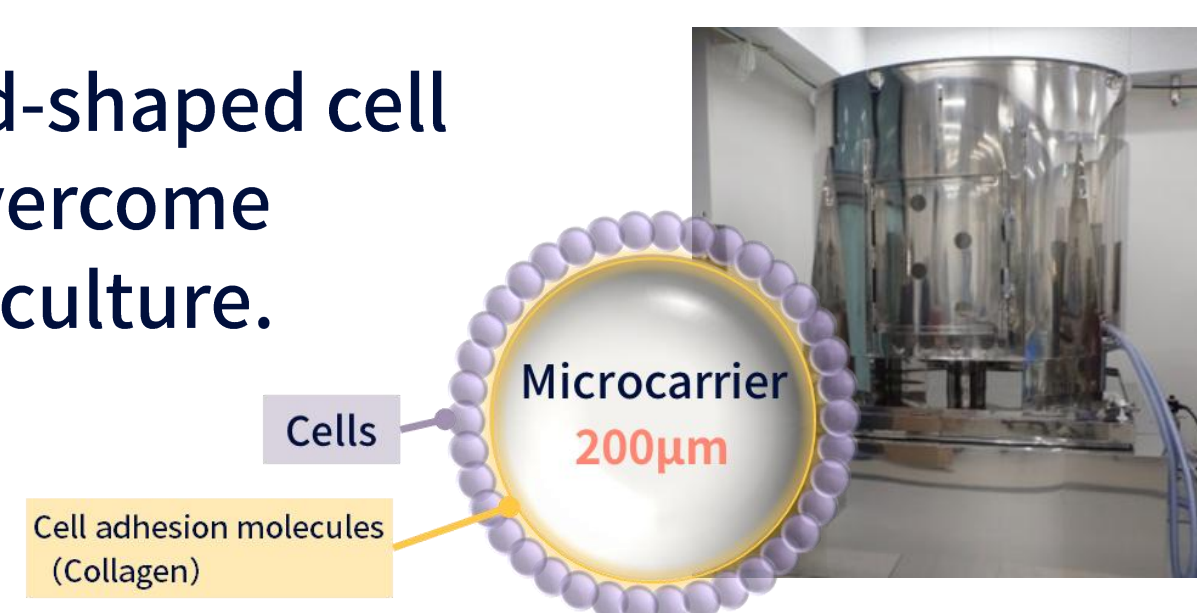
Bead-to-Bead Cell Transfer-Based Passaging Culture of Mesenchymal Stromal Cells Using SCAPOVA™ CL

○Yuka Karino, Toru Itagaki, Tatsuaki Miwa

Introduction

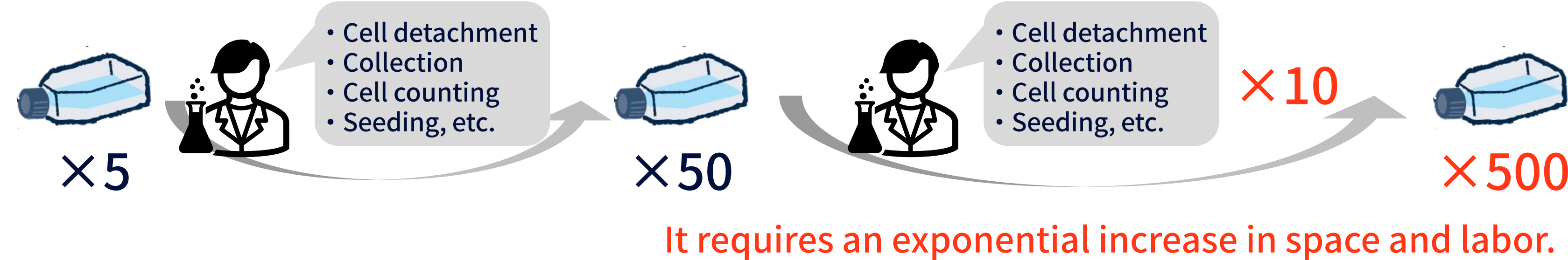
Background

- Microcarriers, which are bead-shaped cell culture scaffolds, can help overcome challenges in large-scale cell culture.
 - ✓ Compact
 - ✓ Easily automated
 - ✓ Cost-effective via labor savings
- 
- The diagram shows a purple spherical microcarrier bead. Small blue circles representing cells are attached to the surface of the bead. A yellow arrow points from a text box to the bead's surface. The text box contains the text: "Cell adhesion molecules (Collagen)".



