General Protocol for SVAPOVA™

Contents

Introduction

This protocol is intended for small-scale cell cultures to use in the conduct of laboratory research. Professor Akira Ito (Nagoya University) and Kuraray jointly developed human Mesenchymal Stem Cell (hMSC) culture system using SCAPOVA™, which is Polyvinal alcohol, PVA, microcarrier produced by Kuraray Co., Ltd.

If you intend to make large-scale cell cultures for industrial purpose, such as mesenchymal stem cell culture for regenerative medicine and clinical purposes, or vaccine production, please contact us at "Contact.LIPG@kuraray.com". Please note these following are general procedures and methods. Please consider optimizing the culture conditions depending on cells which you are using.

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Characteristics of SCAPOVA™

- The material of SCAPOVA™ is hydrogel specifically developed from polyvinyl alcohol, PVA.
- Due to the elasticity and robustness, SCAPOVA™ is less to produce any debris during cell culture.
- SCAPOVA™ is highly transparent microcarrier. Cells on our microcarriers can be clearly observed using phase contrast microscope.
- SCAPOVA™ CL is a PVA microcarrier with Collagen Type I coated surface. Thus, various cells can adhere and grow on SCAPOVA™ CL .
- SCAPOVA™ AS is a PVA microcarrier with activated surface for modifying cell adhesive materials by soaking, such as proteins, peptides, synthetic polymers, and others. SCAPOVA™ AS is under development. Please contact us for the detail.

Table 1. Specifications of SCAPOVA™ CL

Particle size (diameter) [1]	200 - 250 μm
Surface area [2]	Approx. 2600 cm^2/g dry weight
Surface coating	Collagen type I
Relative density [1]	1.03 g/cm^3
Swelling factor [3]	Approx. 10 g wet weight / g dry weight

1. These values were measured in the swollen state.
2. The surface area of swollen microcarriers obtained by 1g of dry SCAPOVA™ CL.
3. The weight of swollen microcarriers divided by the weight of dry SCAPOVA™ CL.

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Reagents and Equipment

SCAPOVA™ can be applied to various types of 3D culture systems.

Erlenmeyer flask + shaking device　　Single-use bioreactor

Figure 1. Schematic illustrations of cell culture systems using SCAPOVA™.

Table 2. General reagents and equipment

reagents and equipment	Example
3D culture system	30 mL single-use bioreactor + 6 channel magnetic stirrer
	Vent cap + Erlenmeyer flask + shaking device
Cell	Various anchorage-dependent cell such as Mesenchymal Stem Cell and Fibroblast
Medium	Medium type depends on type of cell line or culture purpose
Cell dissociation reagent	0.05% or 0.25% Trypsin-EDTA
	Accutase®
Cell strainer	φ70μm or φ100μm cell strainer

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Human Mesenchymal Stem Cell Culture Using SCAPOVA™ CL

The reagents, equipment, and culture conditions in the hMSC culture system are described in tables 3 and 4.

Table 3. Reagents and equipment for hMSC culture system

Product name	Cat. No.	Brand
Single use bioreactor 30mL	BWV-S03A	ABLE Biott
6ch magnetic stirrer	BWS-S03N0S-6	ABLE Biott
hMSC - human mesenchymal stem cells	PT-2501	LONZA
MSCGM™ Mesenchymal Stem Cell Growth Medium BulletKit™	PT-3001	LONZA
Cell Stainer 70 μm	93070	SPL
Accutase®	AT104	Innovative Cell Technologies

Table 4. Parameters for hMSC culture system

Medium volume	30 mL /bioreactor
Amount of SCAPOVA™ CL	37 mg/bioreactor (108 cm^2 / bioreactor)
Seeding cell number	5.4×10^5 cells/bioreactor
Agitation speed for cell adhesion (0-24 h)	15 rpm
Agitation speed for cell expansion (1-7 d)	55 rpm
Bead to bead cell transfer	Cell adhered microcarriers : fresh microcarriers = 1 : 1

Reference

Kaneko M, et al., Expansion of human mesenchymal stem cells on poly (vinyl alcohol) microcarriers. J Biosci Bioeng. 2023 Nov;136(5):407-414.

