

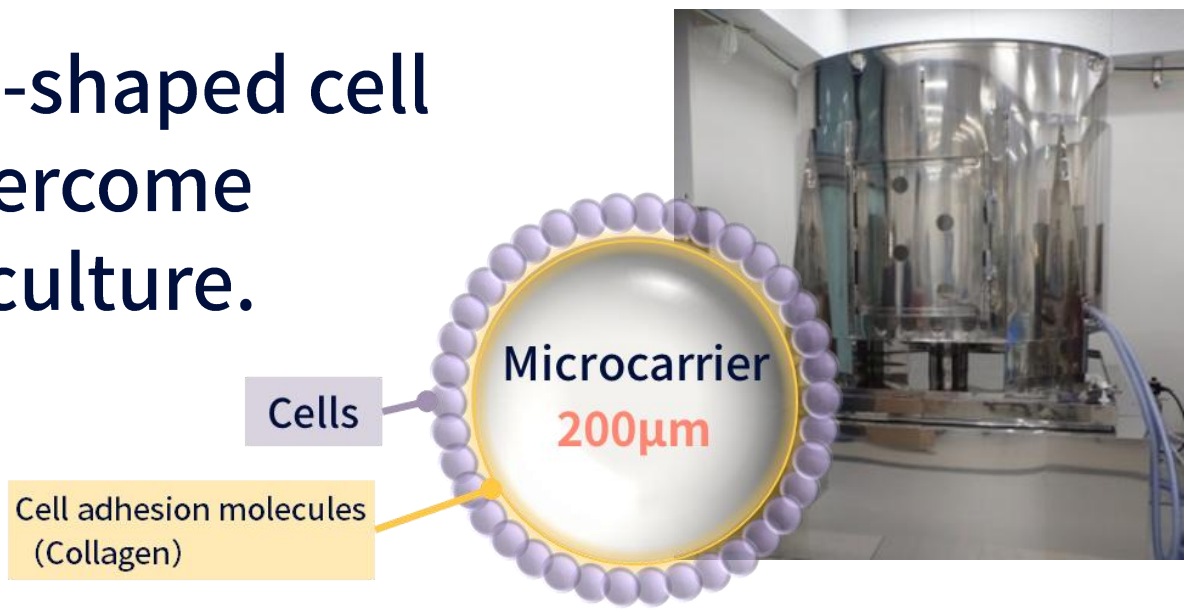
Bead-to-Bead Cell Transfer–Based Passing Culture of Mesenchymal Stromal Cells Using PVA Microcarriers CL

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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



