2

Boyden Chamber Assay

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Summary

The Boyden chamber assay, originally introduced by Boyden for the analysis of leukocyte chemotaxis, is based on a chamber of two medium-filled compartments separated by a microporous membrane. In general, cells are placed in the upper compartment and are allowed to migrate through the pores of the membrane into the lower compartment, in which chemotactic agents are present. After an appropriate incubation time, the membrane between the two compartments is fixed and stained, and the number of cells that have migrated to the lower side of the membrane is determined. Therefore, the Boyden chamber-based cell migration assay has also been called filter membrane migration assay, trans-well migration assay, or chemotaxis assay. A number of different Boyden chamber devices are available commercially. The method described in this chapter is intended specifically for measuring the migration of Madin-Darby canine kidney cells using a 48-well chamber from Neuro Probe, Inc.

Key Words: Boyden chamber assay; migration assay; cell motility; cell migration; chemotaxis; hapatotaxis.

1. Introduction

The Boyden chamber, originally introduced by Boyden for the analysis of leukocyte chemotaxis ***(1)***, is ideally suited for the quantitative analysis of different migratory responses of cells, including chemotaxis, haptotaxis, and chemokinesis. Random cell motility is generally described as chemokinesis, which is distinguished from directed cell motility toward increasing concentrations of soluble attractants, such as growth factors (chemotaxis), or along a concentration gradient of extracellular matrix (ECM) proteins (for haptotaxis *see* **ref. *2***). For the induction of chemotactic or haptotactic response of cells, attractants are added to the lower compartment of the chamber. However, for

From: *Methods in Molecular Biology, vol. 294: Cell Migration: Developmental Methods and Protocols*
Edited by: J-L. Guan © Humana Press Inc., Totowa, NJ

the induction of chemokinesis, equal concentrations of the agent are added on both sides of the membrane.

In addition, the use of the Boyden chamber-based motility assay has other advantages. First, it allows one to have versatility in conducting motility experiments. For example, one can examine the effect of an inhibitor that is specific for an intracellular signaling molecule or a functional blocking antibody that is specific to a cell surface protein on cell motility by adding it to the upper chamber, in which cells are loaded. Actually, this type of experiment has been widely used to examine the potential involvement of a particular intracellular signaling pathway or cell surface protein in cell motility in response to various stimuli ***(3,4)***. Second, the Boyden chamber assay is relatively time saving and allows for cell-motility analysis on a basis without consideration of the effect from cell proliferation. The time allowed for cells to migrate through a porous membrane in the Boyden chamber is generally within a few hours (4–6 h), which is much shorter than the time required for cells to proceed through a cell cycle ***(5)***. Therefore, consideration of the effect of cell proliferation on the results is generally not necessary. Third, the Boyden chamber assay allows for cell motility analysis without consideration of the effect from cell–cell interactions. Cells, in particular epithelial cells, have to release cell–cell contacts for their translocation over a clearly measurable distance. It is already known that cell–cell interactions and cell migration are controlled largely by different mechanisms ***(6–9)***. Because some extracellular factors or genes may be able to stimulate cell migration but fail to disrupt cell–cell interactions, it is reasonable to analyze cell–cell interactions and cell motility separately ***(10)***. The Boyden chamber assay is conducted on a basis largely independent of cell–cell interactions. Next, the Boyden chamber assay allows one to measure the relative cell migration rates of cell populations transiently transfected with genes of interest. With an appropriate marker, such as β-galactosidase, which allows the migrated cells to be visualized by X-gal staining, the relative cell motility of a transiently transfected cell population can be measured ***(11)***. Finally, it is easy for learners to gain acquaintance with the assay and the results are generally very reliable from one experiment to another.