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Preparation of SCAPOVA™ CL

SCAPOVA™ CL are ready to use out of the vial bottle. They swell easily in culture media. SCAPOVA™ CL are gamma irradiated. Two methods are described below.

Method A

1. Tare a 50ml conical tube.
2. Weigh out the required amount of SCAPOVA™ CL into the tube.
   Note: Calculate the desired weight based on the desired surface area (see table 1).
3. Add culture medium into the tube and swell SCAPOVA™ CL for at least ten (10) minutes.
4. Transfer to a bioreactor or an Erlenmeyer flask with all culture media.
   Ex: When you culture cells in 30mL of culture medium, add microcarriers in 20mL of medium, and then add cells in 10mL of medium.

Method B

1. Transfer SCAPOVA™ CL from the bottle into a suitable size container carefully and add PBS (-).
   Ex: 30-50 mL of PBS (-) is prefer for swelling 1g of dry SCAPOVA™ CL.
2. Disperse SCAPOVA™ CL evenly by pipetting and transfer the desired amount of SCAPOVA™ CL into a 15 mL conical tube.
   Note 1: Calculate the desired volume of SCAPOVA™ CL based on the density of them in PBS (-).
   Note 2: In PBS (-), SCAPOVA™ CL tend to stick on the wall of micropipette tips. We recommend coating the wall of the pipette tips with FBS containing medium (pipette 2 or 3 times before use). Glass pipettes can be used instead of plastic tips.

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3. Sediment SCAPOVA™ CL by standing or centrifugation (300×g, five (5) minutes, set the deceleration rate to the slowest) and remove the supernatant.
4. Add culture medium into the tube and transfer to a bioreactor or an Erlenmeyer flask for cell culture.
   Ex: When you culture cells in 30mL of culture medium, add SCAPOVA™ CL in 20mL of medium, and then add cells in 10mL of medium.
5. SCAPOVA™ CL suspended in PBS (-) can be stored at 4°C. Using them within six (6) months is recommended.

Cell Seeding

The procedure of cell culture using bioreactor with SCAPOVA™ described.

1. Transfer SCAPOVA™ with 100-200cm^2 surface area into 30mL-bioreactor.
   Ex: Add SCAPOVA™ (surface area: 108 cm^2) with 20mL of culture medium in 30 mL-bioreactor, then add cells with 10 mL of culture medium.
2. Seed cells to a final concentration of 4×10^4 - 1.2×10^5 cells/mL.
3. Place the bioreactor on the stirrer in incubator. (37°C, 5% CO_2, humidified)
4. To allow cells to adhere to SCAPOVA™ microcarriers, culture cells for several hours in a static or slow agitation (15 rpm).
5. After twenty-four (24) hours, change the agitation speed to 50 rpm.
6. Change the half volume of medium every three (3) or four (4) days.
   (1) Stand the bioreactor for five (5) minutes to sediment microcarriers.
   (2) Remove the half volume of culture medium carefully not to lose microcarriers, then add fresh medium.

Cell Observation

1. Transfer 500 µL - 1 mL of culture medium containing SCAPOVA™ microcarriers from the bioreactor to a culture vessel (ex. 6-well microplate).
2. Observe attached cells on SCAPOVA™ using a phase contrast microscope.

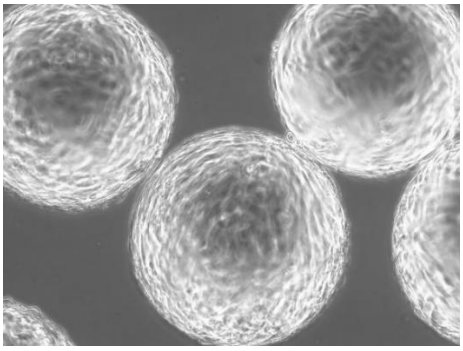

Figure 2. Phase contrast image of fibroblast cells attached on SCAPOVA™ CL.
(This image is obtained with the development stage SCAPOVA™ CL which specification is different from the actual product.)

