Migration and Invasion Assays Using Tumoroid Models

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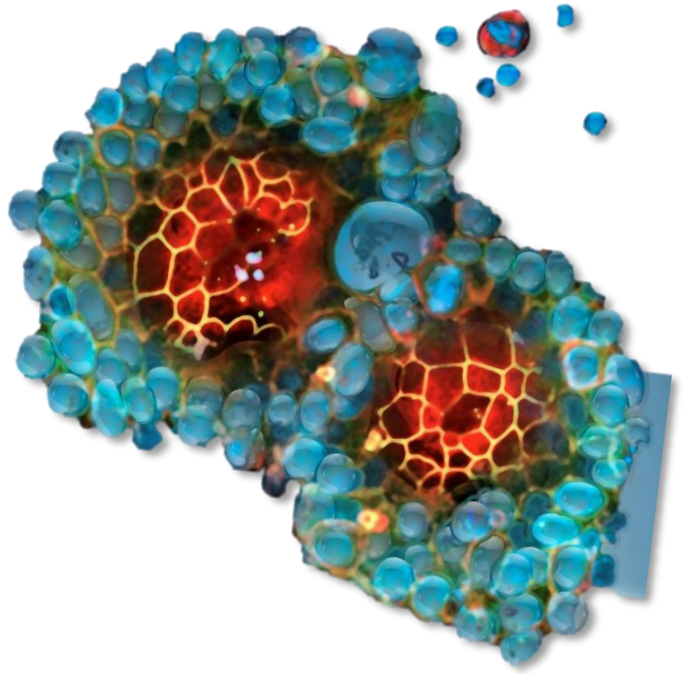
Introduction

Tumoroids, also known as tumoroid lines, cancer organoids, or patient-derived tumor organoids (PDTOs), are patient-derived cancer cells grown as 3D, self-organized multicellular structures.



Scientific literature suggests tumoroids are a promising tool for drug development studies and precision medicine applications because, compared to traditional 2D immortalized cancer cell lines, they:

- Better represent patient characteristics
- Support tumor heterogeneity
- Offer more clinically predictive results

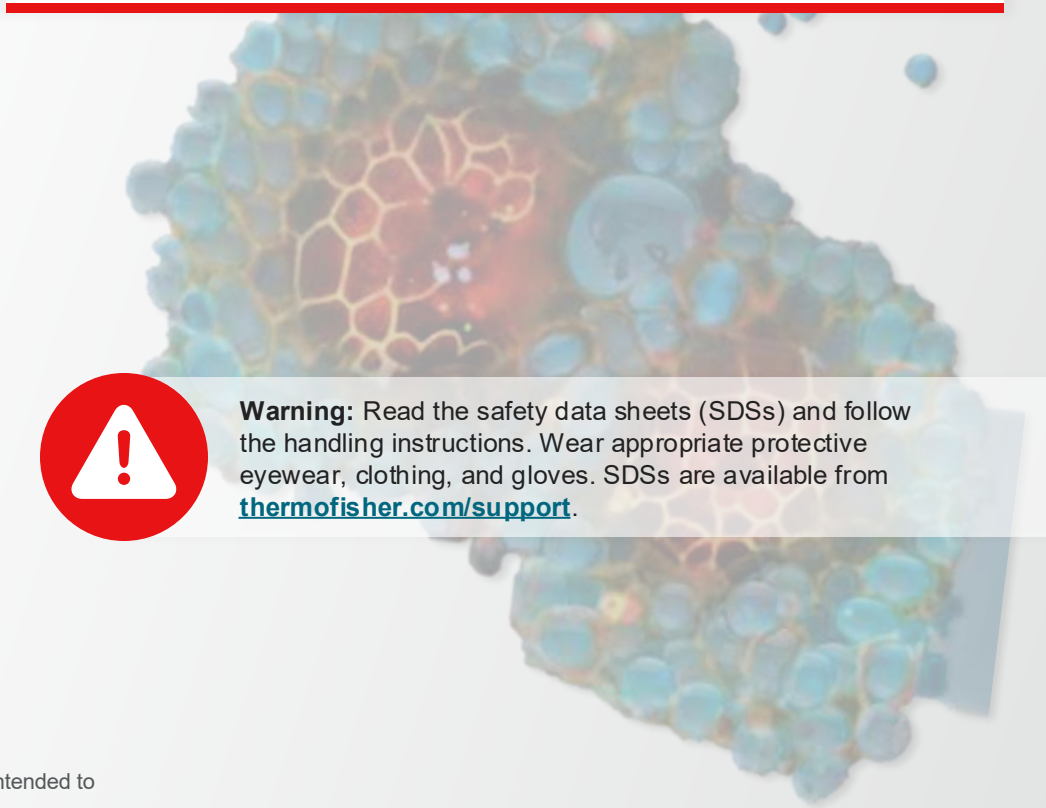
Migration and invasion are critical events in many physiological and pathological processes. For tumor biology, cell migration and invasion are key components of metastasis. This protocol provides a detailed methodology for conducting migration and invasion assays using tumoroids. Guidelines presented here may also be adapted to other formats used for studying cell migration and invasion.