- To culture the number of cells needed for 1 patients : 10^8 - 10^9 cells

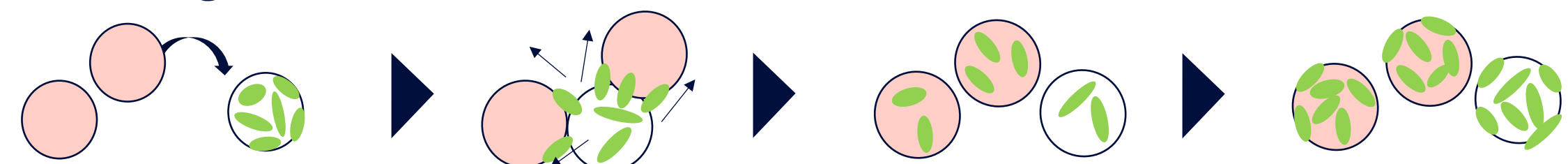
- 2D Culture



Advantages of Microcarrier Culture

Bead-to-bead cell transfer (B to B)

- Cells migrate between microcarriers in microcarrier-based culture



- Cells can be expanded without enzymatic detachment.

□ Microcarrier Culture



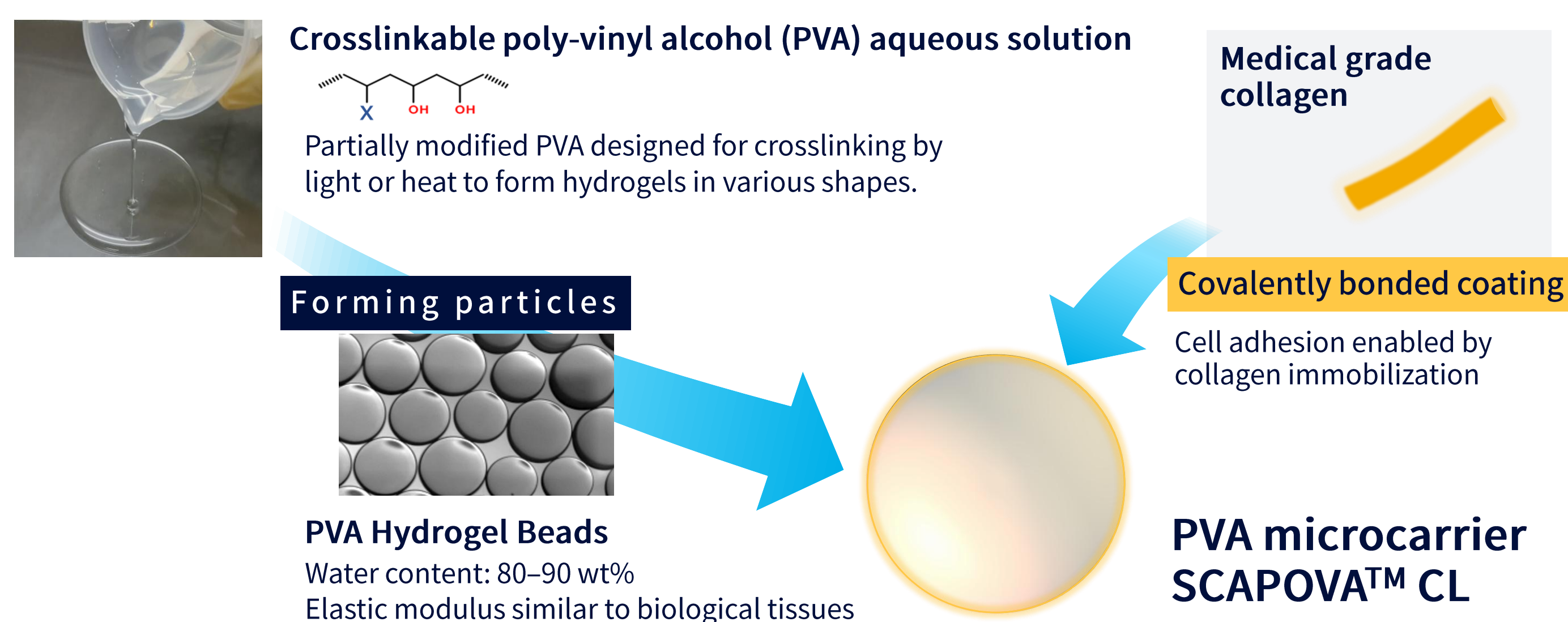
B to B passaging enables reduced labor and space in microcarrier large-scale culture.