Cell Counting

Cell counting is recommended to ensure that cells are growing healthy on the SCAPOVA™.

1. Using pipette, mix SCAPOVA™ microcarriers to disperse uniformly in the bioreactor.
2. Collect 0.5 – 1 mL of culture medium containing SCAPOVA™.
3. Detach cells from SCAPOVA™ and count the cell number.
   (Detail of cell harvesting is described in next section “Cell Harvesting”).

Counting the cell number on SCAPOVA™ without cell harvesting is also possible by NucleoCounter NC-200 / NC-202 (ChemoMetec).

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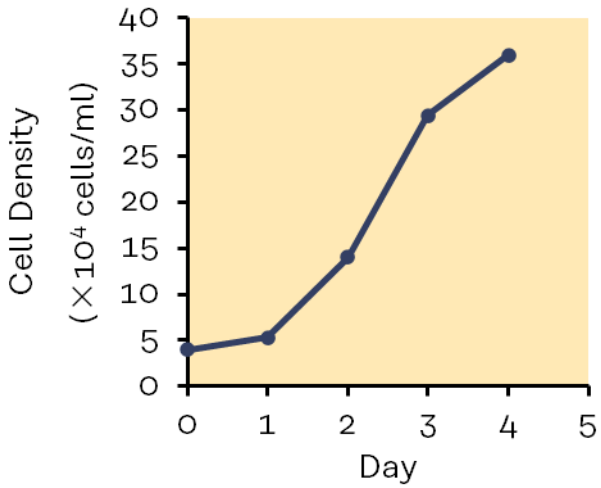

Figure 3. The growth curve of fibroblast cells on SCAPOVA™.
(This data is obtained with the development stage SCAPOVA™ CL which specification is different from the actual product.)

Cell Harvesting

The method using trypsin-EDTA solution is described below. Use suitable cell detachment solution, such as Accutase®, depend on your culture conditions or purposes.

1. Add SCAPOVA™ microcarrier suspension to a 100 µm cell strainer placed on 50 mL conical tube.
2. Wash SCAPOVA™ with 10 mL of PBS (-).
3. Wash out SCAPOVA™ with 5 mL of 0.05% Trypsin-EDTA solution from cell strainer placed upside down on a new 50 mL tube or 60 mm dish.
   Note: Adjust the concentration and volume of trypsin-EDTA solution depending on the cell type and amount of microcarriers.
4. Incubate the tube containing SCAPOVA™ and trypsin-EDTA solution at 37°C in CO_2 incubator with shaking at 85 rpm for five (5) to ten (10) minutes.
   Note: It is also possible to treat with trypsin while stirring in a bioreactor.

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5. Stop the trypsin reaction by adding equal volume (5 mL) of fresh medium.
6. Detach cells from SCAPOVA™ by pipetting for two (2) to twenty (20) times.
7. Separate cells from SCAPOVA™ using cell strainer (70 µm or 100 µm).
8. Rinse SCAPOVA™ with 10 mL of medium.
9. Collect cells by centrifugation (300×g, five (5) minutes).
   Note: Cell detachment should be confirmed by phase contrast microscopy.
10. Remove the supernatant and resuspend the cells in fresh medium.