Required materials not supplied

Item	Cat. No.
Consumables	
Nunc 50 mL Conical Sterile Polypropylene Centrifuge Tubes	339652
Nunc™ Cell Culture Inserts in Carrier Plate Systems	141006
Nunc™ Cell-Culture Treated Multidishes	150628
96 Well Black/Clear Bottom Plate, TC Surface	165305
Reagents and buffers	
Collagen I, rat tail	A1048301
StemPro™ Accutase™ Cell Dissociation Reagent	A1110501
DPBS, no calcium, no magnesium	14190144
OncoPro™ Tumoroid Culture Medium Kit	A5701201
ROCK Inhibitor (Y-27632)	-
Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix	A1413202
Counterstains	
CellTracker™ Red CMTPX	C34552
Trypan Blue Solution, 0.4%	15250061
Equipment	
Varioskan™ LUX multimode microplate reader	VLBL00GD0
EVOS™ M7000 Imaging System	AMF7000
Refrigerated centrifuge	75009750

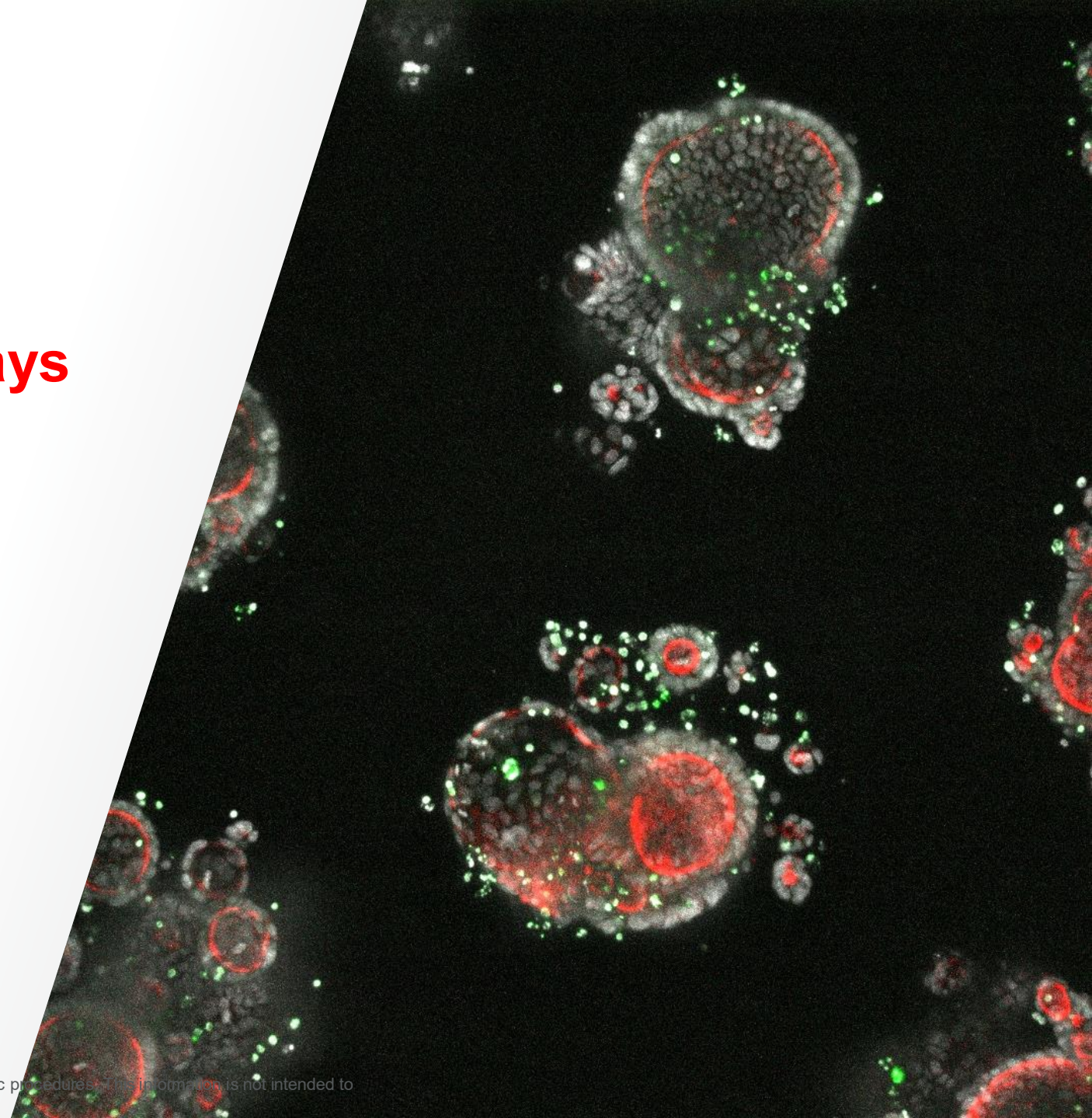
Items with a Cat. No. can be purchased through **thermofisher.com**.

