Three-dimensional Invasion Assay

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[Abstract] The invasive ability of cancer cells is a crucial function for cancer metastasis and the surrounding microenvironment of cancer cells in living tissues is three-dimension (3D). Therefore, to establish an *in vitro* invasion assay in a 3D system to predict cancer invasive ability is valuable in the research for cancer metastasis. Here, we describe a 3D invasion assay for observing the morphology and comparing the invasive ability of cancer cells in artificial 3D environments (Yang *et al*., 2012). Collagen I gels are used to cover on the top of cancer cells attached on coverslip glass dish and medium containing FBS is added as a chemoattractant. After incubation for a suitable time, the cells are fixed and stained. The invasion index can be calculated and the morphology can be imaged with a laser confocal microscope.

Materials and Reagents

1. Cell lines: OECM-1(Huang *et al*., 2004) and FaDu (ATCC® HTB-43™)
2. 0.1% Trypsin-EDTA (Life Technologies, Gibco®, catalog number: 15400)
3. 0.1 mg/ml poly-L lysine (Sigma-Aldrich, catalog number: P9404-25MG)
4. PBS
5. FBS (Thermo Fisher Scientific, catalog number: SH30071.03)
6. PureCor® bovine collagen solution (Advance Biomatrix Inc., catalog number: 5005-B)
7. 1 M NaOH solution
8. 5x RPMI medium
9. 3% Paraformaldehyde (Sigma-Aldrich, catalog number: P6148-500G)
10. 0.5% Triton X100 (Bionovas, catalog number: 56-81-5)
11. Alexa Fluor® 488 Phalloidin (Life Technologies, catalog number: A12379)
12. DAPI (Sigma-Aldrich, catalog number: D8417)
13. 1% BSA in PBS
14. 1.8 mg/ml collagen I mix solution (see Recipes)

Equipment

1. Lab-Tek® chambered #1.0 coverglass system (NUNC, catalog number: 155383)

2. Laser confocal microscope with 60x oil lens (Olympus, model: FV1000)
3. CO_2 incubator

Software

1. Olympus FV10-ASW 1.7 software

Procedure

Day 1

1. Treat Lab-Tek® chambered #1.0 coverglass system with 300 µl of 0.1 mg/ml poly-L lysine solution for one hour at 37 °C.
2. Aspirate the poly-L lysine solution and wash one time with PBS.
3. Trypsinize cells and 2 x 10^5 cells in 500 µl medium were plated on coverglass system for attachment.
4. After attachment time for 3 to 6 h, prepare the appropriate volume of collagen I mix solution (final concentration 1.8 mg/ml) on ice then carefully remove the medium from coverglass system (avoid to wash cells again) and add 500 µl of collagen I mix solution to coverglass system.
5. Cells were incubated at 37 °C, 5% CO_2 for 2 h.
6. Overlay with 400 µl of medium containing with 15% FBS on collagen gels.
7. Incubate at 37 °C, 5% CO_2 for 24 to 48 h.

Day 2 or 3

1. Carefully aspirate medium from wells and rinse wells including collagen gel invaded by cells with PBS once.
2. Carefully pour 400 µl of 3% paraformaldehyde in PBS for 40 min at RT to fix cells.
3. Carefully rinse two times with 400 µl PBS.
4. Permeabilization in 400 µl of 0.5% Triton X-100 in PBS for 40 min at RT.
5. Carefully rinse two times with 400 µl PBS.
6. Incubate cells with 400 µl of 1% BSA in PBS for 40 min at RT.
7. Stain cells with 500 µl of Alexa Fluor® 488 Phalloidin diluted to 1 units/ml in PBS for 90 min at RT.
8. Carefully rinse two times with PBS.
9. Stain cells with 500 µl of 2 µg/ml DAPI in PBS for 30 min.
10. Wash three times with 400 µl PBS and aspirate all PBS.
11. Samples can be stored at 4 °C for 2 weeks or ready to be imaged by a laser confocal

microscope. Imaging and quantification.

12. Use an Olympus FV1000 laser confocal microscope with 60x oil lens to capture images. The volume of observation is xyz = 210 x 210 x 50 μm^3.
13. Confocal Z slices are collected each well at 50 µm from the bottom of the well and z interval is set to 1 µm.
14. Images of sequential Z sections were obtained and reconstructed by Olympus FV10-ASW 1.7 software.

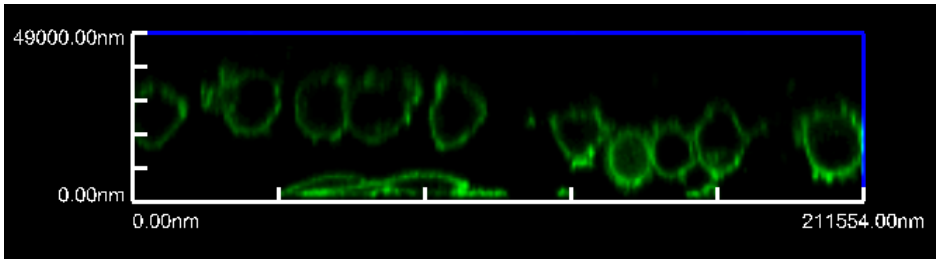

Figure 1. Representative image of FaDu overexpressing Twist1 cells that invaded into collagen after 24 h (*please refer to Reference 1 for the detail*)

15. The invasion index is quantified as the number of cells existing at the distance from the bottom of slide between 30 to 50 µm divided by the total number of cells.
    Note: The cells that are partially fallen into the range of 30-50 µm can also be counted.
16. The data are presented as the percentage of the invasion index of the control sample and representative vertical sections.

Recipes

1. 1.8 mg/ml Collagen I mix solution
   1.7 ml PureCor® bovine collagen solution (3 mg/ml)
   0.6 ml 5x RPMI
   18 µl 1 M NaOH
   Add dH_2O to 3 ml
   All buffers must be on ice before polymerization in the tissue culture incubator. This mix solution must be prepared freshly to use.

Acknowledgments

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