Improving iPS Cell Adhesion Using Novel Disk-Shaped Microcarriers for Large-Scale Culture

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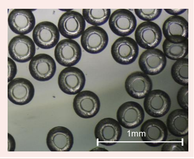
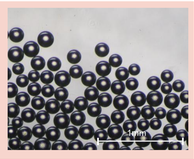
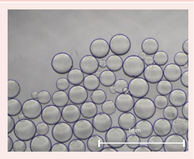
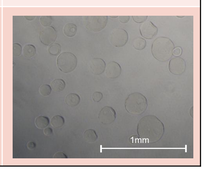
Introduction

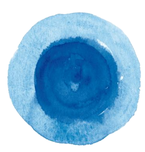
Microcarrier culture enables efficient large-scale expansion within limited surface areas while maintaining the operability of 2D culture and providing a 3D-like environment. However, commercial carriers exhibit low adhesion efficiency for iPS cells. We developed and evaluated disc-shaped carriers to improve cell adhesion.

Materials &methods

	Microcarrier	Manufacturer	Material	Surface Coating	
A	24well plate	Sumitomo Bakelite Co., Ltd.	Polystyrene	iMatrix-511	
B	Disc (Φ300μm-Thickness 100μm)	CSCRIE CO.,LTD	PET	iMatrix-511	
C	CORNING® Microcarrier	Corning Incorporated	Polystyrene USP Class VI	Synthemax®	
D	〈Scapova®〉CL	Kuraray Co., Ltd.	Polyvinyl Alcohol	Collagen	
E	Atelocollagen Microspheres	KOKEN CO., LTD.	Collagen fibrils	Atelocollagen	

＊Only “B” was coated with iMatrix-511 (37℃, 1 hour).
Washed twice with PBS before use.

1. Cell adhesion culture test


iPSCs (CFiS information)


Ultra low 24well plate


Shaker (50r/min)

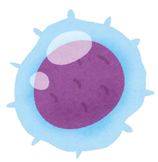
Harvest

Culture on a shaker starting the day after initiating the culture.
The cells were harvested 5 days after seeding.

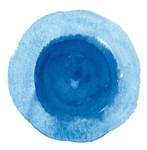
2. iPSC establishment test


Blood
3 donors
(Healthy adults)

TransACT, IL-2 (10 days)


T cells

SeV (3 weeks)


iPSCs

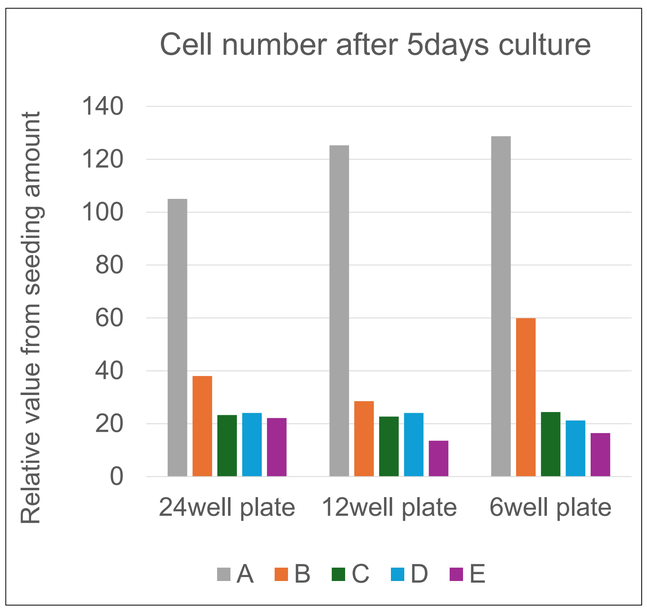

Shaker (50r/min)

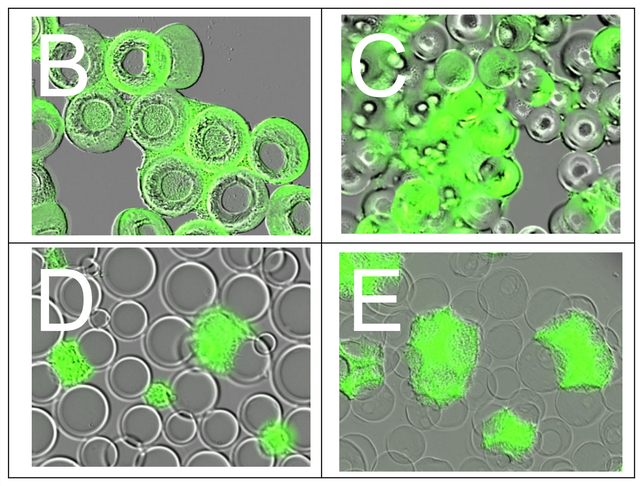
Culture on a shaker starting on day 5 after SeV infection.