Items without a Cat. No. can be purchased from **fishersci.com** or other laboratory suppliers.



Warning: Read the safety data sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. SDSs are available from **thermofisher.com/support**.

Section 1: Wound healing assays to study cell migration



Note

Use similar tumoroid dissociation and plate coating procedures to prepare for 2D random migration assays or microfluidic migration assays, as applicable for the assay of choice

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Coat cell culture dishes with ECM protein

Note

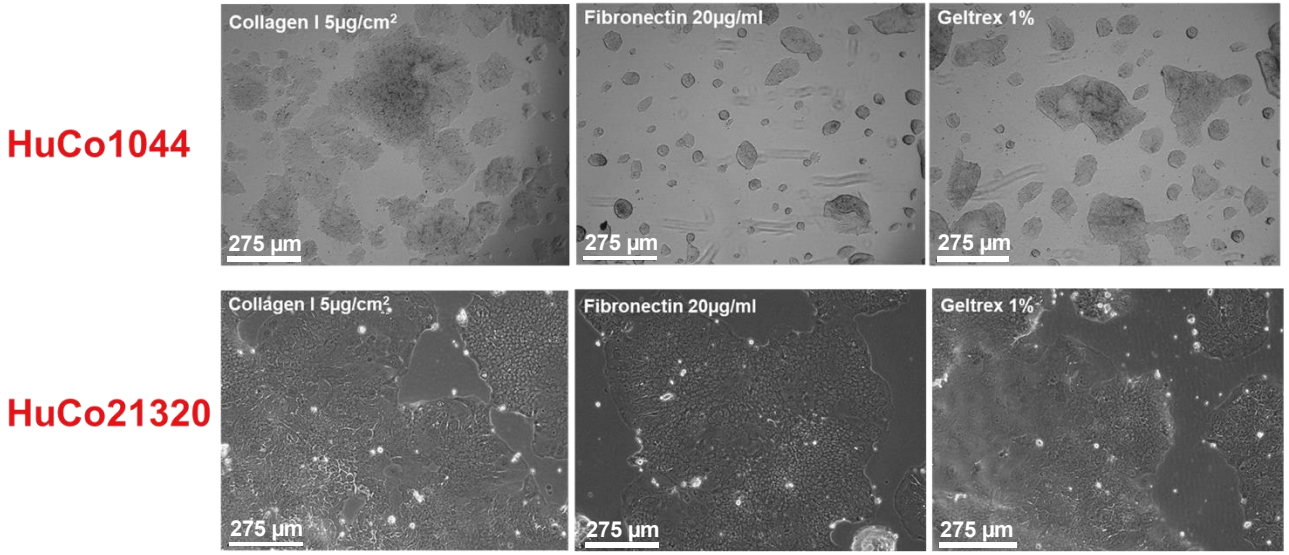
Tumoroids are typically cultured in an extracellular matrix (ECM)-embedded system or in suspension culture supplemented with ECM protein for long-term in vitro propagation. However, many endpoint migration assays require these cells to be plated in a monolayer format. Since tumoroids are grown in serum-free media, they typically require use of an ECM-coated cell culture surface for monolayer culture. This protocol outlines a wound healing assay in a 12-well plate format for colorectal cancer tumoroids.