Aim of the study

- We investigated whether mesenchymal stromal cells (MSCs) could be maintained during B to B passaging to enable efficient scale-up culture using microcarriers.

Method

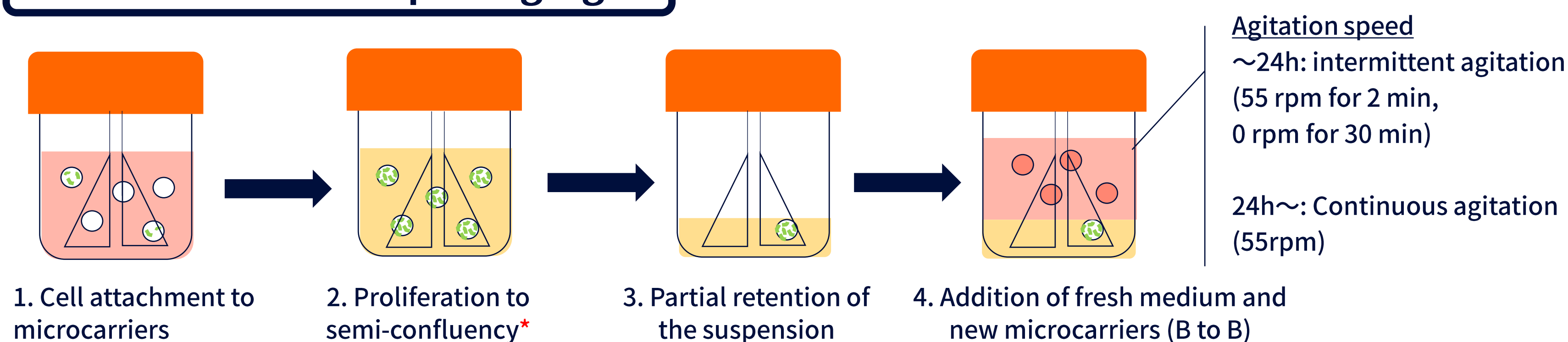
What is SCAPOVA™ CL ?



