Reconstructed dermal tissue

Summary

The dermal tissue is reconstructed in vitro with primary human fibroblasts and their self-secreted extracellular matrix. The cells are seeded on tissue-culture treated, 24-well plates containing custom-cut filter paper support rings (inner diameter: 10 mm, outer: 14.5 mm, Whatman grade 4), with a seeding density of 30,000 cells per well (1.6 x 104 cells/cm2). They are grown at 37 °C and 5 % CO2 in rich growth medium (DMEM, high glucose, GlutaMAX Supplement, pyruvate, Gibco) supplemented with 10 % fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin and 50 µg/ml ascorbic acid, with the growth medium replaced every two to three days. The cells initially form a confluent monolayer, adhering to the bottom of the well and the porous filter paper. Over the following weeks, they self-assemble into a three-dimensional connective tissue model with several layers of fibroblasts embedded in a native, collagen-rich extracellular matrix [1]. By week four, the reconstructed dermal tissues have a dense ECM, a thickness of 50 ± 10 µm, remain anchored to the support ring and can be detached from the bottom of the well and manipulated by tweezers [2].

Materials

Primary human fibroblasts

Whatman Grade 4 filter paper (ref: Whatman 1004-185)

DMEM Growth medium, high glucose, GlutaMAX (ref: Thermo Fisher 10566016)

Fetal Bovine Serum (ref: Sigma-Aldrich F7524)

Antibiotics, Penicillin and Streptomycin (Diluted from 10X, ref: Eurobio Scientific CABPES01)

Ascorbic acid (CAS Number: 134-03-2, ref: Sigma-Aldrich A7631)

PBS without Ca and Mg (ref: Eurobio CS1PBS01)

Trypsin-EDTA 0.05% (Diluted from 10X, ref: Thermo Fisher 15400054)

24-well plate, tissue culture treated (ref: Falcon 353047)

Equipment

Biological safety cabinet, class 2 (SafeFAST Top, FASTER)

CO_2 Incubator (Binder world)

Centrifuge (Eppendorf 5702 R)

Procedure

Prepare culture plate and supports

Cut filter paper into rings (inner diameter 10 mm; outer ~14 mm), and sterilize them. They are now referred to as supports.

Add one support into each well of a sterile 24-well plate. [Verify there's only one per well; they tend to stick to each other]

Wash with PBS. Add ~1 ml of PBS per well and let it stay till you prepare the cells.

Aspirate the PBS just before seeding the cells.

Prepare cell suspension for seeding

(30,000 cells/ml in DMEM + 10% SVF + P/S + Ascorbic Acid 50 µg/ml). 25 ml for one plate and 50 ml for two plates.

Cultivate primary human fibroblasts to ~70% confluency (37 °C, 5% CO_2, DMEM + 10% SVF + P/S, in 75 cm^2 flask)

Centrifuge cells

Aspirate the growth medium

Wash with 10 ml PBS to remove serum which deactivates trypsin (without Ca and Mg [They favorize cell adhesion]).

Add trypsin (at least 1 ml for 75 m^2 flask)

Make sure trypsin covers all surface

Incubate 5 min (37 °C, 5% CO_2)

Gently shake to help cell detachment

Add growth medium [Serum contained in the medium deactivates trypsin]. 9 ml of DMEM + 10% SVF + P/S for 75 m^2 flask.

Flush forcefully to detach cells from bottom of flask

Mix with pipette

Transfer to a Falcon 15 ml centrifugal tube

Take 10 µl of cell suspension to count cells

Place them between the slide and the coverslip of a Malassez counting cell. [Gently eject the suspension at the edge. It will expand by capillarity]

Count the cells in the squares of the chamber. [Count cells that are partly outside the square on 2 out of the 4 edges]

The number of cells is given by dividing the number counted by the number of squares and then dividing by the volume of the squares.

N/25 * 10^5 cells/ml

Centrifuge for 5 min at 1,200 rpm, Room Temperature

Resuspend the cells in N/25 * 10^6 / (30,000 * final volume) [=N*0.053 ml for 25 ml]

Take 1 ml from above cell suspension and transfer into a Falcon 50 ml tube.

Complete with 24 ml of DMEM + SVF 10% + P/S

Add 50 µg/ml ascorbic acid [125 µl of 10 mg/ml solution for 25 ml]

Seed cells

Aspirate the PBS from the 24 well plate

Transfer 1 ml of prepared cell suspension into each well

Place in incubator (37 °C, 5% CO_2)

Change growth medium

Replace growth medium (DMED + SVF 10% + P/S + Ascorbic acid 50 µg/ml) every two days approx. [Mondays, Wednesdays and Fridays].

The fibroblasts proliferate and excrete an extracellular matrix (collagen fibers and more) in the following weeks. The engineered dermal sheet reaches its optimal manipulability 3 to 4 weeks after seeding, reaching a thickness of around 50 µm.

References

[1] M. Madi, M.-P. Rols, and L. Gibot, “Efficient In Vitro Electropermeabilization of Reconstructed Human Dermal Tissue,” *J. Membr. Biol.*, vol. 248, no. 5, pp. 903–908, Oct. 2015, doi: 10.1007/s00232-015-9791-z.

[2] G. Albérola, E. Bellard, J. Kolosnjaj-Tabi, J. Guard, M. Golzio, and M.-P. Rols, “Fibroblasts transfection by electroporation in 3D reconstructed human dermal tissue,” *Bioelectrochemistry*, vol. 157, p. 108670, Jun. 2024, doi: 10.1016/j.bioelechem.2024.108670.