

A quantitative cell migration assay using ThinCert™ cell culture inserts

Introduction

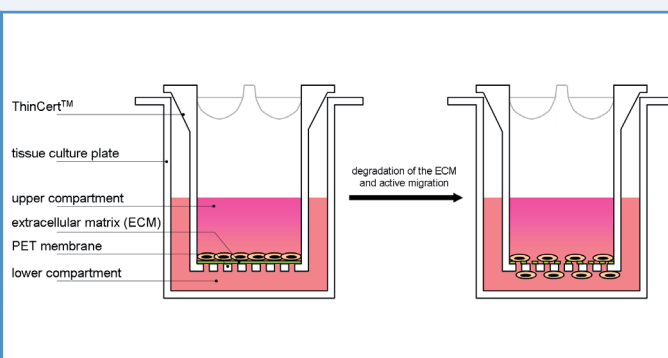
Cell migration plays an important role in physiological and pathological processes as diverse as embryonic development, wound healing, immune response, inflammation, and tumorigenesis. The availability of methods to study cell migration is of great importance to allow a better understanding of the underlying biological mechanisms. Moreover, since the correlation between the *in vitro* migratory potential of tumor cells and their *in vivo* invasive properties was reported (Klemke et al., 1998), cell migration assays have gained widespread acceptance in the screening of anti-cancer drugs.

A broad spectrum of assays to analyze chemokinetic and chemotactic cell migration has been developed in previous years (reviewed in Entschladen et al., 2005). Among them, the Filter assay is one of the most frequently used *in vitro* assays. First published in 1962 by Stephen Boyden (Boyden, 1962), the Filter assay is still employed in cell migration studies. This assay involves a two-compartment system where cells may be induced to migrate from an upper compartment through a porous membrane into a lower compartment, thus following the gradient of a chemoattractant (Figure 1). Relative to the size of the investigated cells, the size of the pores in the membrane must be small enough to avoid the passive passage of cells, but large enough to allow their active migration. Customarily, 3 µm-pores are applied for leukocytes and 8 µm-pores for epithelial and tumor cells. Modifications of the Filter assay include variants where the porous membrane is coated with an extra-cellular matrix (invasion assays), or where it carries a cell monolayer (transepithelial migration assays).

With ThinCert™ cell culture inserts Greiner Bio-One offers a high quality solution for multiple applications in cell biology, to include cell migration and invasion studies. ThinCert™ cell culture inserts are available in several well formats (6, 12, 24 well) and with PET membranes featuring various pore sizes (e.g. 3.0 and 8.0 µm) and densities. Here, an application protocol for the quantification of migratory cells with ThinCert™ cell culture inserts is provided. The several application examples discussed here indicate the excellent suitability of ThinCert™ cell culture inserts for cell migration studies and the reproducibility of the obtained results.

Figure 1: Experimental setup to study cell invasion *in vitro*.

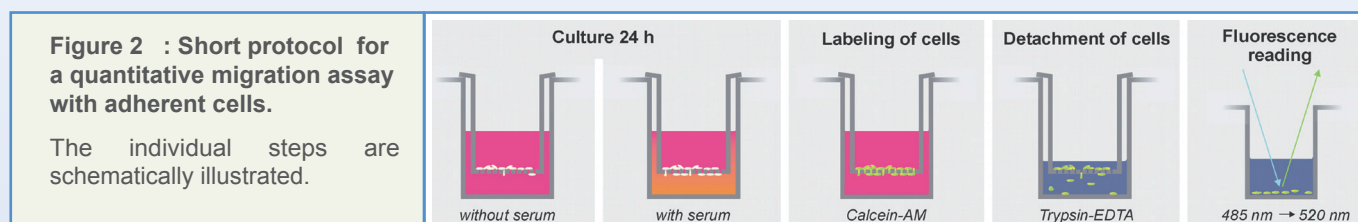
A ThinCert™ cell culture insert is placed in the well of a multiwell cell culture plate, thus forming a migration chamber. The migration chamber consists of an upper and lower compartment with a porous PET membrane in-between. Cells may actively migrate from the upper to the lower compartment.



Principle of the Assay

ThinCert™ cell culture inserts are placed in a multiwell cell culture plate, thereby forming two compartments: the upper compartment of the insert and the lower compartment of the plate well. Both compartments form the migration chamber, separated by the porous PET membrane (Figure 1). Cells sown on the PET membrane in serum-free medium may be induced to actively migrate through the PET membrane into the lower compartment with media containing serum or another chemoattractant. Adherent cells remain attached to the under-side of the PET membrane. After fluorescently labelling all cells with Calcein-AM, the migratory cells are detached from the underside of the PET membrane. Finally, the migratory cells are quantified in a standard fluorescence microplate reader. An overview on the individual steps of the migration assay is given in figure 2.