1. Add Collagen I, rat tail solution to 12-well cell culture plate at 5 µg/cm^2 and incubate at room temperature for 1 hour.

2. Carefully aspirate solution from the wells and rinse three times with equal volumes of sterile 1X DPBS.

Note: Plates may be used immediately or air stored at 4°C for future use.

Note: The selection of coating material may vary depending on the cell type and affects cell attachment and morphology (see below). To ensure reliable and consistent results, optimize the coating type and concentration prior to use.



Dissociate tumoroids and seed cells in monolayer format

1. Transfer tumoroids and medium from the flask or well plate to a 15 mL conical tube.
2. Wash with cold DMEM/F12 + GlutaMAX™ and add wash to the 15 mL conical.

   Note: Adjust wash volumes based on culture vessel size and use multiple 15 ml tubes if necessary.
3. Repeat the previous step with a second wash.
4. Centrifuge for 5 min at 400 RCF at 4°C.
5. Use a serological pipette to aspirate the supernatant without disturbing the cell pellet.
6. Add 12 mL of ice cold DPBS (-/-) to the 15 mL conical tube on top of the cell pellet and triturate ≥ 10 times to fully resuspend the cells.

   Note: Be careful not to overflow the 15 mL conical tube or draw solution into pipet filler.
7. Centrifuge for 5 min at 400 RCF at 4°C.
8. Prepare an appropriate volume of StemPro™ Accutase™ Cell Dissociation Reagent + 10 µM Y-27632 solution by supplementing StemPro Accutase Cell Dissociation Reagent 1:1000 with 10 mM Y-27632. 2 mL of StemPro Accutase + Y are needed to passage a T-25 flask.
9. Use a serological pipette to aspirate the supernatant, without disturbing the cell pellet.
10. Use a micropipette to aspirate the rest of the supernatant, removing as much of the DPBS (-/-) as possible without disturbing the cell pellet.
11. Add 2 mL of StemPro Accutase + Y solution to the conical tube on top of the cell pellet.
12. Use a micropipette set at 800 µL to triturate ≥ 10 times to fully resuspend the cells.
13. Place cells in water bath at 37°C for 10 minutes. Do not use a bead bath. Every ~2 minutes, swirl tube vigorously in order to resuspend cells in solution.

Dissociate tumoroids and seed cells in monolayer format

14 After the 10-minute incubation, use a micropipette set at 800 µL to triturate the cells in the StemPro Accutase + Y.

15 Collect a sample of the cell suspension for counting (see Step 17 below).

16 Add 8 ml of OncoPro medium supplemented with 10 µM Y-27632 to tube containing cells to dilute Accutase and stop dissociation process.

17 Proceed to count the number of viable cells according to your method of choice.

18 Centrifuge cell solution for 5 min at 400 RCF at 4°C. Aspirate supernatant.

19 Resuspend cells to density of 175,000 cells/mL in complete OncoPro medium supplemented with 10 µM Y-27632.

20 Seed 175,000 cells/well (50,000 cells/cm²) onto the collagen coated 12-well plate to achieve 80–90% confluency after 24 hours.

Note: The number of cells required for a confluent monolayer depends on the cell type and the surface area of the plate. Adjust as needed based on observed tumoroid growth rate and vessel configuration used for the assay.

21 Add additional medium to overlay cells if needed.

Perform scratch assay

1. Once cells reach confluence, carefully remove the culture medium from the well. Using a 10 µL pipette tip, create a scratch in the cell monolayer.

   Note: Keep the pipette tip perpendicular to the plate surface while making the scratch to ensure consistency. It is crucial to maintain uniform scratch size across experimental conditions to minimize variability.