Result 1

	Surface Area	Diameter	24well (2cm²)	12well (4cm²)	6well (9.6cm²)
A	200mm²/well	17.25mm			
B	0.14mm²/disc	0.3mm	1400 discs	2800 discs	7000 discs
C	0.09mm²/bead	0.16mm	2222 beads	4444 beads	10666 beads
D	0.16mm²/bead	0.23mm	1250 beads	2500 beads	9625 beads
E	0.2mm²/bead	0.25mm	1000 beads	2000 beads	4800 beads

Each microcarrier was prepared in an area equivalent to 24 wells, 12 wells, or 6 wells and placed in a 24-well Ultra low plate.

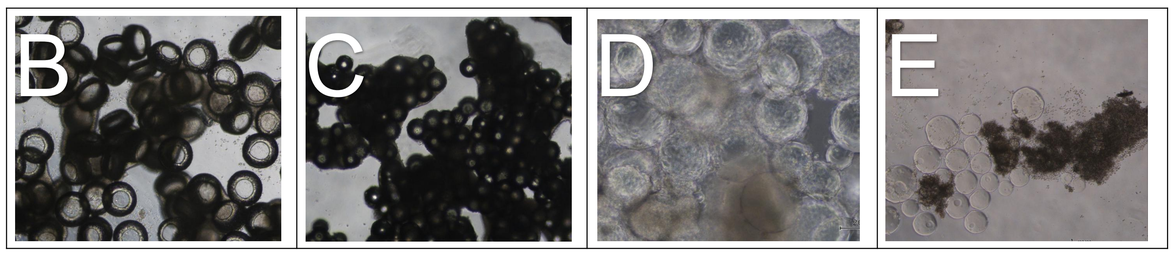


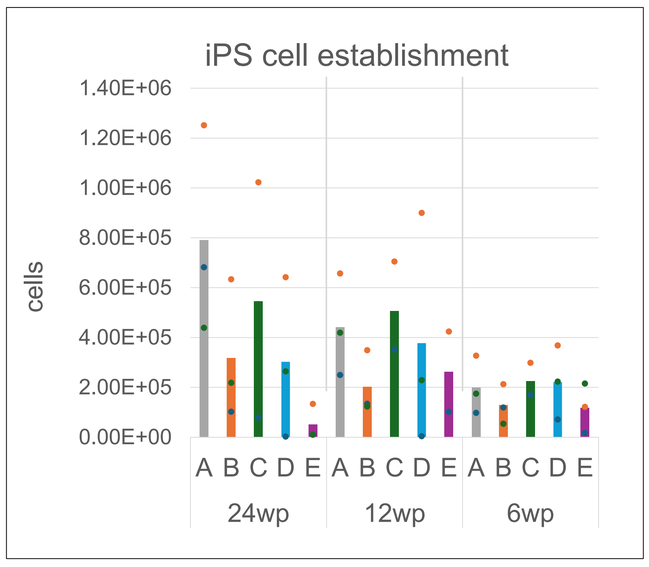


iPS cells on the beads exhibit a 3D morphology rather than a typical adherent (2D) appearance.

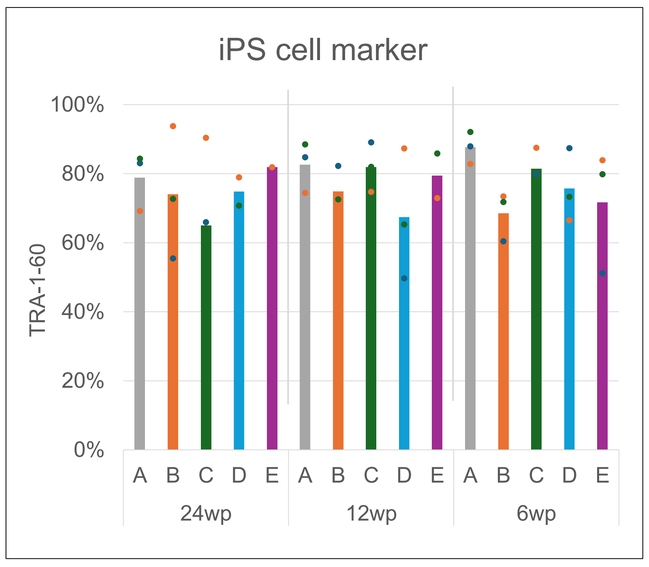
iPS cells on the disc-shaped carriers proliferated more effectively than those on the beads, although still lower than that observed on 24-well plates.

Result 2





Number of cells established per 2.00E+04 T cells. Approximately 3weeks later after infection with SeV, the cells were collected and counted.



The expression of TRA-1-60 was confirmed by FCM at the same time of harvest.

iPS cells were successfully established using both the disc-shaped carriers and the beads. Cells that formed a 3D–like structure showed lower expression of iPS cell markers.

Discussion

Compared to microcarriers, disks showed better 2D adhesion. However, even with disks, 3D cell aggregates were observed. These 3D aggregates exhibited low expression of iPS markers. At present, it is difficult to create a carrier that is completely 2D.
In this study, only plates were used, so future investigations using closed culture systems are necessary.

Acknowledgements

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