

3D culture invasion assay

● Cells:

HaCaT or SCC cell lines, 3×10^6 cells/ well

Human dermal fibroblasts, 10 cm dish (1~2 x 6 well plates)

● Medium:

HaCaT: MCDB153 ($\text{Ca}^{2+}=0.1$ mM) / 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin / EGF (10 ng/ml) / 5% dialyzed FBS

Fibroblasts, TE13: DMEM / 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin / 10% FBS

3D culture medium: MCDB153 : DMEM= 1: 1 / 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin / 10% FBS/ EGF 10 ng/ml

if necessary

◎ bovine pituitary extract (Gibco 0.1 mg/ml)

insulin 5 $\mu\text{g/mL}$

hydrocortisone 1.4 μM

putrescine 60 μM

ethanolamine 0.1 mM

phosphoethanolamine 0.1 mM

sodium selenite 25 nM

EDTA-PBS: 0.02% EDTA-2Na / PBS, autoclaved

Trypsin solution: 0.01% trypsin / 0.02% EDTA-2Na / PBS

● Collagen gel

10 x concentrated MEM (10 x MEM)

Collagen gel (Cellmatrix type IA, Nitta Gelatin Inc)

Collagen gel neutralizing buffer: 0.05N NaOH / 2.2% NaHCO_3 / 200 mM HEPES

● Others

10 cm dish, 6-well plate, 50 ml tube

Disposable pipets (5 ml, 10ml, 25ml), 21G needle

Cell strainers (Becton Dickinson)

<Day 1>

1. Human dermal fibroblasts (confluent in 10 cm dish) are trypsinized and suspended in 2 ml of FBS.
2. Mix 16 ml of type I collagen gel (Cellmatrix type I-A), 2 ml of 10 x MEM, and 2 ml of collagen gel neutralizing buffer on ice (in 50 ml tube).
3. Suspended fibroblasts (step 1) are added to Sol.2 and mix well.
4. The mixed solution is placed into 6 well plates (3 mL/well) and is hardened for 30 min in a CO₂ incubator at 37°C in humidified air.
5. HaCaT (TE13) cells are trypsinized and dispensed onto each gel at a density of 3×10^6 cells in 3 mL of 3D culture medium
6. Incubated overnight

<Day 2>

7. The hardened gels are detached from the plates using 21G needle.

<Day 3~7>

8. Incubation is continued for 1 week until the size of the contracted gel stabilized. Culture medium is renewed every other day.

<Day 8>

9. Cell strainers (Becton Dickinson) are placed upside-down into a 6 well plate, after removal of the strainer handles using scissors, and 3D culture medium is poured into the wells until the nylon mesh of the strainers was covered.
10. The contracted-gel discs are then placed on the mesh so that the HaCaT cells laid on the top of gel discs and the fluid level was adjusted to just below the upper edge of the gel. It is important that the gel was steeped sufficiently in the fluid, while at the same time its surface is exposed to air.

<Day 9~15>

11. Throughout the experiment, half of culture fluid is renewed every other day. After 1 week of an air-liquid interface culture, the gel discs are fixed in a phosphate-buffered formalin solution, embedded in paraffin and vertical sections were stained with hematoxylin and eosin.