A number of different Boyden chamber devices are available commercially. They basically vary in sample size and quantitation method. In general, cell migration is quantified by simply enumerating migrated cells under a light microscope or by measuring optical density values of stained cell extracts. Once an appropriate chamber has been chosen, the migration experiments will need to be optimized for any given cell type and attractants. Some of the important factors to be considered are the number of cells to load on the chamber, the pore size of the membrane, the type and concentration of the attractant, and the incubation time. Note that the ECM protein can be coated on the mem-

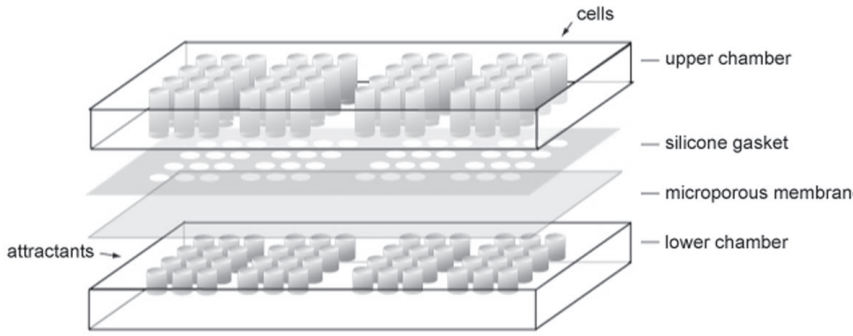

Fig. 1. Components of Neuro Probe standard 48-well chemotaxis chamber.

brane or directly loaded into the lower chamber in its soluble form. In our experience, the results derived from these two loading methods are similar. It is likely that the soluble form of the ECM protein will coat the membrane to form a matrix during the course of the experiment. If a soluble attractant such as growth factor is used, the ECM protein should still be loaded to allow cells to attach. Collagen and fibronectin are usually chosen respectively for epithelial cells and fibroblasts to attach. The following protocol is intended specifically for measuring the migration of Madin–Darby canine kidney (MDCK) epithelial cells using a 48-well chamber from Neuro Probe, Inc., and soluble collagen as an attractant. However, this could easily be adopted to analysis of other cell types in response to other stimuli.

2. Materials

1. Standard 48-well chemotaxis chamber (*see* **Fig. 1**), including: a lower chamber, a silicone gasket, an upper chamber, and thumb nuts (Neuro Probe; Cabin John, MD).
2. Poretics® polycarbonate: polyvinylpyrrolidone-free, 8-µm pore size, 25 × 80 mm (cat. no. K80SH58050; Osmonics; Livermore, CA).
3. MDCK cells (ATCC; cat. no. CCL-34).
4. Dulbecco's modified Eagle's medium (DMEM), high glucose, pH 7.4.
5. Fetal bovine serum (FBS).
6. Versene: 0.2 g of ethylene diamine tetraacetic acid (0.53 m*M*) and 0.01 g of phenol red in 1 L of phosphate-buffered saline, pH 7.4.
7. 2.5% (w/v) Trypsin (cat. no.15090-046; Gibco Invitrogen): dilute 1:25 in Versene before use.
8. Collagen from calf skin (cat. no. C9791; Sigma-Aldrich): dissolve collagen at 1 mg/mL in 0.1 *N* acetic acid. Allow to stir at room temperature until dissolved (takes 1–3 h). Keep collagen stock at 4°C.

9. Methanol.
10. Giemsa stain, modified solution (cat. no. GS500; Sigma-Aldrich): dilute 1:10 in distilled H_2O before use.
11. Petri dishes.
12. Glass slide and cover glass (32 × 24 mm).
13. Nail polish.
14. Terg-A-Zyme® (cat. no. 1304; Alconox; New York, NY): an enzyme active detergent available from Fisher. Dissolve 8 g/L in distilled H_2O.

3. Methods

3.1. Preparing the Cells

1. Seed 5×10^5 MDCK cells per 60 mm-dish in DMEM supplemented with 10% FBS and penicillin-streptomycin and allow them to grow until 50–70% confluency, which usually takes 18 to 24 h.
2. Remove the medium and wash the cells twice with Versene. Add 1 mL of Versene containing 0.05% trypsin and allow the culture to stand at 37°C for 10 to 15 min. Add 3 mL of DMEM with 10% FBS, pipet the cells off the dish, and transfer them to a 15-mL centrifuge tube.
3. Pellet the cells by centrifugation at 150–200*g* for 5 min. Remove the medium, add 5 mL of DMEM, and centrifuge again.
4. Remove the medium and resuspend the cells in 1.5 mL of DMEM. Count and adjust the cells to 5×10^5 cells/mL in DMEM (*see* **Notes 1** and **2**). If necessary, add the appropriate concentration of an inhibitor or antibody to the cell suspension.