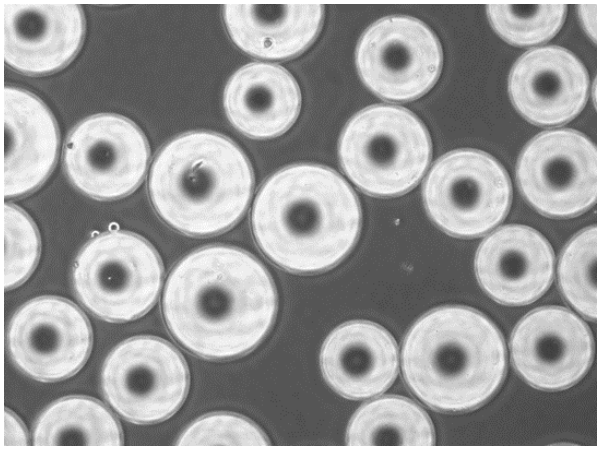

Figure 4. Phase contrast image of SCAPOVA™ CL microcarriers after Trypsin-EDTA treatment.
(This data is obtained with the development stage SCAPOVA™ CL which specification is different from the actual product.)

Option: Bead-to-bead cell transfer

Using SCAPOVA™, bead-to-bead cell transfer can be possible. Adding fresh microcarriers into cell attached microcarriers, the cells transfer onto fresh microcarriers without trypsin-EDTA treatment. Bead-to-bead cell transfer is easy subculture method, but the efficiency depends on cell types. Representative method is described below.

1. Grow up cells on SCAPOVA™ over 80% confluent.
2. Collect one-sixth (1/6) to one-half (1/2) volume of cell attached microcarriers and transfer into a new bioreactor.
3. Add fresh microcarriers swollen in fresh media to the bioreactor. The final density of the fresh and cell attaching microcarriers is same with normal SCAPOVA™ culture.
   For example: 1/6 cell attaching + 5/6 fresh or 1/2 cell attaching + 1/2 fresh microcarriers.
4. Culture cells as shown in “Cell Seeding” section, 4-6.

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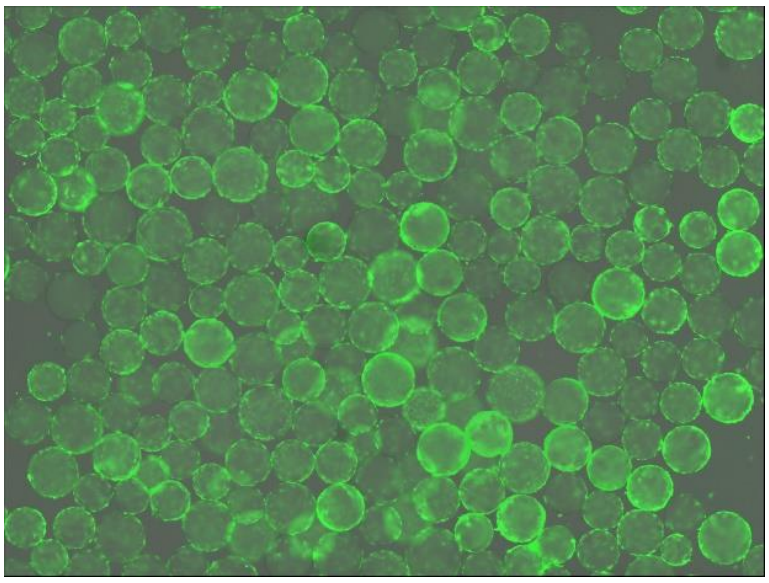

Figure 5. Fluorescent image of SCAPOVA™ CL after bead-to-bead cell transfer.

Fresh microcarriers were added to fibroblast cells attached microcarriers after four (4) days culture. After additional six (6) days culture, live cells were stained with Calcian-AM (Green). Cell attachment to most of microcarriers indicates that bead-to-bead cell transfer is occurred. (This data is obtained with the development stage SCAPOVA™CL which specification is different from the actual product.)

Kuraray Co., Ltd.
Tokiwabashi Tower, 2-6-4, Otemachi, Chiyoda-ku
Tokyo, Japan

Contact

Life Innovation Promotion Group, Research and Development Division
E-mail　Contact.LIPG@kuraray.com

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E-mail　Contact.LIPG@kuraray.com