2. Gently rinse the well once with basal medium to remove detached cells. Add 500 µL of OncoPro basal medium containing 10 µM Y-27632 to the well.

   Note: Use of basal medium is recommended to minimize cell proliferation during the assay. The addition of Y-27632 helps prevent apoptosis.

3. Using a phase contrast microscope, capture an image of the scratch at 0 hours.

   Tip: Mark reference points near the scratch on the bottom of the dish to ensure accurate re-imaging of the same field later.

4. Place the plate in a 37°C incubator for 12–48 hours, depending on the tumoroid line used.

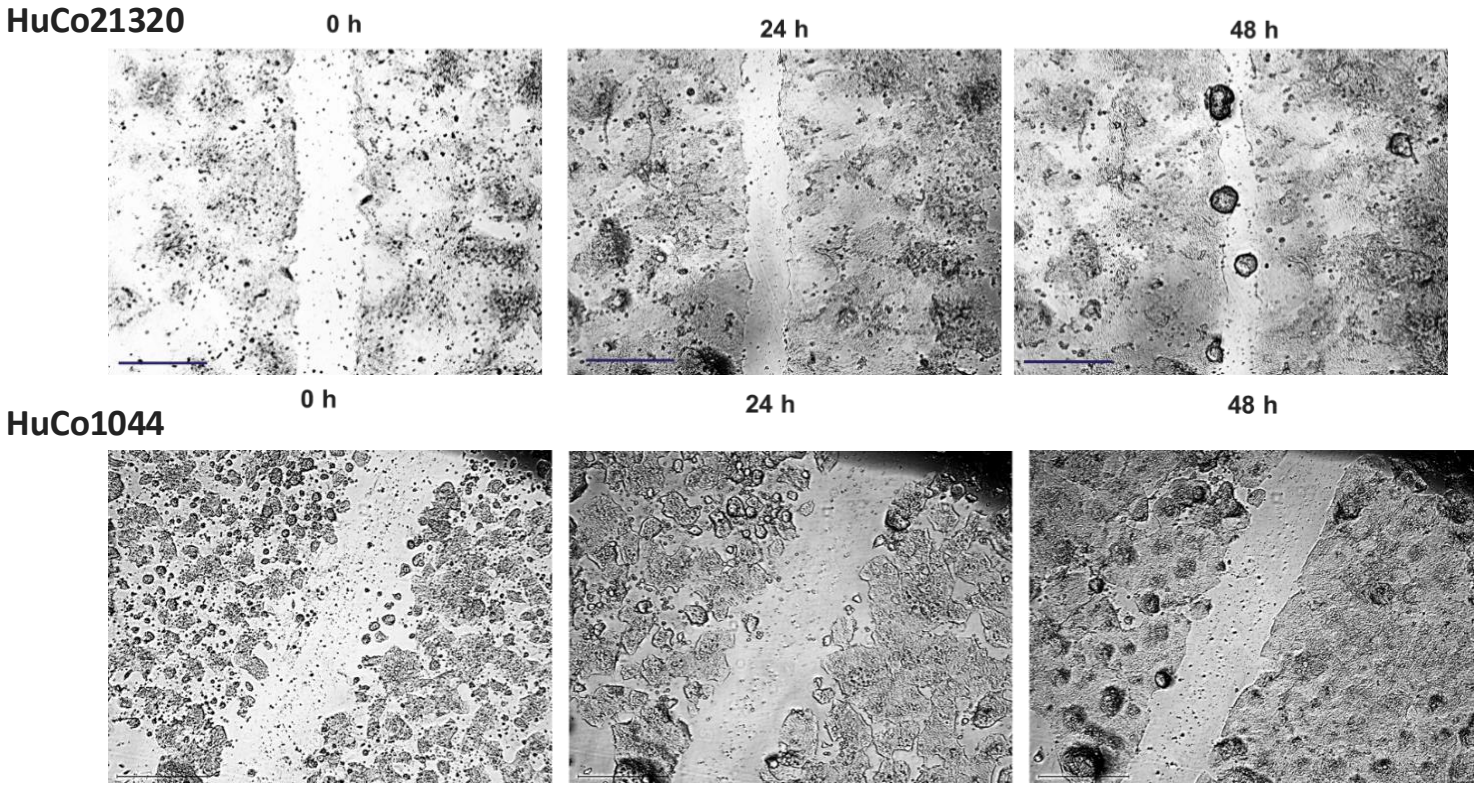
   Note: Cultures can be imaged at multiple time points or monitored continuously using a microscope equipped with a stage top incubator if required.

