Surface modification protocol for SCAPOVA™ AS

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Introduction

SCAPOVA™ AS is a microcarrier for cell culture with an activated surface for modifying cell adhesive materials, such as proteins, peptides, synthetic polymers, and others. This protocol describes general surface modification protocols for the preparation of microcarriers for cell culture on a small scale. It is recommended to optimize the modification conditions according to the proteins or other coating materials to be used for surface modification. For more information on cell culture methods using microcarriers, please contact us from the following website.
https://www.kuraray.com/microcarriers/

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Characteristics of SCAPOVA™ AS

- Any cell-adhesive protein or cell culture coating material can be modified on the surface of SCAPOVA™ AS, making it suitable for adhesion and growth of a variety of cells.
- Made of highly resilient poly(vinyl alcohol) (PVA) hydrogel, no debris are generated during shaking or stirring the cell culture.
- High transparency of SCAPOVA™ AS allows easy microscopic observation of adherent cells.
- Since no animal-derived components are used for surface modification, the use of animal origin free coatings allows for completely animal origin free cell culture.

Table 1: Characteristics of SCAPOVA™ AS

Particle size $(D)_{50}$	**Approx. 140- 190 µm *1**
surface area	Approx. 1400-1800 cm^2 /g dry weight *2
surface modification	Various proteins, culture coating materials
specific gravity	1.03 g/cm *1
degree of swelling	Approx. 4-5 g wet weight / g dry weight *3

*1. Particle size and specific gravity are values for SCAPOVA™ AS swollen with phosphate buffered saline (PBS).
*2. Surface area is the value obtained when 1 g of SCAPOVA™ AS in its dry state is swollen with PBS.
*3. The degree of swelling ratio, is the measure of how much a material swells relative to its dry state. The ratio of the difference between the initial weight of a dry material (i.e., SCAPOVA™ AS) and the weight of the material (i.e., SCAPOVA™ AS) when fully swollen, to the initial weight of the dry material (i.e., SCAPOVA™ AS).

Surface Modification Protocols

SCAPOVA™ AS can be modified with a variety of extracellular matrix proteins, including collagen, fibronectin, vitronectin, and laminin-511 fragments, and other cell adhesive materials.

A general protocol for surface modification of SCAPOVA™ AS is shown below.

1. Weigh the required amount of SCAPOVA™ AS into a centrifuge tube, microtube, or another appropriate container.
2. Suspend SCAPOVA™ AS in dry state by directly adding a protein solution of 30–50 times the weight of SCAPOVA™ AS.
   e.g.) Add 3–5 mL of coating solution to 100 mg of SCAPOVA™ AS in dry state.
   Note: Adding protein solution after swelling SCAPOVA™ AS may reduce the modification efficiency. SCAPOVA™ AS may float when the protein solution is added. In this case, please shake it gently or mix it with a vortex mixer to disperse it in the solution before proceeding to the next step.
3. Mix well for at least 1 hour using a tube rotator or shaker.
   Note: If you use the protein solution, be careful not to allow the coating solution to foam.
4. After mixing, settle SCAPOVA™ AS by centrifugation or standing, and then remove the supernatant.
5. Add fresh PBS and wash particles using a vortex mixer or similar.
6. Repeat steps 4 and 5 three times.
7. After surface modification, SCAPOVA™ AS can be stored in PBS at 4°C for about 1 week.

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Examples of Protein Modification and Cell Culture

Here we show the examples of protein modification to SCAPOVA™ AS according to the above protocol and culturing cells with it.

Protein modification

1. SCAPOVA™ AS (ca. 100 mg, surface area: 162 cm^2) was weighed into 15 mL centrifuge tubes.
2. Protein solutions (50–150 µg/mL) were added directly to SCAPOVA™ AS in dry state.
3. Mixing for 1 hour using a tube rotator.
4. Sediment SCAPOVA™ AS by centrifugation (2600 x g, 5 min) and remove the supernatant.
5. PBS (3 mL) was added, and particles were washed using a vortex mixer.
6. 4, 5 were repeated three times.
7. After modification of proteins, SCAPOVA™ AS was stored in PBS at 4°C and used for cell culture.