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

2D Culture

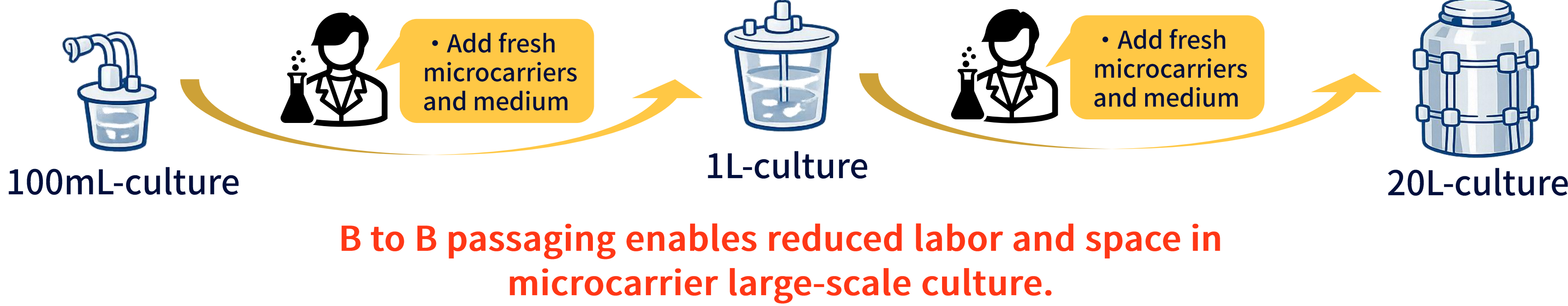


Advantages of Microcarrier Culture

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

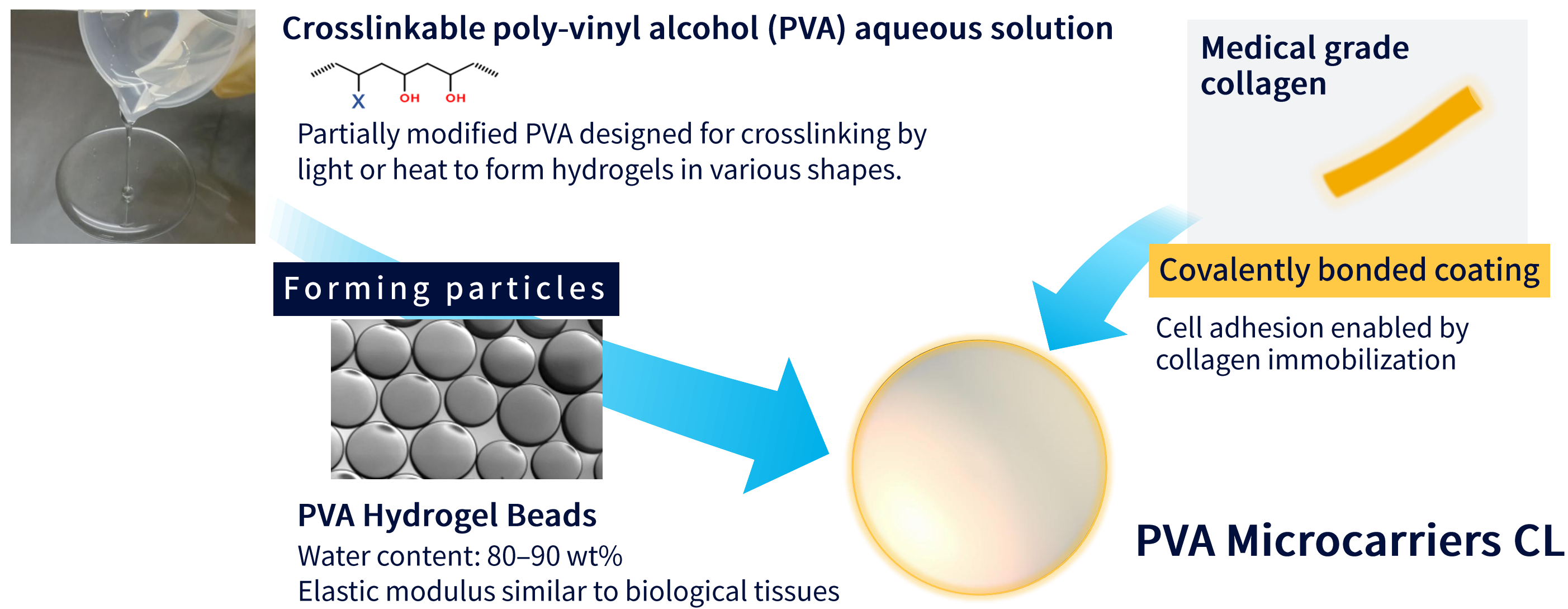


Aim of the study

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