5. After incubation, capture the final image of the scratch using the previously marked reference points. The acquired images can be further analyzed quantitatively if needed.

Troubleshooting

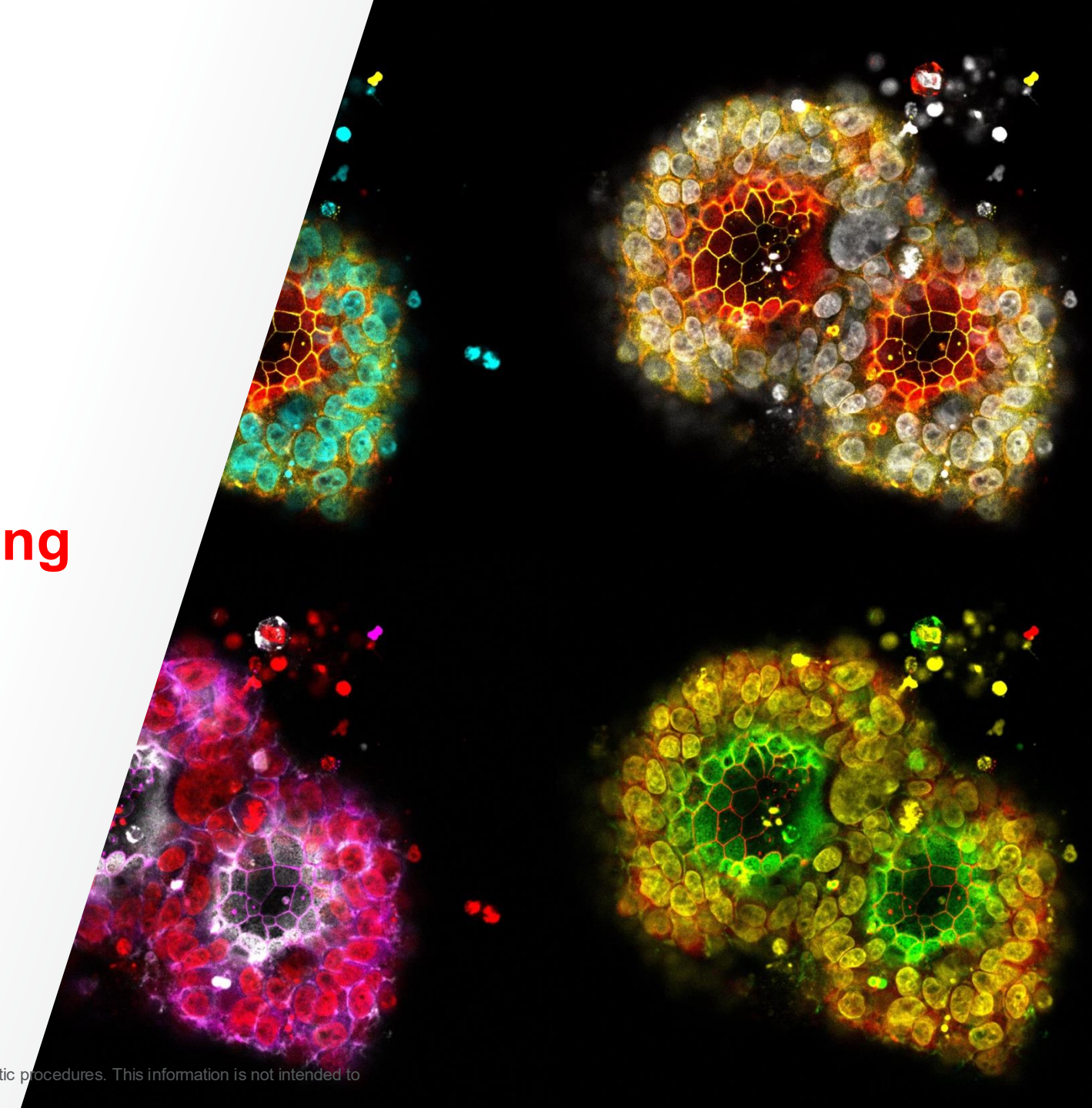
Problem/question	Solution/answer
Cells not attached or not forming monolayer	Seed dissociated tumoroid cells on different ECM proteins and over a range of coating concentrations in initial optimization experiments to select a suitable protein for enabling cell adhesion. Optimize seeding density to promote monolayer formation within desired time frame following cell plating.
Monolayer is uneven	Filter dissociated tumoroid cells through a 40 µm mesh cell strainer prior to counting to remove non-dissociated cell clumps that could interfere with even monolayer formation.
Scratch wound formed is inconsistent	Be fast while making a scratch. Use the lid of the plate as a reference scale to make a uniform scratch.

