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Animal-origin-free cell culture of human adipose-derived stem cells utilizing PVA Microcarriers SCAPOVA™ AS

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Introduction

We have developed polyvinyl alcohol (PVA)-based microcarriers, SCAPOVA™, for mass cell culture in regenerative medicine. SCAPOVA™ has the following features, (1) high cell proliferation rate, (2) high robustness and safety, and (3) ease of handling. SCAPOVA™ AS, which is one of the variants of SCAPOVA™, can be coated with any cell adhesion molecules simply by mixing with these solutions. It can be also adopted for animal-origin-free cell culture.

In general, serum-free cell culture using microcarriers tends to cause aggregation of cells and microcarriers, which makes it difficult to ensure the cell uniformity. To address this issue, we have developed an animal-origin-free cell culture methods utilizing SCAPOVA™ AS, which minimized the aggregation of cells and microcarriers by adding aggregation inhibitor.

Materials and Methods

Human adipose-derived stem cells (ADSCs) were cultured on SCAPOVA™ AS after culture on the flasks. Materials used in cell cultures were described in Table 1.

In a 30 mL single use bioreactor, 30 mL of Culture Medium and 41.6 µL of iMatrix-511 solution were mixed well. SCAPOVA™ AS, weighed as 208 cm^2, was added into the bioreactor. The bioreactor was placed on a magnetic stirrer and agitated overnight at 55 rpm.

ADSCs were maintained on T75 flasks under serum-free, undifferentiated culture conditions (37°C, 5% CO_2, under saturated water vapor). After several days, ADSCs were detached with 0.025% Trypsin-EDTA. ADSCs were seeded in the bioreactor containing SCAPOVA™ AS with iMatrix-511 coating, at a concentration of 1×10^6 cells/reactor. They were incubated in agitated culture (37°C, 5% CO_2, under saturated water vapor). To promote ADSC-adhesion to SCAPOVA™ AS, intermittent agitation (agitated at 55 rpm for 2 min, settled for 30 minutes) for 24 hours was adopted. After 24 hours, agitation speed was maintained at 55 rpm.

AggreGuard™, a cell aggregation inhibitor, was added to the medium at a concentration of 0.5 U/mL after 1 day culture. In this study, ADSCs were cultured for 4 days with and without AggreGuard™, and aggregation of cells and microcarriers and cell numbers were compared.

Results and Discussions

Visual observation of the bioreactors on day 4 incubating showed that the group with AggreGuard™ has no large aggregations. AggreGruard™ was suggested to be effective in inhibiting cell aggregation (Figure 1). In addition, we sampled 500 µL from each bioreactor and observed with a phase-contrast microscopy and fluorescence microscopy (Figure 2). The images show that the addition of AggreGruard™ reduces aggregations of SCAPOVA™ AS and ADSCs.

Cell counts were measured NucleoCounter® NC-202™ on day 4. The number of cells with and without AggreGuard™ increased 7-fold from cell seeding (Table 2). These results indicate that AggreGuard™ has no effect on cell proliferation.

Conclusion

- ADSCs were cultured serum-free on iMatrix-511-coated SCAPOVA™ AS, and cell numbers increased 7-fold in 4 days.
- AggreGuard™ inhibited aggregation without inhibiting cell proliferation in serum-free cultures in SCAPOVA™ AS.

Table 1. Reagents and equipment

Description	Material	Supplier
HADSC – Human Adipose-Derived Stem Cells Cat. No. PT-5006	Cell	Lonza K.K., Switzerland
Corning® CellBIND® T75 flask Product number: 3290	Flask	Corning Inc., USA
30 mL single use bioreactor Product code: BWV-S03A	Bioreactor	ABLE Corp., Japan
Magnetic stirrer Product code: BWS-S03N0S-6C	Stirrer	ABLE Corp., Japan
StemDesign™ MSC Culture Medium AF Product number: M101-AF-500	Medium	Solallis Bio Inc., Japan
iMatrix-511 Product code: 892012	Cell adhesion molecules	MATRIXOME Inc., Japan
Trypsin-EDTA Product number: 25200-072	Dissociation reagent	Thermo Fisher Scientific Inc., USA
AggreGuard™ Product ID: AGR500-001	Cell aggregation inhibitor	Cellrev Co., Ltd., UK
NucleoCounter® NC-202™ Product number: 900-2020	Cell counter	ChemoMetec Inc., USA

