Extravillous Trophoblast Migration and Invasion Assay

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[Abstract] Extravillous trophoblast (EVT) migration and invasion through the decidualized endometrium is essential to successful placentation. SGHPL-4 cells, an EVT cell line derived from first trimester placenta, is a widely used model of cytotrophoblast differentiation into an invasive phenotype. Here we describe a quantitative cell migration assay that can be modified to also measure cell invasion. SGHPL-4 cells were seeded into BD Fluoroblok cell culture inserts constructed with an 8 µm porous membrane and allowed to migrate towards epidermal growth factor, a known chemoattractant for EVTs. To assess EVT invasion, Fluoroblok inserts were first coated with Matrigel, a basement membrane matrix. SGHPL-4 cells were labeled with calcein AM and cells that had invaded and/or migrated across the membrane were quantified by a bottom-reading fluorescence plate reader. The advantage of the Fluoroblok inserts over other migration/invasion assays is that they allow nondestructive detection of migrated cells.

Materials and Reagents

1. SGHPL-4 cells (Kindly provided by Dr. Guy Whitley, St. George's University of London)
2. Ham's F10 Nutrient Mix (Life Technologies, Invitrogen™, catalog number: 11550-043)
3. Fetal bovine serum (FBS)
4. Dulbecco's Phosphate-Buffered Saline (DPBS) without Ca^{2+} and Mg^{2+} (Life Technologies, Invitrogen™, catalog number: 14190)
5. TrypLE Express (Life Technologies, Invitrogen™, catalog number: 12604013)
6. Matrigel, Growth Factor Reduced, Phenol Red Free (BD Biosciences, catalog number: 356231)
7. Recombinant Human Epidermal Growth Factor (hEGF) (BD Biosciences, catalog number: 354052)
8. BD Falcon HTS FluoroBlok Inserts (BD Biosciences, catalog number: 35112)
9. Calcein AM (Life Technologies, Invitrogen™, catalog number: C3100MP)

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10. Hank's balanced salt soution (HBSS) (Life Technologies, Invitrogen™, catalog number: 14025)

Equipment

1. Centrifuges
2. 37 °C, 5% CO_2 Cell culture incubator
3. Inverted Fluorescent Microscope
4. Fluorescent plate reader

Procedure

DAY 1

1. For Invasion Assay, pre-Coat Fluoroblok Filter (8 µm porous membrane)
   a. Prechill Fluoroblok inserts, companion plates and pipet tips to help maintain Matrigel in the liquid state.
   b. Place desired number of prechilled inserts into a 24-well companion plate.
   c. Add 50 µl of 1:10 Matrigel (diluted in HamF10) to each transwell insert.
   d. Incubate at 37 °C, 3 h.
2. Serum starve cultures (70-75% confluent) for 24 h in 0.5% FBS/HamF10
   a. Aspirate media.
   b. Wash with 7 ml warm DPBS (without Ca^{2+} and Mg^{2+}).
   c. Add 12 ml warm 0.5% FBS/HamF10.
   d. Incubate cells for 24 h at 37 °C.

DAY 2

1. Prepare cells (Upper Chamber)
   a. Rinse cells once with 10 ml DPBS (without Ca^{2+} and Mg^{2+}); add 3 ml TrypLE Express and incubate at 37 °C for 3-5 min; add 7 ml 0.5% FBS/HamF10 → 10 ml total.
   b. Count cells using a hemacytometer.
   c. In a 50 ml conical tube, centrifuge cells at 300 *x g* for 10 min.
   d. Remove supernatant and resuspend cells in 0% FBS/HamF10 to obtain a cell suspension concentration of 1.2 x 10^6 cells/ml (or 1,250 cells/µl).
   e. Cap tube and store at room temperature till ready to load in chamber.
2. Prepare the chemoattractant (Treatments in Bottom Chamber)
   a. Dilute desired chemoattractant in 0% FBS/HamF10. You will need 800 µl per well.
   b. Prepare 10 ng/ml EGF as positive control.

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c. Add 800 µl of chemoattractant to the bottom of each well. Avoid bubbles.

3. Assemble invasion chamber
   a. Using a forceps, carefully remove insert from empty well.
   b. Add 200 µl of cells (2.5 x 10^5 for Invasion Assay or 5 x 10^4 for Migration Assay) to Matrigel-coated (for Invasion Assay) or uncoated insert (for Migration Assay).
   c. Lower the insert at an angle into the well containing the chemotactic substance. Check for bubbles by looking under the plate. If there are bubbles, remove insert and try again.
   d. Incubate at 37 °C for 12 h for Cell Migration Assay or 20-22 h for Cell Invasion Assay.

DAY 3

1. After invasion period, label invaded cells (on lower side of filter) with Calcein AM. For each well,
   add 2 µl of Calcein AM to 500 µl of HBSS.
2. Carefully aspirate the media from the insert, without disturbing the Matrigel layer.
3. Transfer the insert to a fresh well containing Calcein AM/HBSS solution.
4. Incubate at 37 °C for 1 h in the dark.
5. Read plate on fluorescent plate reader at 520 nm or take pictures using an epifluorescent microscope.

Acknowledgments

This protocol is adapted from Angelova *et al.* (2012).

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