Representative results



Colorectal cancer tumoroids (HuCo21320 and HuCo1044) grown as monolayers on collagen I, rat tail coated 12-well plates were used for an *in vitro* scratch assay. Images were captured at 0, 24 and 48 h after incubation using an EVOS M7000 microscope with a 10x objective. Scale bar = 650 µm.

Section 2: Invasion assays using cell culture inserts



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Coat cell culture inserts

Note

For the invasion assay, the cell culture insert membrane is coated with an ECM protein to establish a physiological barrier. The selection of ECM is cell type-dependent, with Geltrex utilized in this protocol for colorectal tumoroids. The ECM protein type and concentration should be optimized prior to use.

1. Thaw Geltrex matrix overnight at 4°C before use. Dilute 1 part of Geltrex with 6 parts of cold OncoPro basal medium to prepare the coating solution.

2. Carefully add 100 µL of the diluted Geltrex solution to the membrane of 24-well cell culture inserts (8 µm pore size) to ensure uniform coating.

3. Incubate the coated inserts at 37°C for 2 hours to facilitate proper polymerization and ECM stabilization.

Note: Coat the inserts on the day of the experiment for optimal results. Maintain a set of uncoated wells as negative controls to ensure accurate experimental comparisons.

Dissociate tumoroids and seed on cell culture insert

1. Transfer tumoroids and medium from the flask or well plate to a 15 mL conical tube.
2. Wash with cold DMEM/F12 + GlutaMAX™ and add wash to the 15 mL conical.

   Note: Adjust wash volumes based on culture vessel size and use multiple 15 ml tubes if necessary.
3. Repeat the previous step with a second wash.
4. Centrifuge for 5 min at 400 RCF at 4°C.
5. Use a serological pipette to aspirate the supernatant without disturbing the cell pellet.
6. Add 12 mL of ice cold DPBS (-/-) to the 15 mL conical tube on top of the cell pellet and triturate ≥ 10 times to fully resuspend the cells.

   Note: Be careful not to overflow the 15 mL conical tube or draw solution into pipet filler.
7. Centrifuge for 5 min at 400 RCF at 4°C.
8. Prepare an appropriate volume of StemPro™ Accutase™ Cell Dissociation Reagent + 10 µM Y-27632 solution by supplementing StemPro Accutase Cell Dissociation Reagent 1:1000 with 10 mM Y-27632. 2 mL of StemPro Accutase + Y are needed to passage a T-25 flask.
9. Use a serological pipette to aspirate the supernatant, without disturbing the cell pellet.
10. Use a micropipette to aspirate the rest of the supernatant, removing as much of the DPBS (-/-) as possible without disturbing the cell pellet.
11. Add 2 mL of StemPro Accutase + Y solution to the conical tube on top of the cell pellet.
12. Use a micropipette set at 800 µL to triturate ≥ 10 times to fully resuspend the cells.
13. Place cells in water bath at 37°C for 10 minutes. Do not use a bead bath. Every ~2 minutes, swirl tube vigorously in order to resuspend cells in solution.

Dissociate tumoroids and seed on cell culture insert

14 After the 10 minute incubation, use a micropipette set at 800 µL to triturate the cells in the StemPro Accutase + Y.

15 Collect a sample of the cell suspension for counting (see Step 17 below).

16 Add 8 ml of OncoPro medium supplemented with 10 µM Y-27632 to tube containing cells to dilute Accutase and stop dissociation process.