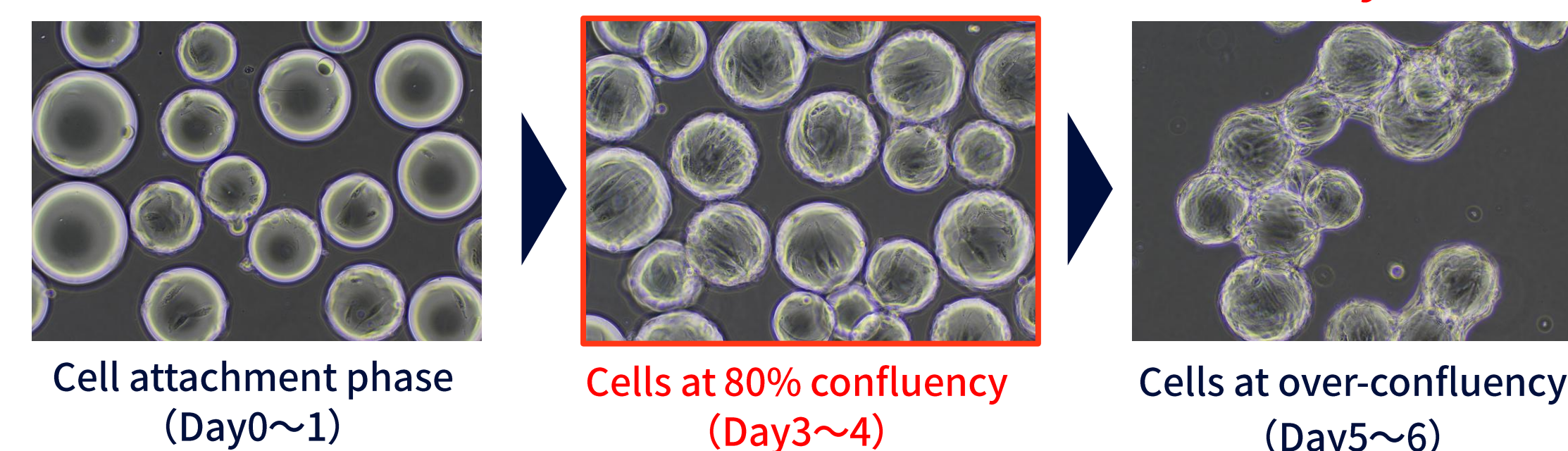
Culture Conditions

Bioreactor	30 mL single use bioreactor (ABLE Corp.)
Particle quantity of SCAPOVA™ CL	6.9 cm ² /mL (208 cm ² /reactor)
Cell type	HADSC – Human Adipose-Derived Stem Cells (LONZA K. K.)
Cell seeding density	5 × 10 ³ cells/cm ² (1.0 × 10 ⁶ cells/reactor)
Culture medium	ADSC-1 (KBM) + 1% Penicillin / streptomycin
Cell count measurement	NucleoCounter® NC-202™ Count & Viability of Cells Grown on Microcarriers
Staining	Microcarrier : iFluor® 594 succinimidyl ester Cells : Calcein AM