Method

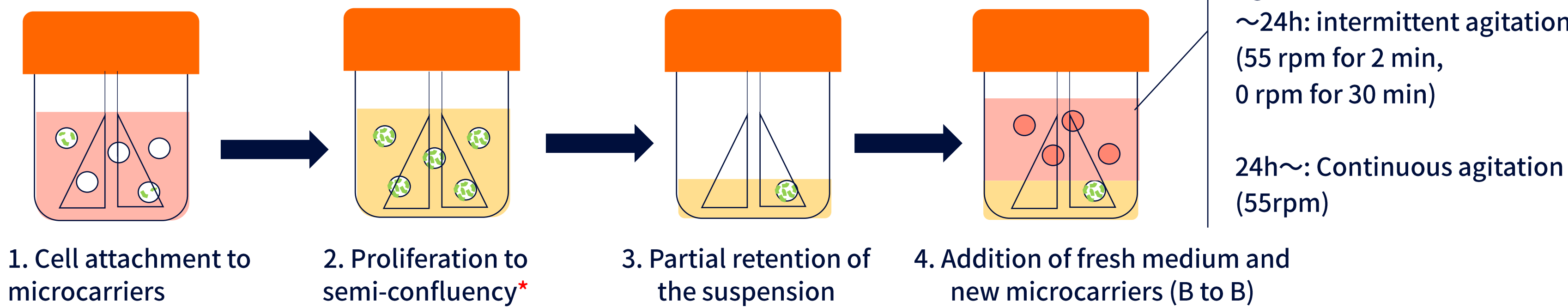
What is PVA Microcarriers CL ?



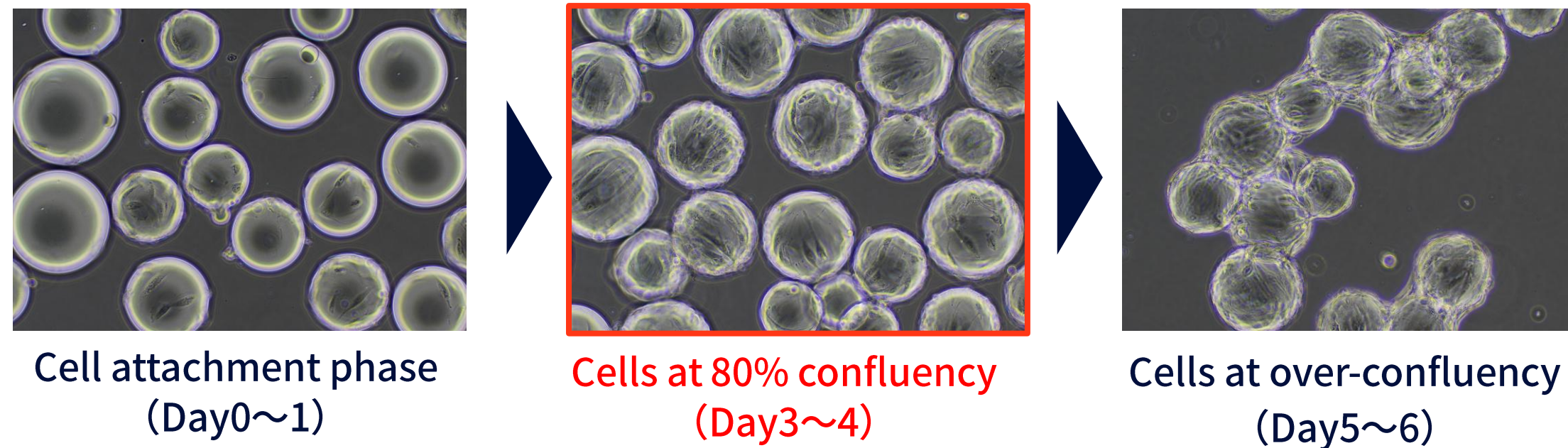
Culture Conditions

Bioreactor	30 mL single use bioreactor (ABLE Corp.)
Particle quantity of PVA Microcarriers 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 passing