1. Prepare cell cultures according to standard cell culture procedures
2. Starve cells overnight in serum-free medium with 0.2 % BSA
3. Harvest cells and wash them twice in PBS
4. Resuspend cells in serum-free medium with 0.2 % BSA to an appropriate final cell concentration (e.g. 10^6 / ml)
5. Place 24 well ThinCert™ cell culture inserts in the wells of a CELLSTAR® 24 well cell culture plate
6. Add 600 µl culture medium with or without chemoattractant to each well of the cell culture plate
7. Add 200 µl cell suspension to each cell culture insert
8. Incubate the cell culture plate for 3 - 24 h in a cell culture incubator at 37 °C and 5 % CO₂
9. Remove the cell culture medium from each well of the cell culture plate and replace it by 450 µl serum-free culture medium with 8 µM Calcein-AM¹
10. Incubate for 45 min in a cell culture incubator at 37 °C and 5 % CO₂
11. Remove the culture medium from the cell culture inserts
12. Transfer the cell culture inserts into a freshly prepared 24 well cell culture plate containing 500 µl prewarmed Trypsin-EDTA per well
13. Incubate for 10 min in a cell culture incubator at 37 °C and 5 % CO₂, agitate the plate from time to time
14. Discard the cell culture inserts and transfer 200 µl of the Trypsin-EDTA solution (now containing the migratory cells) from each well into a black flat bottom 96 well plate
15. Read fluorescence in a fluorescence plate reader at an excitation wavelength of 485 nm and an emission wavelength of 520 nm



¹ Calcein AM is a cell-permeant dye that can be used to determine cell viability in most eukaryotic cells. In live cells the non-fluorescent calcein AM is converted to green-fluorescent calcein, after acetoxymethyl ester hydrolysis by intracellular esterases.

Material and Methods

Material and culture media		
Item	Manufacturer	Cat.-No.
DMEM medium	Biochrom AG	F0435
L-Alanyl-L-Glutamine	Biochrom AG	K0302
Trypsin/EDTA solution (0.05%/0.02%)	Biochrom AG	L2143
Black 96 well polystyrene strip plate	Greiner Bio-One GmbH	756 076
CELLSTAR® 24 well cell culture plate	Greiner Bio-One GmbH	662 160
ThinCert™ 24 well cell culture insert with 8 µm pores	Greiner Bio-One GmbH	662 638
Fetal calf serum	Invitrogen Life Technologies	10 270-106
Calcein-AM solution (4mM in anhydrous DMSO)	Sigma	C1359-100UL
Bovine serum albumin (30%)	Sigma	A7284-10ML

Methods²

Cell cultures were prepared and maintained according to standard cell culture procedures. Cell culture media were supplemented with 4 mM L-Alanyl-L-Glutamine. The cell lines HT1080, HeLa and NIH3T3 were cultured in DMEM medium with 10 % fetal calf serum (FCS). The night before the migration experiment the cells were deprived in serum-free DMEM medium containing 0.2 % bovine serum albumin. Cells were harvested, washed twice in PBS, and resuspended in serum-free DMEM medium with 0.2 % BSA to obtain an appropriate final concentration (e.g. 10^6 cells/ml). 24 well ThinCert™ cell culture inserts with 8 µm pores and translucent PET membranes were placed in the wells of a CELLSTAR® 24 well cell culture plate. 600 µl of serum-free DMEM medium with 0.2 % BSA and varying concentrations of FCS was added to each well of the cell culture plate (lower compartment). 200 µl of the cell suspension was added to each cell culture insert, and the plate with inserts was incubated for 20 h in an incubator at 37 °C and 5 % CO₂. Subsequently, the cell culture medium was removed from each well of the cell culture plate and replaced with 450 µl DMEM medium containing 0.2 % BSA and 8 µM Calcein-AM. The plate with inserts was incubated for 45 min in a cell culture incubator at 37 °C and 5 % CO₂. Thereafter, the culture medium was removed from the ThinCert™ cell culture inserts³, and the ThinCert™ cell culture inserts were transferred in the wells of a freshly prepared 24 well plate containing 500 µl Trypsin-EDTA per well. This plate was incubated for 10 min in a cell culture incubator at 37 °C and 5 % CO₂ with sporadic agitation. The ThinCert™ cell culture inserts were discarded, and 200 µl of the Trypsin-EDTA solution (now containing the detached migratory cells) was transferred from each well of the 24 well plate into a new well of a flat-bottom black 96 well plate. Finally, the migratory cells were quantified in the black 96 well plate with a fluorescence plate reader (Tecan Safire) at an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

² The following procedure is optimized for adherent cells only and may be adapted to suit other cell types.

³ Phenol red in the culture medium may interfere with the subsequent fluorescence reading. The culture medium must therefore be removed from the cell culture insert.

Results and Discussion

Migrations of different cell types along gradients of fetal calf serum (FCS) were analyzed after application of the above-mentioned protocol. When 2×10^5 HT1080 cells were sown onto the ThinCert™ membrane, a concentration-dependent migration along different gradients of FCS could be observed. Similar high migration rates were observed along the 0 % → 5 % and 0 % → 10 %-FCS gradients, whereas weaker gradients (0 % → 0.5 % FCS and 0 % → 2 % FCS) yielded lower migration rates (Figure 3).