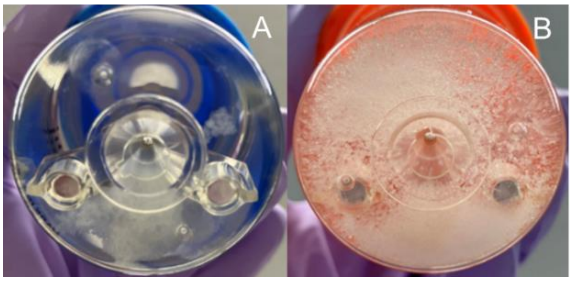

Figure 1. Visual observations on day 4 without AggreGuard (A), with AggreGuard (B)

Table 2. Cell counts on day 4

AggreGuard (U/mL)	Day0 (cells/cm^2)	Day4 (cells/cm^2)
0	5.0×10^3	3.4×10^4
0.5	5.0×10^3	3.6×10^4

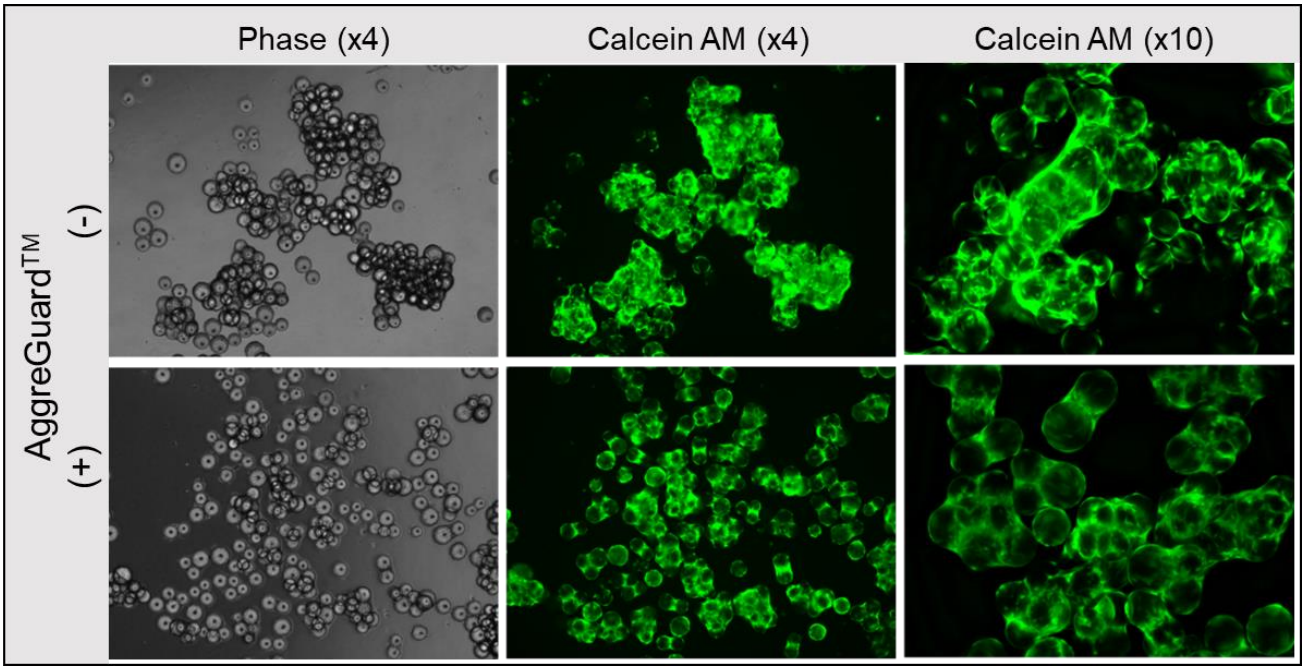

Figure 2. Phase-contrast microscopy and Calcein AM staining of ADSCs on SCAPOVA™ AS without AggreGuard™ (top panels), with AggreGuard™ (bottom panels)

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