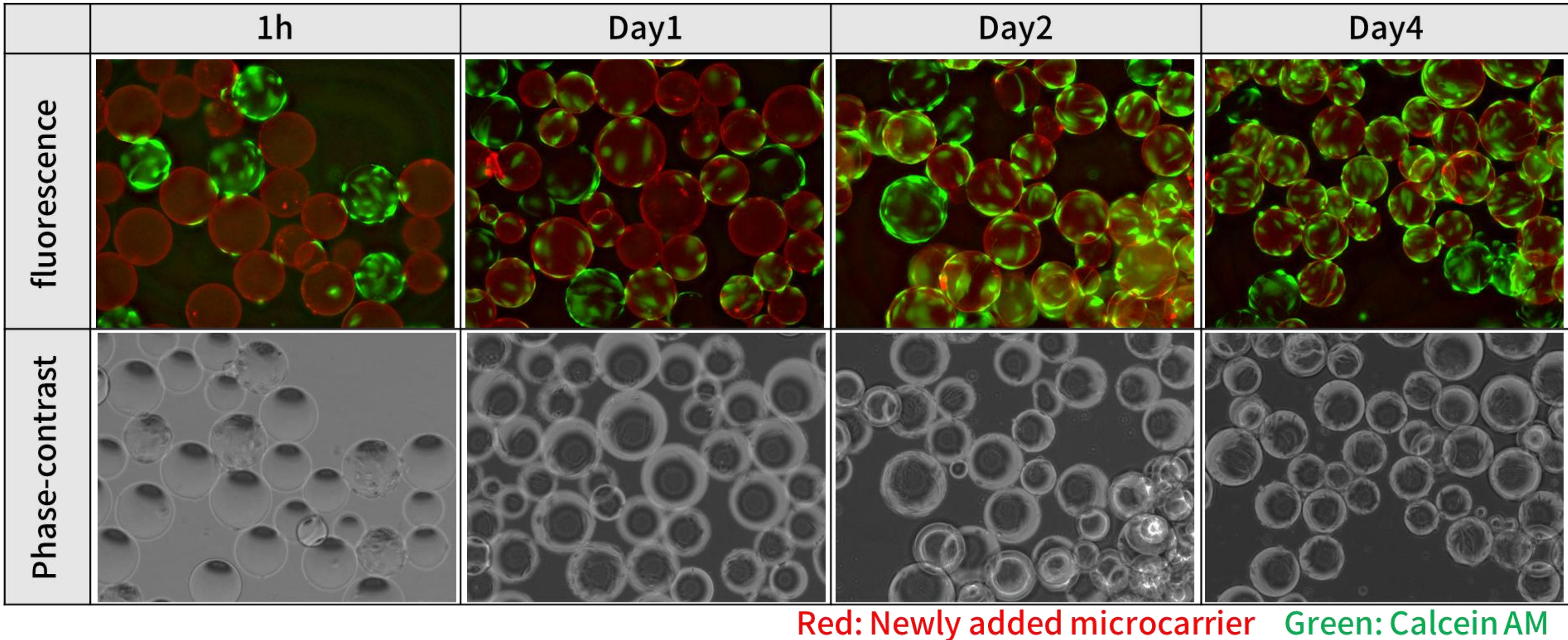


* B to B passing timing:
add new microcarriers before cells reach 100% confluency.