Furthermore, the migration rates of HeLa and NIH3T3 cells were assessed in the absence or presence of FCS gradients. The presence of 0 % → 10 % FCS gradients yielded a 10 and 20 - fold increase in the number of migratory HeLa and NIH3T3 cells, respectively, as compared to the control experiments without serum (Figure 4).

Figure 3: Migration of HT1080 cells over 20 h in response to varying concentrations of fetal calf serum.

Each data point represents the average of four single experiments. Error bars indicate the standard deviation. RFU: relative fluorescence units.

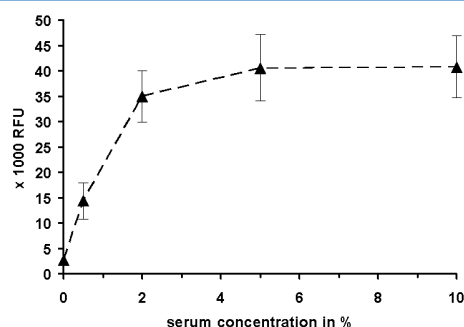
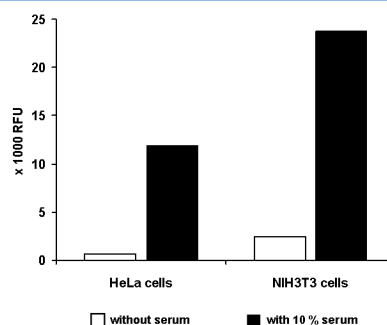


Figure 4: Migration of HeLa and NIH3T3 cells through 8 µm pores over 20 h in the absence and presence of fetal calf serum.

RFU: relative fluorescence units.



Throughout a wide range of cell populations (2.5×10^4 to 30.0×10^4 cells/migration chamber), the number of migratory HT1080 cells displayed a linear correlation with the total number of cells in the migration chamber (Figure 5). It is recommended that cell concentrations to be applied in a cell migration assay be selected from within this range.

Finally, two different production lots of ThinCert™ cell culture inserts were used to perform cell migration assays with HT1080 cells along 0 % → 10 %-FCS gradients. A high reproducibility of results was observed, from among both individual ThinCert™ cell culture inserts as well as two different lots of manufacture used to conduct the experiments (Figure 6).

Figure 5: Migration of HT1080 cells over 20 h in response to 10 % fetal calf serum.

The number of migratory cells (relative fluorescence units, RFU) correlates linearly with the total amount of cells in the cell culture insert. Each data point represents the average of four single experiments. Error bars indicate the standard deviation.

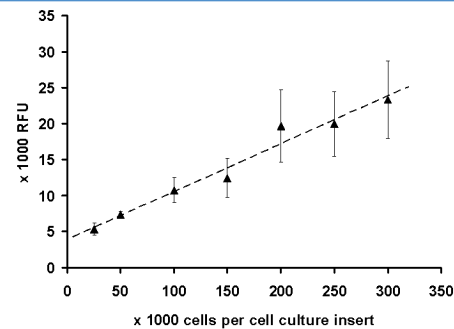
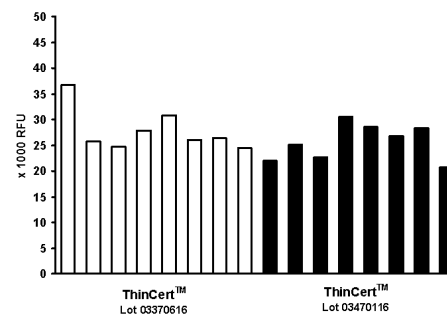


Figure 6: Reproducibility of migration experiments with ThinCert™ cell culture inserts.

Migration experiments were performed using 2×10^5 HT1080 cells per cell culture insert and 10 % fetal calf serum. Only little variation of the amount of migratory cells was observed among individual experiments and the two different lots of inserts.



Conclusion

The examples described here illustrate the excellent suitability of ThinCert™ cell culture inserts for cell migration assays. The adapted protocol allows for both ease of quantification of the migratory cells and excellent reproducibility of the obtained results. This protocol provides a ready-to-use solution for cell migration studies, in addition to serving as a basis for use in advanced applications such as transepithelial migration or *in vitro* cell invasion studies.

References

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- Entschladen F., Drell T.L. 4th, Lang K., Masur K., Palm D., Bastian P., Niggemann B., Zaenker K.S. (2005) Analysis methods of human cell migration. *Exp Cell Res.* Jul 15;307(2):418-26.
- Klemke R.L., Leng J., Molander R., Brooks P.C., Vuori K., Cheresh D.A. (1998) CAS/Crk coupling serves as a “molecular switch” for induction of cell migration. *J Cell Biol.* Feb 23;140(4):961-72.