Method of B to B passaging

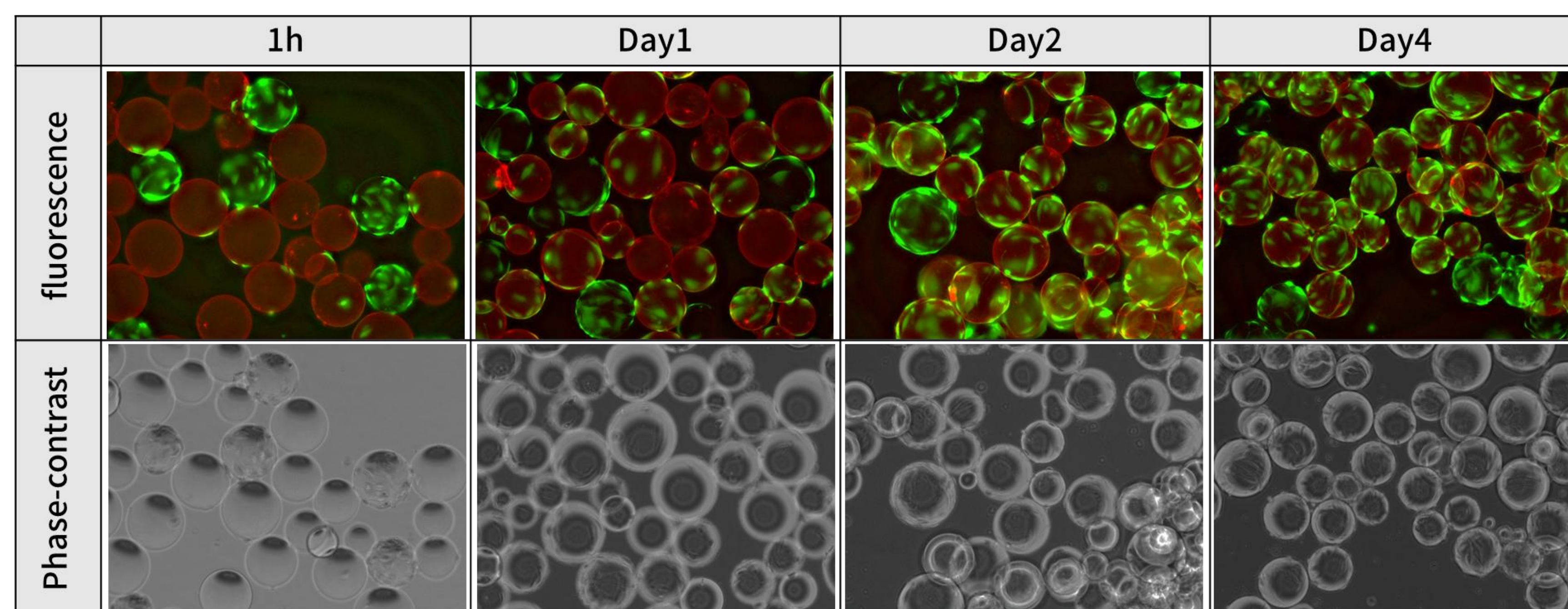


*B to B passing timing:
add new microcarriers before cells reach 100% confluency.



