Transwell (Boyden Chamber) Cell Migration Assay

Quan Kang, 6/6/2004; commented by T.-C. He

I. Reagents and Materials

1. Transwell Unit: Corning-Costar 3494 Transwell. PTFE membrane and polystyrene plate, 12 wells plate, 3.0μm pore size membrane, pre-coated with collagen types I and IV. (Fisher Cat # 07-200-561).
2. BSA media: DMEM, 0.1%BSA (**Note: you can add P/S to the media for long-term storage**).
3. Q-tips (cotton swabs), 10%formalin, Hematoxylin, Permont (Fisher).

II. Transwell (Boyden Chamber) Assay Using Soluble Chemoattractents

1. Pre-warm BSA media and Complete media at 37^{o}C
2. Prepare desired concentration of chemoattractant solution with BSA media (**without FBS**), for 12 wells plate, 1ml/well will be enough.
3. Harvest migratory cells (e.g., in T-25): Trypsinize with 1ml Trypsin and resuspend cell in suspension with 5ml complete media.
4. Spindown ½ speed for 5min (table top clinical centrifuge), remove the media, wash the cell pellet in BSA media once; resuspend and adjust the concentration of the cell pellet to 5X10^{5}/ml with BSA media. For 12well plate, you will need 0.5-1ml/ transwell. (**Note: cells in T25 flask are trypsinized and resuspended in a total of 6ml BSA media; and 0.2ml to 0.5ml be sufficient for one 12mm transwell insert**).
5. Assemble transwell insert in the chamber of 12 wells transwell plate (contain 1ml chemoattractant solution), make sure the solution touches the bottom surface of membrane and get rid of bubbles.
6. Add the migratory cell mix (in 0.5ml to 1.0ml total volume) into the Transwell insert; allow cells to migrate for 4 to 5 hours in the 37^{o}C, 5%CO_2 incubator.
7. Remove insert from the chamber. Turn chamber upside-down and **gently** submerge in PBS several times to remove unattached cells.
8. Fix cells by submerging insert in 10% formalin for 10min. Then wash with PBS once.

9. Stain cells by soaking insert in hematoxylin for 30min (or overnight), then wash the insert **gently** several times with tap water to remove excess stain.
10. Remove cells from unmigrated (top) side by **gently** scrapping this side with a wet Q-tip/cotton swab. Make sure wiping all cells out in this (top) side. Allow the membrane to dry.
11. Cut out membrane from insert with the migrated side down to a glass slide. Add a few drops of Permont onto the membrane and cover it with cover slide evenly.
12. Count migrated cells on a grid under high power (e.g., x20 or x30). You usually need to count **5 to 10 high power fields** (HPFs) to determine the average cell number migrated per HPF.

III. Transwell (Boyden Chamber) Assay Using Monolayer Cells Expressing Soluble Chemoattractents

Note: This protocol is designed to test whether pre-seeded cells in the bottom chamber (a.k.a., Chemoattractant Producing Cells, CPCs) can express certain secreted factors to attract cells in the top chamber (i.e., Transwell insert).

1. Plate CPCs in the bottom chamber (i.e., 12-well Transwell Plate) (**Note: In fact, the regular 12-well plate can accommodate the Transwell insert as well**) the day before the migration assay. Depending on your experiments, you can use 293 or HCT116 cells as CPCs because they are easy to be transfected. We usually use **HCT116** cells for **adenovirus infections** (e.g., with AdBMPs, or AdWnt3A, etc) using the following schedule: *plate HCT116 cells in complete medium in early morning (i.e., 8:00AM) for 4-6 hours; add adenovirus for additional 4-6 hours; at the end of the day (i.e., >6:00PM), wash the infected cells with BSA media twice, and add **1.0ml BSA media** to each well; incubate at 37C 5%CO2 overnight*.
2. In next morning (e.g., around 10-11AM), pre-warm BSA media and Complete media at 37^{o}C incubator (**Note: it is more desirable that aliquots of the above media are pre-equilibrated in the 37C 5%CO2 incubator**).
3. Harvest migratory cells (e.g., in T-25): Trypsinize with 1ml Trypsin and resuspend cell in suspension with 5ml complete media.

4. Spindown ½ speed for 5min (table top clinical centrifuge), remove the media, wash the cell pellet in BSA media once; resuspend and adjust the concentration of the cell pellet to $5X10^5$/ml with BSA media. For 12well plate, you will need 0.5-1ml/ transwell. (**Note: cells in T25 flask are trypsinized and resuspended in a total of 6ml BSA media; and 0.2ml to 0.5ml be sufficient for one 12mm Transwell insert**).
5. Assemble transwell insert in the chamber of 12 wells transwell plate (containing 1ml chemoattractant medium produced by the bottom layer CPCs) (**Note: do not change the overnight medium already in the well**); Make sure the solution touches the bottom surface of membrane and get rid of bubbles.
6. Add the migratory cell mix (in 0.5ml to 1.0ml total volume) into the transwell insert; allow cells to migrate for 4 to 5 hours in the 37^{o}C, 5%CO_2 incubator.
7. Remove insert from the chamber. Turn chamber upside-down and gently submerge in PBS several times to remove unattached cells.
8. Fix cells by submerging insert in 10% formalin for 10min. Then wash with PBS once.
9. Stain cells by soaking insert in hematoxylin for 30min (or overnight); then wash the insert **gently** several times with tap water to remove excess stain.
10. Remove cells from unmigrated (top) side by **gently** scrapping this side with a wet Q-tip/cotton swab. Make sure wiping all residual cells out in this (top) side. Allow the membrane to dry.
11. Cut out membrane from insert with the migrated side down to a glass slide. Add a few drops of Permont onto the membrane and cover it with cover slide evenly.
12. Count migrated cells on a grid under high power (e.g., x20 or x30). You usually need to count **5 to 10 high power fields** (HPFs) to determine the average cell number migrated per HPF.