17 Proceed to count the number of viable cells according to your method of choice.

18 Centrifuge cell solution for 5 min at 400 RCF at 4°C. Aspirate supernatant.

19 Resuspend cells at a concentration of 1e6 cells/mL in complete OncoPro medium supplemented with 10 µM Y-27632 and 5 µM CellTracker Red CMTPX dye. Incubate the cell suspension at room temperature for 15 minutes to allow dye uptake. Immediately proceed to seed the labeled cells.

20 Carefully aspirate excess ECM solution from the cell culture insert.

21 From the prepared pre-stained cells (step 19), take 200 µL containing 2 × 10^5 cells and carefully seed onto each Geltrex-coated 24-well inserts.

22 Incubate the inserts at 37°C for 10 minutes to allow the cells to settle properly.

23 Gently add 650 µL of culture medium (with or without chemoattractant) to the bottom well.

Chemoattractant condition: Complete OncoPro medium containing 10 µM Y-27632.

No chemoattractant condition: OncoPro basal medium containing 10 µM Y-27632.

Note: Choice of chemoattractant and its concentration is based on cell type and experiment setting.

24 Incubate the inserts at 37°C for 36 h.

Note: Incubation time is tumoroid-line dependent.

Quantify invaded cells

Note

Invasive cancer cells can degrade the ECM layer and pass through the pores of the insert membrane. Colon tumoroids are non-adherent in the absence of ECM coating. Upon invasion, they remain in suspension in the media in the lower chamber. Since they are pre-stained with a fluorescent cell tracker dye, they can be collected, and relative invasion can be quantified by measuring their fluorescence using a fluorescence plate reader.

1. Carefully remove the non-invaded cells from the top of the insert. Then, collect the medium from the bottom chamber (which contains the invaded cells) and centrifuge at 200 RCF for 5 min to pellet the cells. Resuspend the pellet in 200 µL of medium and transfer the resuspended cells into a black 96-well plate.

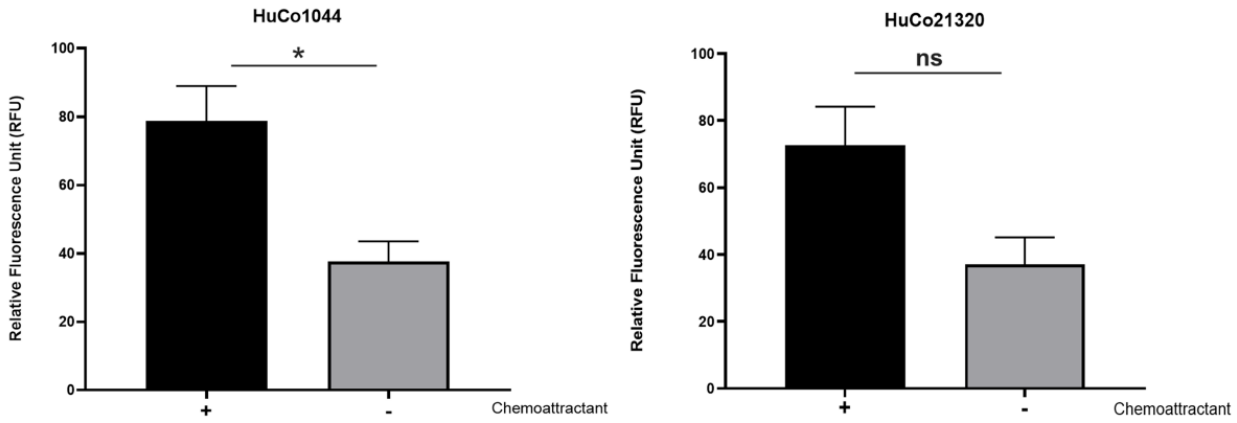
   Note: Use of black walled plates reduces fluorescent signal crosstalk and background signal.

2. Using a Varioskan LUX multimode plate reader, detect fluorescence at excitation 577 nm / emission 602 nm for CellTracker Red CMTPX signal.

   Note: Include control wells containing only medium and CellTracker dye to quantify background signal.

3. Once the fluorescence values are obtained, subtract the background values (to obtain corrected value) and plot the graph.

Representative results



Relative fluorescence value of invaded cells after 36 hrs in the absence (–) or presence (+) of chemoattractant. RFU was measured from 3 different wells in 2 independent experiments. Values are mean ± SEM. The corresponding p-values (*, $p < 0.05$) were obtained by unpaired students' t-test.

Thank you