A quantitative cell invasion assay using ThinCert™ cell culture inserts

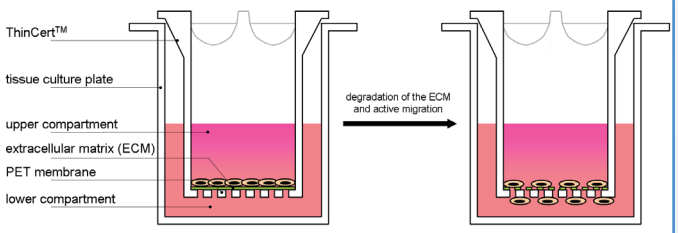
Introduction

Malignant tumors undergo a series of changes when progressing to the formation of metastasis. Crucial steps in this process are the detachment of tumor cells from the primary tumor, the degradation of the basal lamina and the successive invasion into the surrounding tissue. Many genes and factors have been involved in the tumor invasion, including adhesion molecules, cytoskeletal proteins and proteases (reviewed in Wang and Zhang, 2005). To investigate the function of such factors in physiological and pathological cell invasion a series of in vitro and in vivo approaches has been established in the past (reviewed in Crnic and Christofori, 2004). A classical in vitro assay to study cell migration and invasion is the Filter assay or Boyden chamber assay' (Boyden, 1962), where cells migrate along a chemoattractant gradient from an upper compartment through a porous membrane into a lower compartment. To determine the invasive potential of the cells the membrane can be coated with an extracellular matrix (ECM), thus mimicking the basal lamina (Albini et al., 1987).

Here, we provide an application protocol for a quantitative invasion assay performed with ThinCert™ cell culture inserts in a 24 well format. Thereby, the cells of interest are sowed onto the ECM coated PET membrane of the cell culture insert. Invasive cells are able to degrade the ECM and migrate to the underside of the membrane into the lower compartment that contains a chemoattractant (Figure 1). Subsequently the cells are fluorescently labelled, dislodged from the membrane and quantified in a fluorescence plate reader.

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

A ThinCert™ cell culture inserts 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 migration from the upper to the lower compartment. If the membrane is coated with an extracellular matrix, the invasive potential of the cells can be determined.



Material and Methods

Material and culture media		
Item	**Manufacturer**	**Cat.-No.**
DMEM medium	Biochrom AG	F0435
L-Alanyl-L-Glutamine	Biochrom AG	K0302
RPMI medium	Biochrom AG	F1295
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

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Germany (Main office): Greiner Bio-One GmbH, info@de.gbo.com I **Austria:** Greiner Bio-One GmbH, office@at.gbo.com
Belgium: Greiner Bio-One BVBA/SPRL, info@be.gbo.com I **Brazil:** Greiner Bio-One Brasil, office@br.gbo.com I **China:** Greiner Bio-One Suns Co. Ltd., office@cn.gbo.com
France: Greiner Bio-One SAS, infos@fr.gbo.com I **Japan:** Greiner Bio-One Co. Ltd., info@jp.gbo.com I **Netherlands:** Greiner Bio-One B.V., info@nl.gbo.com
UK: Greiner Bio-One Ltd., info@uk.gbo.com I **USA:** Greiner Bio-One North America Inc., info@us.gbo.com

Material and culture media		
Item	**Manufacturer**	**Cat.-No.**
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
Water (tissue culture grade)	Sigma	W3500-100ML

Coating of the ThinCert™ cell culture inserts

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. Extracellular matrix from Engelbreth-Holm-Swarm mouse tumors (~20 mg/ml) was thawed overnight at 4°C and diluted 1:40 with cold water to yield a protein concentration of 0.5 mg/ml. 60 µl of the ECM solution was added to each ThinCert™ cell culture insert and dried overnight under laminar air flow. Non-coated ThinCert™ cell culture inserts were used in control experiments.

Preparation of the cells

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 and NIH3T3 were cultured in DMEM medium with 10 % fetal calf serum (FCS). MCF7 cells were cultured in RPMI medium with 10 % FCS. The night before the migration experiment the cells were deprived in appropriate serum-free culture medium (DMEM for HT1080 and NIH3T3 cells, RPMI for MCF7 cells) containing 0.2 % bovine serum albumin (BSA). Cells were harvested, washed twice in PBS and resuspended in serum-free culture medium with 0.2 % BSA prior to adjustment to a final concentration of 10^6 per ml.

Sowing of the cells and incubation

600 µl serum-free culture medium with 0.2 % BSA and 10 % FCS was added to each well of the 24 well plate (lower compartment). 200 µl of the cell suspension was added to the pre-coated ThinCert™ cell culture inserts, and the plate with inserts was incubated for 20 h in a cell culture incubator at 37°C and 5 % CO_2. To determine the background migration some wells of the 24 well plate were prepared with only serum-free medium.

Labelling, detachment and quantification of the cells

Cell culture medium was removed from each well of the 24 well plate (lower compartment) and replaced with 450 µl culture medium containing 0.2 % BSA and 8 µM Calcein-AM[1]. The plates with inserts were incubated for 45 min in a cell culture incubator at 37°C and 5 % CO_2. Thereafter, the culture medium was removed from the ThinCert™ cell culture inserts[2], and the ThinCert™ were transferred to 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 % CO_2 with sporadic agitation. The ThinCert™ were discarded, and 200 µl of the Trypsin-EDTA solution (now containing the detached migratory cells) from each well of the 24 well plate was transferred to a 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.

[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.

[2] 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.

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Calculation of the results

To determine the invasive properties of the studied cell lines the invasion index (I %) was calculated according to the following formula:

$$I\ \% = \frac{(RFU_1 - RFU_0)}{(RFU_2 - RFU_0)} \times 100\ \%$$

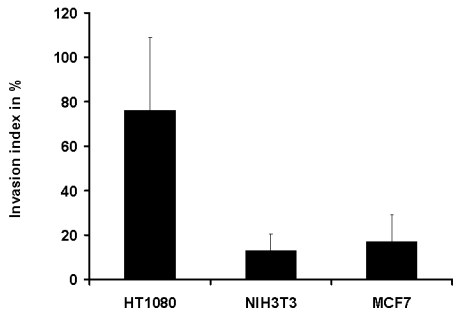
In this formula RFU1 represents the relative fluorescence units obtained from cells that migrated through an ECM coated membrane towards 10% FCS. RFU2 denotes the relative fluorescence units obtained from cells that migrated through an uncoated membrane towards 10% FCS, and RFU0 signifies the relative fluorescence units obtained from cells that passively passed through an uncoated membrane in the absence of FCS.

Results and Discussion

Here, the invasion profiles of the cell lines HT1080, MCF7 and NIH3T3 were assessed using the above described protocol. The cell lines HT1080 and MCF7 are tumor cell lines with invasive and non-invasive properties, respectively. In contrast, NIH3T3 cells are non-invasive non-tumor cells. In the performed invasion assays NIH3T3 an MCF7 cells revealed low invasion indices, whereas HT1080 cells portrayed a high invasion index (Figure 2). These results reflect the specific invasive properties of the investigated cell lines and demonstrate the suitability of ThinCert™ cell culture inserts for invasion assays. The established protocol may be used to study the mechanisms of tumor cell invasion and to develop substances that alter the metastatic properties of tumor cells.

Figure 2: Invasion profiles of different cell lines.

For the invasion experiments 24 well ThinCert™ cell culture inserts were coated with 30 µg extracellular matrix. The data represent the average invasion index obtained in three independent experiments. Error bars indicate the standard deviation.



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Further Readings

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