Protocol

Transwell filter migration assay

Version 1.0

Background The transwell filter migration assay is a relatively simple cell migration assay. It is suitable for the screening of compounds to determine their chemoattractant or inhibitory effects on cell migration, and provides a rapid indication of net cell population movement responses to putative chemoattractants or cell migration inhibitors. A variety of motile cell types (including freshly isolated and cultured leukocytes) can be used in this system.

Procedure:

Note: This is a simple and abbreviated protocol, detailing only the practical aspects of carrying out a transwell filter migration assay. However, there are a number of important variables that need to be considered in the selection of a suitable transwell system and in the appropriate design of an experiment. For details of these issues, please refer to www.neuroprobe.com and our (forthcoming) review.

A. *Testing for chemoattractants*

1. Spin and wash cells twice in Geys' Balanced Salt Solution + 1 mg/ml fatty acid-free BSA (Geys/BSA). Reconstitute cell pellet in a small volume of Geys/BSA and count.

2. Resuspend cells to a density of 4 x 10^6/ml in Geys/BSA (this gives 10^5 cells in 25 μl). Note that it is possible to use cells at 2 x 10^6/ml (5 x 10^4 cells in 25 μl) if cells are limited in number and/or difficult to isolate.

3. The chemoattractant(s) to be tested are diluted to appropriate concentrations in Geys/BSA. Chemoattractants are typically tested at multiple concentrations (spanning the nM range), and each condition is assayed in triplicate. It is advised to prepare 120 μl of sample for each triplicate condition.

4. The migration plate is a 96-well disposable chemotaxis chamber fitted with a polycarbonate filter membrane containing pores of a specified diameter (ranging from 2-

14 μm). 29 μl of a given sample is added to each of 3 wells (*see Key Point 3). No bubbles should be present in the wells upon filling.

5. The framed filter is carefully aligned and securely fitted over the top of the multiwell plate.

6. 25 μl of the prepared cell suspension (10^5 cells) is placed on the top of each well. The lid is placed over the samples so as to prevent evaporation.

7. The assembled chamber is incubated at 37 C, 5% CO_2 for between 45 min and 4 hours (it may be necessary to perform a timecourse of migration in order to determine the optimal incubation time). Any remaining cell suspension is incubated at 37 C along with the chamber; these cells will be used to construct a standard curve (Step 11).

8. After incubation, the cells are gently removed from the top of the filter with a pipette, 20 μl of ice-cold 20 mM EDTA in simple PBS is added to the top of each well and incubated at 4 C for 15 min.

9. The filter is carefully flushed with Geys/BSA (to wash off the EDTA droplets) and then removed.

10. Cells that have migrated into the lower compartment of the migration chamber are stained using the vital dye MTT. 3 μl MTT (at 5 mg/ml in RPMI-1640 without phenol red) is added to each well and incubated at 37 C for 1-2 hours.

11. An 8-point standard curve of cells is prepared in order to accurately quantify the number of migrated cells. This consists of a two-fold dilution series of cells (top standard = number of cells added to each well), diluted in Geys/BSA. A zero (no cells) must be included. 25 μl of each standard is added in triplicate to a 96-well plate. 3 μl MTT is added to each of these wells, and incubated alongside the migration plate (Step 10) for 1-2 hours.

12. The liquid is carefully aspirated from the wells, leaving the stained cells at the bottom of the wells. The converted formazan blue dye is solubilised using 20 μl DMSO per well. Absorbance of the converted dye is measured at a wavelength of 595 nm using an ELISA plate reader. The number of cells migrated in each well is determined by interpolation of the standard curve.

B. Testing for inhibitors of cell migration

Inhibitors of cell migration are identified by their ability to prevent migration to a known chemoattractant. The method of testing for inhibitors of cell migration is similar to that

for testing chemoattractants, with the primary exception that the putative inhibitor is added in **equal concentrations** to both the top and bottom compartments of the migration chamber, while the chemoattractant is restricted to the bottom compartment as previously. On a practical level, the required modifications to protocol A are as follows:

1. Cells should be resuspended to a density of 4.45×10^6/ml (or 2.22×10^6/ml).

2. Chemoattractant and test compound stocks should be made up to 10x the desired final concentration(s) in Geys/BSA. The test compound and chemoattractant are then diluted 10-fold (to a final volume of 120 µl per triplicate condition) to generate the samples for the bottom compartments of the chamber. The test compound is also diluted 10-fold into the cell suspension (10 µl compound + 90 µl cells per triplicate condition), to ensure that it is present at equal concentrations in both the top and bottom compartments.

KEY POINTS:

* **1.** If using cultured cell lines, ensure that the culture and maintenance conditions are standardised, and always use the cells at the same point on their growth curve for every assay in a series. We routinely subculture our Jurkat and THP-1 cells to 4×10^5/ml the day before performing a transwell migration experiment.

* **2.** Do not incorporate RPMI or FCS into the migration assay buffer, as substances present in both RPMI and FCS have been shown to influence cell migration.

* **3.** It is crucial to add exactly the right volume of sample to the wells, in order to ensure that the wells are completely and appropriately filled. It is advised to calibrate a pipette specifically for the purpose of filling chamber wells. For details of the suggested calibration procedure, visit the Neuroprobe website www.neuroprobe.com.

* **4.** Do not attempt to load more than 48 wells (half a plate) at a time, as evaporation of sample from the lower wells may occur if too long is taken to load the samples. This may result in an improper seal when the filter membrane is fitted over the plate. If more than 48 wells are required in an experiment, split the wells over 2 separate plates and load the plates sequentially.

Materials

The transwell systems are manufactured by Neuroprobe, Gaithersburg, MD, USA (www.neuroprobe.com). The UK distributor is Receptor Technologies (www.receptortechnologies.co.uk). We use ChemoTx plates (Neuroprobe 101-8) and 30 µl clear plates (Neuroprobe MP30).

Geys' Balanced Salt Solution (Sigma G-9779)
Fatty acid-free BSA (Sigma A-8806)
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma M-5655)
RPMI-1640 without phenol red (Sigma R-8755)
DMSO (Sigma D-5879)

Assay buffer Geys + 1 mg/ml fatty acid-free BSA
EDTA + simple PBS 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, adjusted to pH 7.2. Add 20 mM EDTA.
Vital dye 5 mg/ml MTT dissolved in RPMI-1640 without phenol red. The solution is filter sterilised through 0.22μm filter to remove any undissolved MTT. Aliquoted to 100 µl aliquots and frozen which can be used for each set of triplicate wells. This solution is light-sensitive and so needs to be aliquoted as quick as possible in darkened conditions.

Source References

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References where we have used this protocol

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Reckless J, Tatalick LM, Grainger DJ (2001) *Immunology* **103**(2):244-54.
Fox, DJ, Reckless J, Warren SG, Grainger DJ (2002) *J. Med Chem* **45**(2):360-370

DISCLAIMER

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