Result

1. Time-dependent observation of B to B

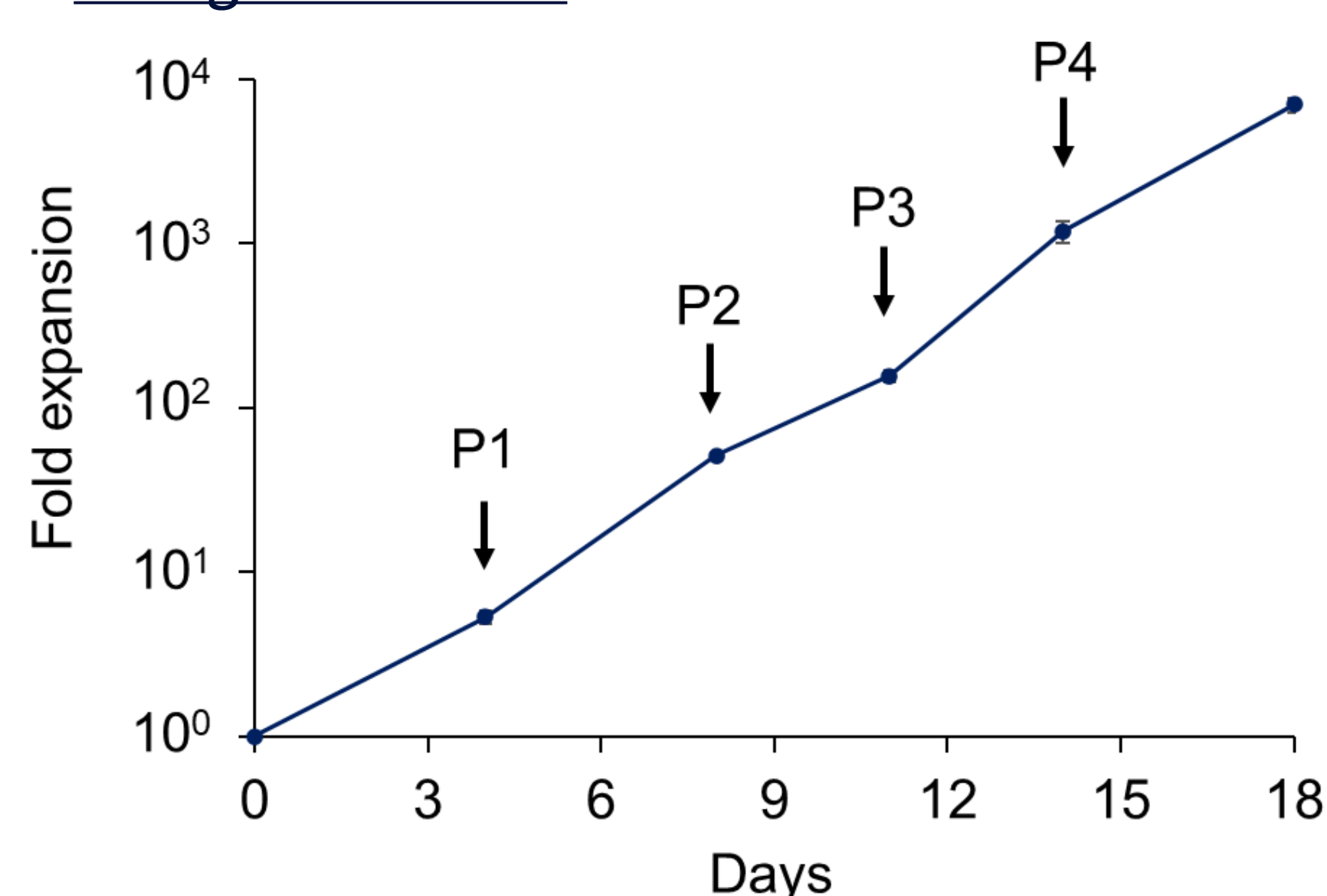


Red: Newly added microcarrier Green: Calcein AM

- Fresh microcarriers were added to MSC attached microcarriers and cultured for 4 days.
- MSCs migrated and uniformly attached to and proliferated across all microcarriers.