3.2. Loading and Assembling the Chamber

1. Dilute collagen stock in DMEM to 10 µg/mL before loading (*see* **Note 3**). Add 30 µL of the diluted collagen or control reagents to each well of the bottom chamber. The volume should be a slight positive meniscus when the well is filled. If not, re-set the pipetor and load again. It is recommended to use a manual p200 pipetor for loading (*see* **Note 4**).
2. Use forceps to handle polycarbonate membranes. Cut off 1 mm of the corner of a membrane. Lift the membrane by the end using two forceps, and orient it to the chamber so the cut corner corresponds to the Neuro Probe trademark on the lower right corner of the chamber (*see* **Note 5**). Gently place the membrane (shiny side facing up) over the wells of the chamber. Avoid too much movement of the membrane after it has been placed on the wells.
3. Place the silicone gasket over the membrane with cut corner on the lower right.
4. Place the top chamber over the gasket with the Neuro Probe trademark oriented to the lower right corner. Push the top chamber down against the bottom chamber with even pressure on all sides with one hand; with the other hand tighten the thumb nuts.
5. Resuspend the cells by gentle mixing and load 50 µL of cell suspension to each well of the top chamber. Hold the pipetor vertically so that the end of the pipet tip rests against the side of the well just above the membrane. Eject the fluid out

of the pipet tip with a rapid motion to avoid trapping bubbles in the bottom of the well.
6. Wrap the whole chamber in plastic and incubate it at 37°C and 5% CO_2 for 6 h.

3.3. Staining the Membrane

1. Transfer 20 mL of methanol to a Petri dish.
2. Remove the thumb nuts while pressing down the chamber evenly on all sides.
3. Disassemble the chamber, lift the membrane with forceps, and immediately flow it on methanol at room temperature for 10 min to fix cells. The side of the membrane with the migrated cells faces down.
4. Remove the membrane from methanol and allow it to air dry (few min).
5. Dilute 2 mL of Giemsa stain in 20 mL of distilled H_2O in a Petri dish.
6. Flow the membrane (the side with migrated cells facing down) on the diluted Giemsa stain solution for 1 h to stain the cells (*see* **Note 6**).
7. Destain the membrane by briefly (few seconds) rinsing it in distilled H_2O.
8. Drain the excess H_2O from the membrane and place it (the side with migrated cells facing down) on the Petri dish cover. Use a damp cotton swab to wipe the unmigrated cells from the top of the membrane.
9. Cut the membrane into four pieces, each contains 12 wells. Keep track of its orientation by cutting the lower right corner.
10. Attach the membrane with a little amount of nail polish to a glass slide and cover it with a square cover glass.

3.4. Counting the Cells

1. Survey the stained membrane under a light microscope at 50X and 200X magnification and select at least three wells for each experimental group on which the migrated cells are well stained and evenly distributed.
2. Take micrographs using a digital camera (Nikon COOLPIX995) connected to the microscope at 50X magnification. Each micrograph covers a well on the membrane *see* **Note 7**).
3. Remove the camera from the microscope and connect to a computer using the USB cable. Acquire the images from the memory card of the digital camera and view the images with the aid of the software Adobe Photoshop® (Adobe Systems; San Jose, CA). Select the function "view/show" to add grids on the image and count the number of cells on the monitor (*see* **Fig. 2**). Alternative, adjust the size of the image for printing on an A4 size paper and then count the number of cells on the printed image.
4. Calculate the value (mean ± standard error) of the migrated cells from at least three wells for each experimental group.

3.5. Cleaning the Chamber

1. After disassembling the chamber, immediately rinse all parts in running tap H_2O for 30 min. Rinse the chamber components several times with distilled H_2O and let them air dry at room temperature.