Results

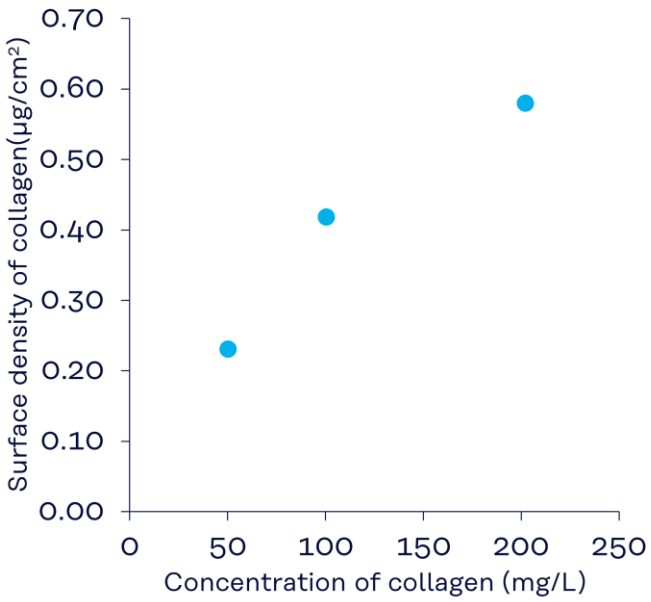

Figure 1. Concentration dependence of modified collagen density

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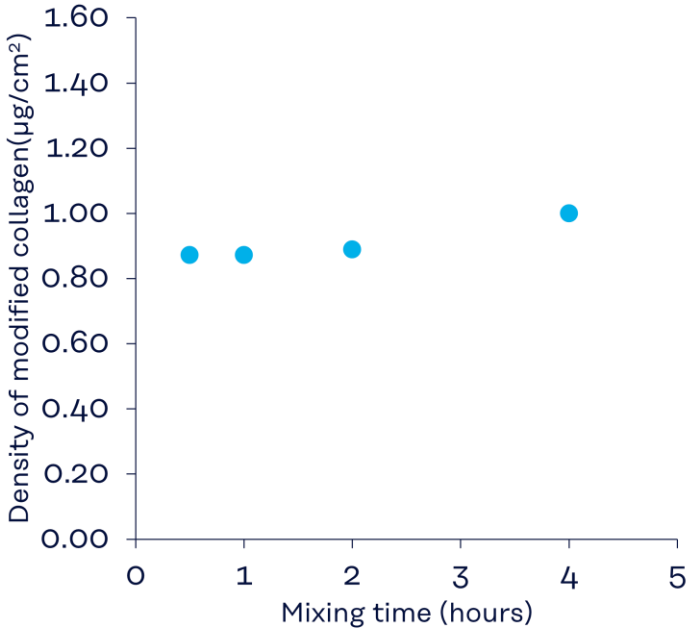

Figure 2. Time dependence of modified collagen density

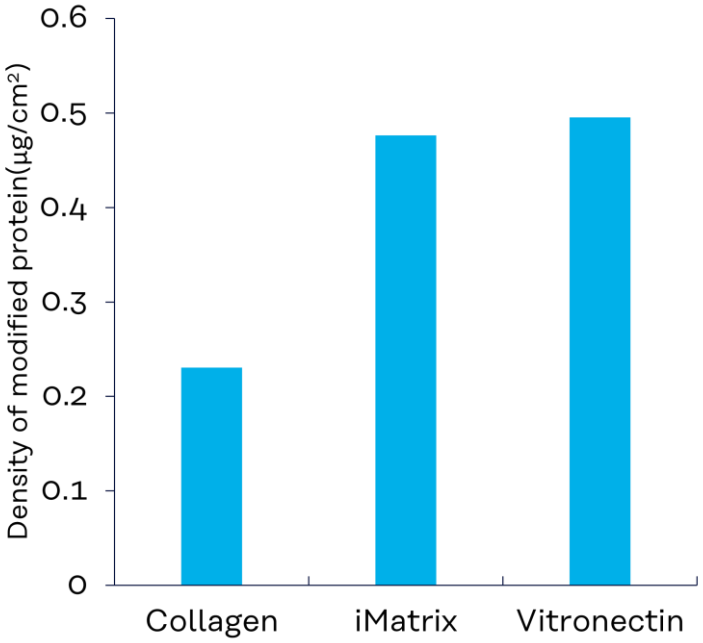

Figure 3. Differences in the density of modified protein by protein species

Cell culture

Here we show how to culture NIH-3T3 cells with shaking in 125 mL triangular flasks.

1. The supernatant of SCAPOVA™ AS dispersion prepared above was removed and transferred to a 125 mL triangular flask with culture medium (30 mL).
2. A suspension of 8.1 x 10^5 cells was added to the flask.
3. Incubated with shaking (80 rpm) at 37°C under 5% CO_2 and saturated water vapor.
4. After 4 days of incubation, separate the cell-attached microcarriers from the unattached cells using a cell strainer.
5. Cells were detached from the microcarriers by adding 5 mL of 0.25% Trypsin-EDTA solution and shaking (80 rpm) for 5 minutes at 37°C.
6. Cells and microcarriers were separated using a cell strainer, and then the number of cells counted.

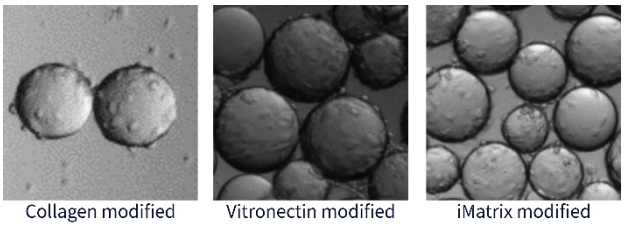

Figure 4. Images of cell-attached microcarriers after 4 days culture

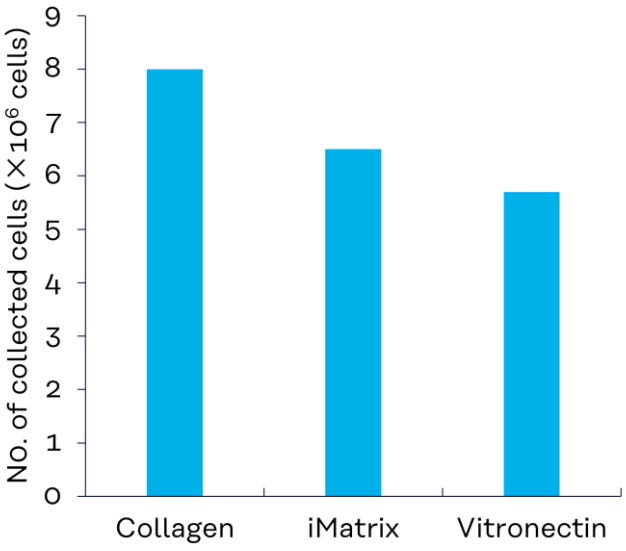

Figure 5. Numbers of collected cells after 4 days culture

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