3. 18-day culture of MSCs by B to B passaging

MSC growth curve



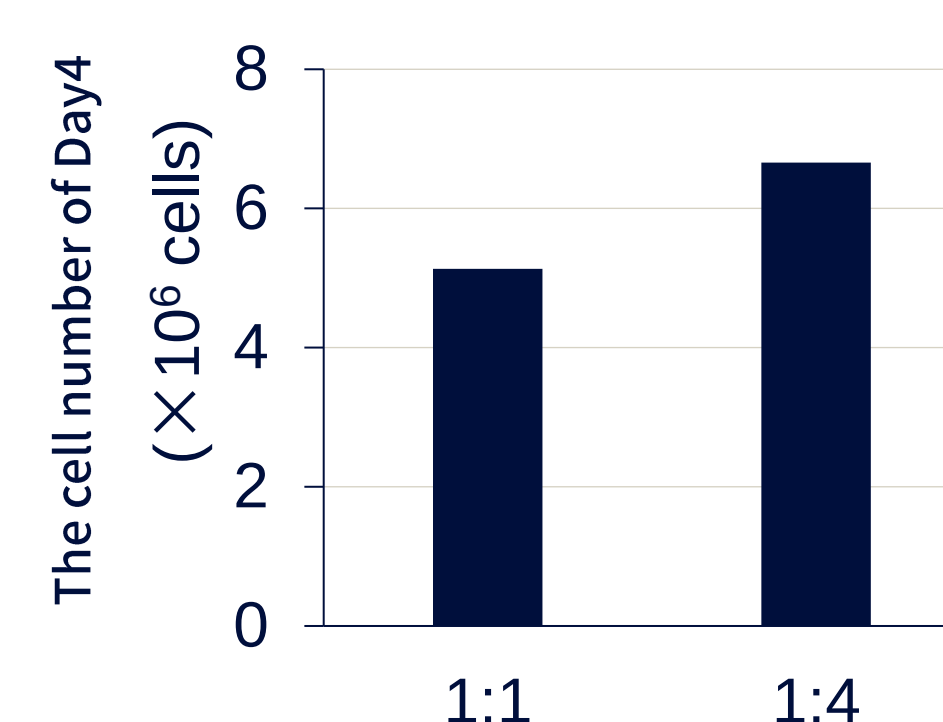
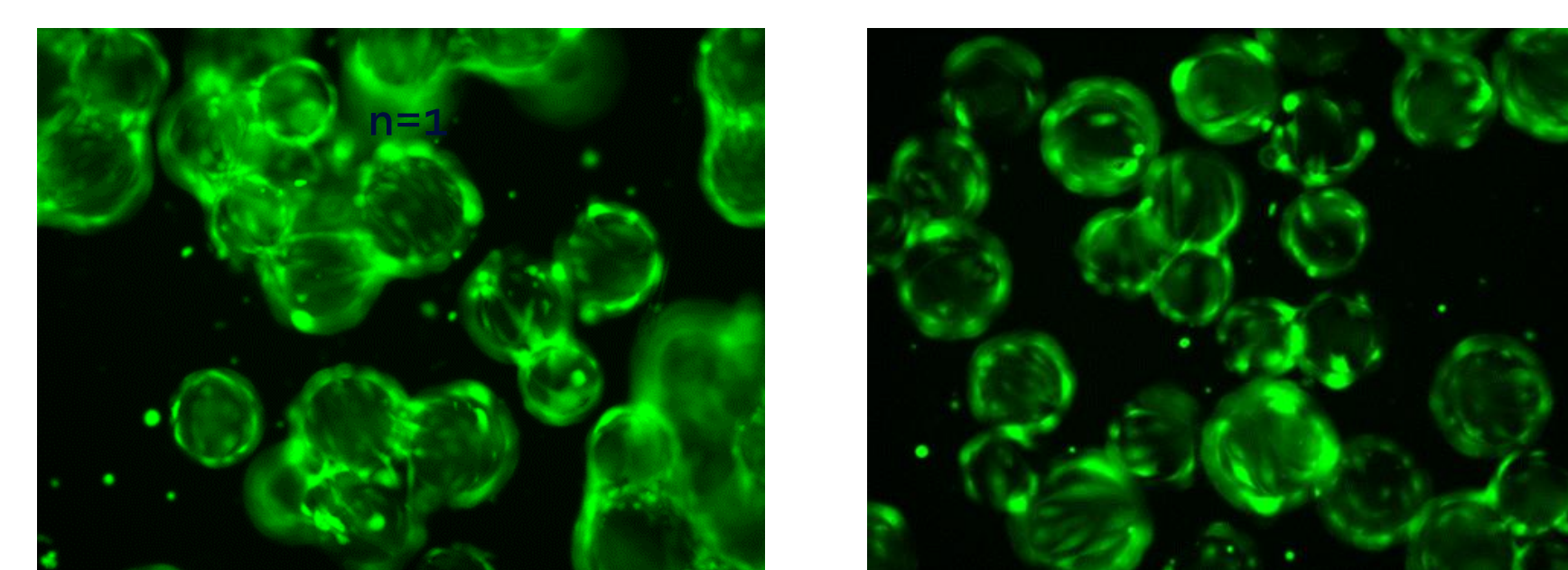
MSC surface marker expression (n=2)

MSC Markers	Positive cells (%)
CD73	100.0
CD90	99.9
CD105	99.3
CD45, CD34, CD11b, CD19 HLA-DR	0.4

- MSCs maintained a constant proliferation rate during 18 days of culture.
- MSC surface markers were maintained.
- Theoretically, MSCs can be expanded up to 10,000-fold within 18 days.

2. Evaluation of the ratio of added microcarriers

(MSC attached microcarriers) : (Newly added microcarriers)=
1:1 1:4



- MSCs were cultured with added microcarriers at ratios of 1:1, 1:4.
- At the 1:4 ratio, no large aggregates were formed, and the cell number was higher on Day4.

Conclusion

- Using SCAPOVA™ CL, MSCs were cultured by B to B passaging. As a result, MSCs migrated and showed uniform attachment and proliferation across the microcarriers.



- When the ratio of MSC-attached microcarriers to newly added microcarriers was set to 1:4, MSCs proliferated without forming aggregates.
- B to B passaging enabled MSC culture without cell detachment, theoretically achieving a 10,000-fold expansion within 18 days.
- The surface marker expression of the cultured MSCs was maintained.