Result

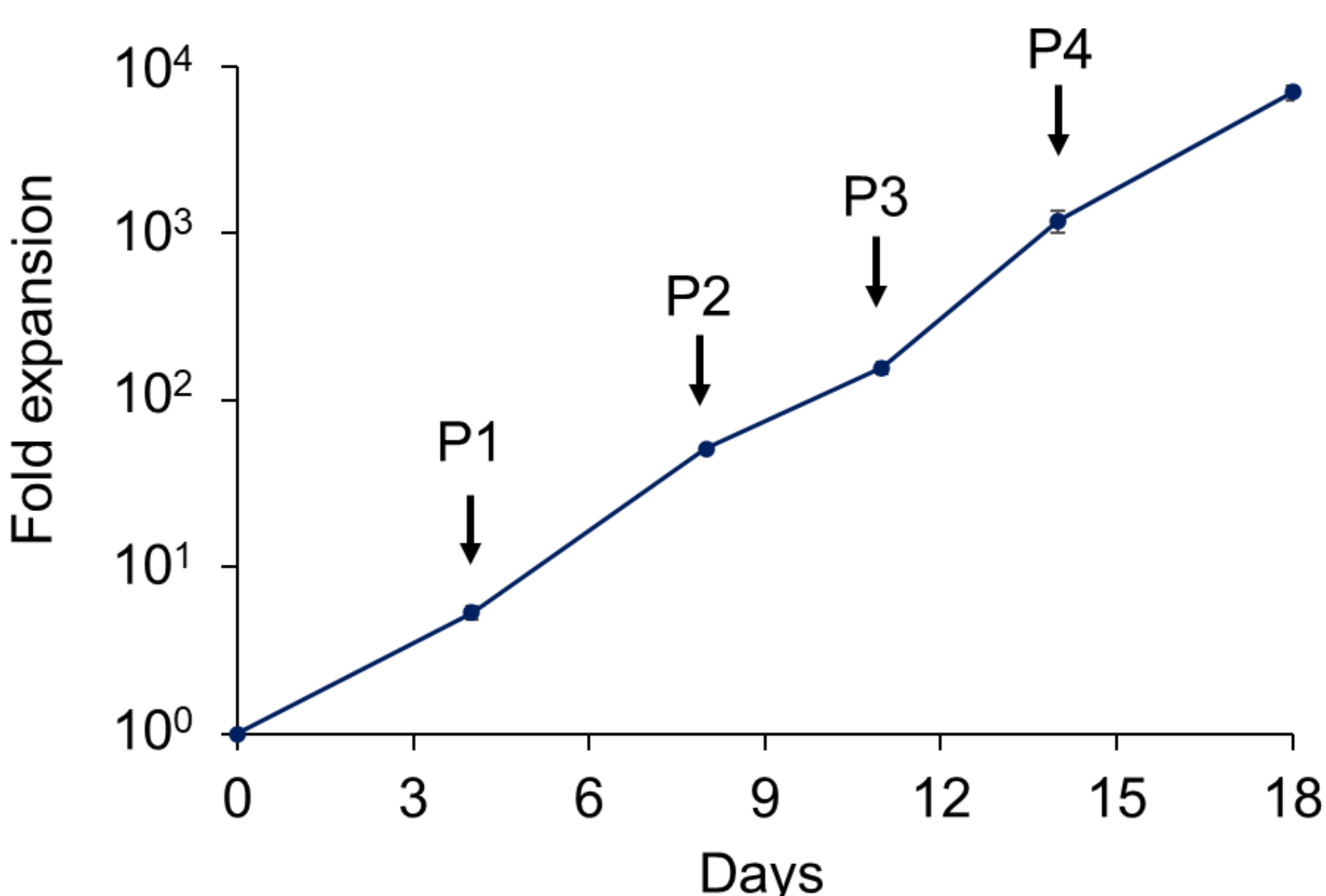
1. Time-dependent observation of B to B



- 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 passing

MSC growth curve



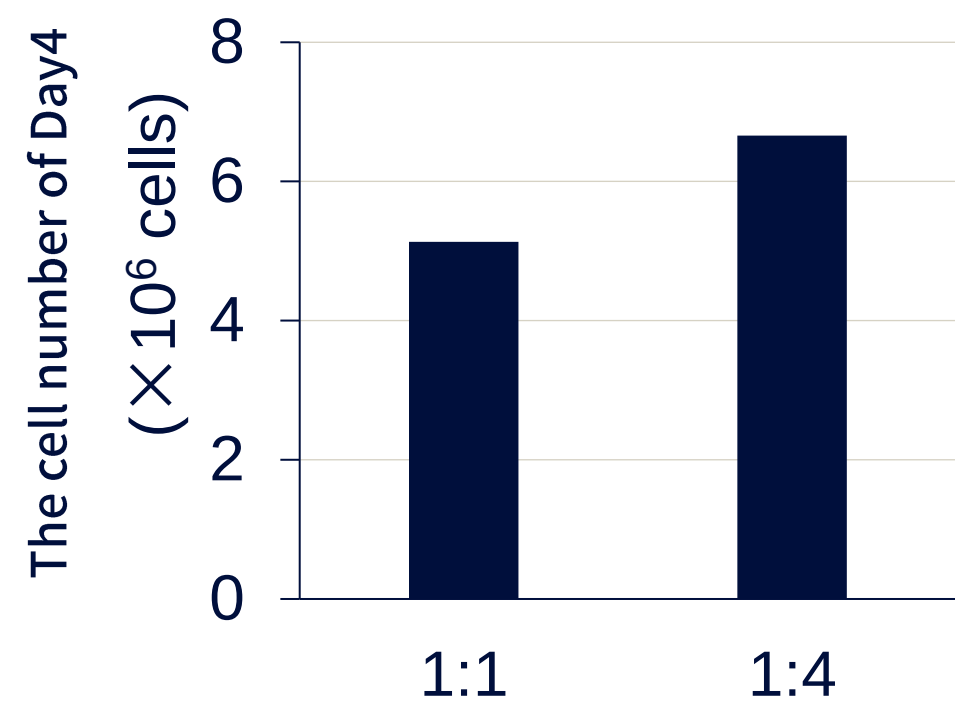
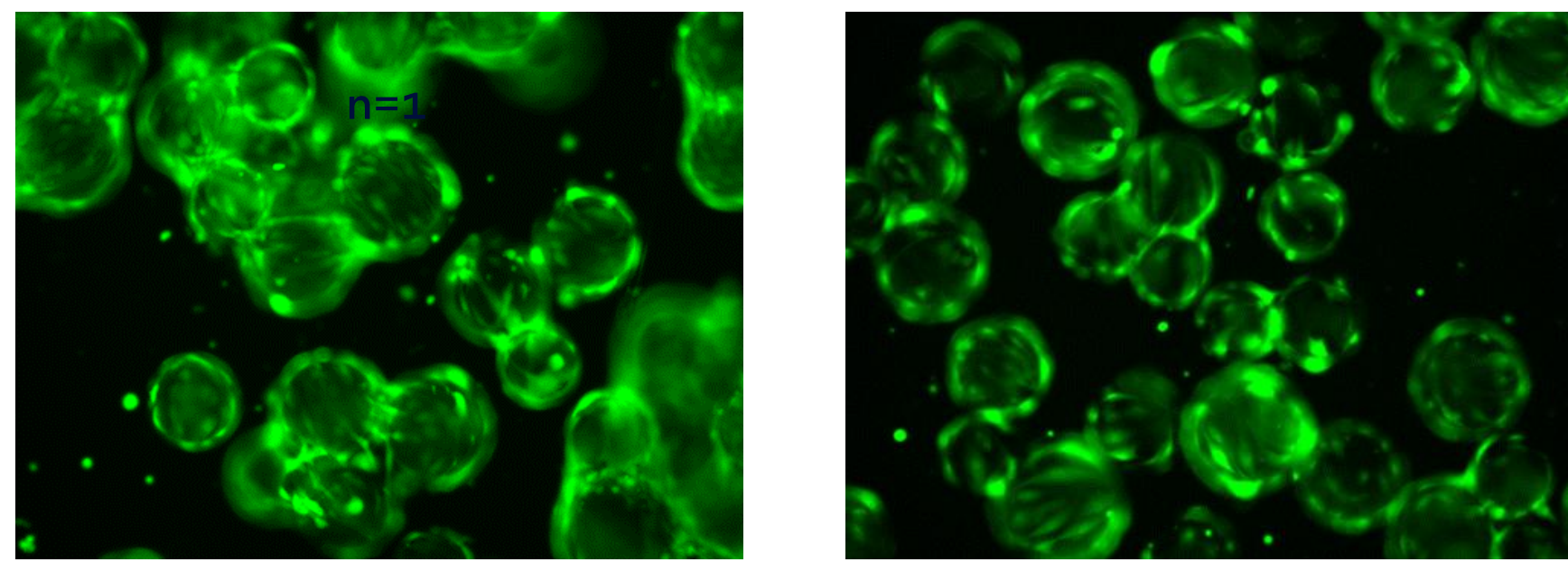
MSC surface marker expression (n=2)

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 PVA Microcarriers CL, MSCs were cultured by B to B passing. 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 passing 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.

PVA Microcarriers HP