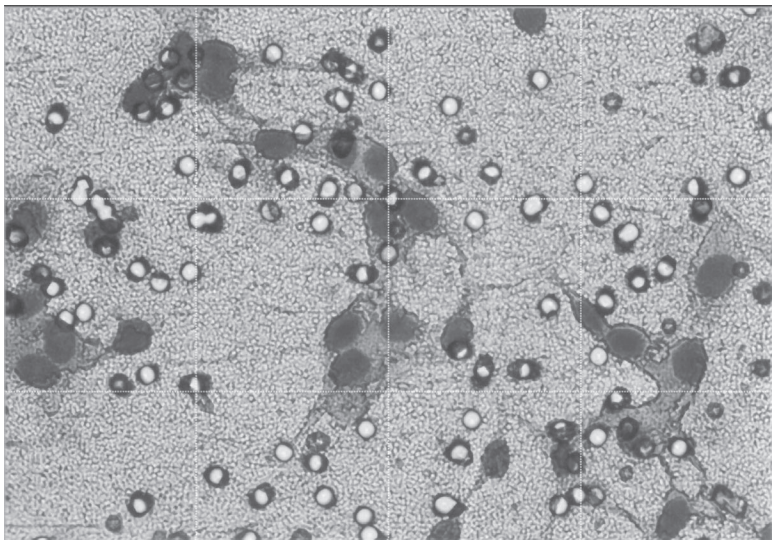

Fig. 2. A representative micrograph of a stained membrane taken by a digital camera under a light microscope.

2. For periodic cleaning, soak all the chamber components in Terg-A-Zyme® solution at 55°C for 1 h. Wash them thoroughly under running tap H_2O for 30 min and rinse them several times with distilled H_2O. Soak them in distilled H_2O overnight and allow them to air dry (*see* **Note 8**).

4. Notes

1. It is recommended that less than six experimental groups are planned for a chamber, which allows at least eight wells for an experimental group. This ensures at least three representative wells that can be selected for quantification for each experimental group. In addition, more experimental groups will take more time for preparation, which causes cells in different experimental groups to stay in suspension with varied time, which may affect cell motility.
2. Because the volume varies from one pipetor to another, it is better to prepare a little more sample for loading. For example, if eight wells per experimental group are planned for loading, prepare enough sample for loading 10 wells. The volume of cell suspension (5×10^5 cells/mL) to load on a well of the upper chamber is 50 µL, that is, 2.5×10^4 cells per well. Make 2.5×10^5 cells in 0.5 mL of DMEM for loading 10 wells.
3. Collagen is less soluble in neutral pH than in low pH. After diluting collagen in DMEM, immediately load it to the lower chamber.
4. To avoid trapping bubbles in the wells of the chamber, the liquid should not be expelled completely from the pipet tip. In addition, for the lower chamber, it is

important to load the correct volume of attractants, which should form a slight positive meniscus when the well is filled. For loading the upper chamber, hold the pipetor vertically so that the end of the pipet tip is against the side of the well just above the membrane and expel the liquid quickly from the pipet tip.

5. Ensure to keep track of the orientation of the membrane by cutting it on the right lower corner.
6. Instead of immersing the membrane in Giemsa stain, we let the membrane flow on the stain solution. This allows only the side of the membrane with the migrated cells to be stained.
7. With the aid of a digital camera and computer, the total number of stained cells in a well can be counted accurately and objectively. Directly counting the cells in selected fields from a well under a microscope at high magnification is not recommended.
8. It is important to immediately immerse all parts of the chamber in water after dissembling the chamber and wash them thoroughly to prevent the accumulation of debris. It is recommended that after approximately five experiments, the chamber components are cleaned with Terg-A-Zyme® (*see* **Subheading 3.5.2.**). Never autoclave the chamber or immerse it in water hotter than 60°C.

Acknowledgments

The author would like to thank Po-Chao Chan and Chun-Chi Liang for technical assistance. Work in the author's laboratory is supported by the National Science Council, Taiwan.

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Cell Migration
Developmental Methods and Protocols
(Ed.)J.-L. Guan
2005, XVI, 364 p. 155 illus., 5 in color., Hardcover
ISBN: 978-1-58829-382-4
A product of Humana Press