forum
No. 8, 2007
Technical Notes and Applications for Laboratory Work

Content

ThinCert™ cell culture products – innovative solutions for cell-based assays and tissue culture

Ethical, scientific and financial concerns lead to an increasing importance of cell-based assays and three-dimensional cell culture in lieu of live-animal experimentation. Therefore, new cell culture devices have been developed that allow the establishment of an *in vitro* environment with maximum retention of native functionality. With respect to these developments, ThinCert™ cell culture inserts with porous PET membranes are significant because they form a two-compartment system to readily mimic a variety of *in vivo* situations, such as:

- migration and relocation of cells (**Fig. 1A**),
- interaction and communication of physically separated cell populations (**Fig. 1B**),
- formation of tight cell-cell junctions,
- vectorial transport between two lumens (**Fig. 1C**),
- tissue growth and differentiation at the air-liquid-interface (**Fig. 1D**).

greiner bio-one

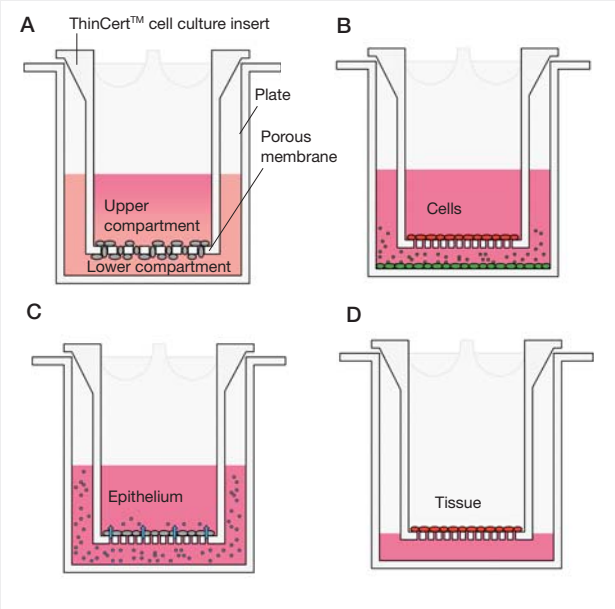

Figure 1: Overview on applications of ThinCert™ cell culture inserts
A, B, C: Schematic drawings illustrating experimental setups for migration and invasion assays (A), co-cultivation (B) and transport studies (C). D: Tissue growth at the air-liquid-interphase.

1. Characteristics of ThinCert™ cell culture inserts

ThinCert™ cell culture inserts combine consistent high quality with a user-friendly format. For example, the inserts are specifically designed to entail an eccentric position in the wells of a multiwell plate thereby facilitating pipette access to the lower compartment (Fig. 2A). The insert housing and PET membrane are combined by a thermobonding process which creates a very robust seal that prevents any membrane leakage. Nevertheless, the insert membrane can be cut out and easily subjected to downstream processing, such as sectioning and mounting on microscopy slides (Fig. 2B). The capillary pores of the ThinCert™ membrane are produced by a precise track-etching process which results in an even pore distribution and in highly uniform pore diameters (Fig. 2C and D).

2. Cell-based assays in ThinCert™ cell culture inserts

The following application examples illustrate the usage of ThinCert™ cell culture inserts in diverse cell-based assays. A selection guide for the appropriate insert type for your application of choice is provided in Table 1.

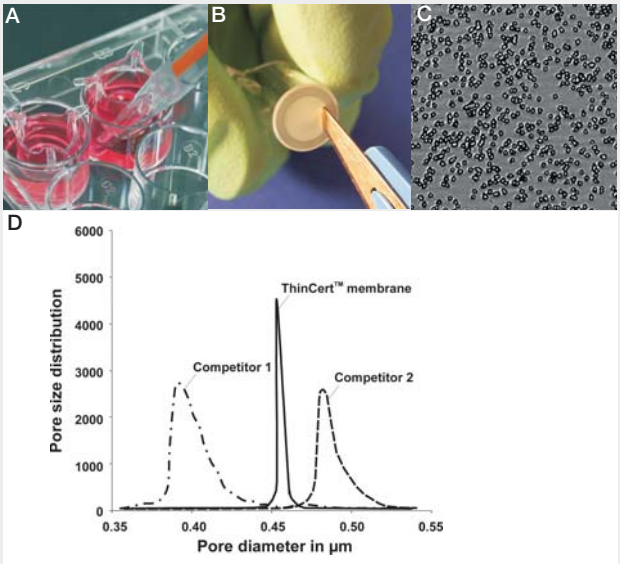

Figure 2: Characteristics of ThinCert™ cell culture inserts
A: Specific design of ThinCert™ cell culture inserts with easy pipette access to the lower compartment. B: Detachment of a ThinCert™ membrane with a scalpel. C: Microscopic view of a porous ThinCert™ membrane with even pore distribution. D: Pore size distribution of different porous membranes with nominal pore sizes of 0.4 µm.

2.1 Migration and invasion

Cell migration plays a significant role in physiological and pathological processes during embryonic development, wound healing, immune response, inflammation, and tumorigenesis. The filter assay or 'Boyden chamber assay' (Boyden, 1962) is a classical *in vitro* model used to study cell migration. This assay is performed in cell culture inserts with porous membrane supports (e.g. ThinCert™) and involves cell

migration from the upper compartment through the pores of the membrane towards a chemo-attractant source in the lower compartment.

To determine the invasive potential of cells, the membrane may also be coated with an extracellular matrix (ECM), which mimics the basal lamina (Albini et al., 1987). Usually ThinCert™ cell culture inserts with 8.0 µm pores are used in such assays.

Invasive cells (e.g. HT1080) and non-invasive cells (e.g. MCF7, NIH3T3) may be distinguished based on their invasion index, which is defined as the ratio of the number of cells migrating in the presence and absence of an ECM coating (Fig. 3A).

Several procedures may be conducted in order to quantify migratory cells in the filter assay. For example, cells may be stained with crystal violet, non-migratory cells may be removed from the upper compartment with a cotton swab and migratory cells may be counted with a microscope (Hiscox et al., 2006). Other procedures involve cell staining with fluorescent dyes such as DAPI or Calcein-AM , the detachment of migratory cells from the underside of the membrane using Trypsin-EDTA and their subsequent quantification using a fluorescence plate reader.

2.2 Co-Culture

Co-culture describes a rather heterogeneous field including applications as diverse as the investigation of immune cell interactions, the stimulation of cell proliferation, the maintenance of cell differentiation and the restoration of heterocellular functions *in vitro* (e.g. blood-brain-barrier). With ThinCert™ cell cultures inserts, cells may be seeded in the upper and lower compartment, which keeps them physically separated at all stages of the co-culture. The pores of the ThinCert™ membrane, however, allow the exchange of molecules between the two compartments and hence between the two cell populations.

MCF7 cells cultivated on ThinCert™ cell culture inserts with translucent membranes and 0.4 µm pores may receive a growth promoting stimulus from primary human fibroblasts cultivated on the underside of the membrane. This enhanced proliferation is indicative of an increased fraction of proliferative, Ki67 expressing cells in proportion to the total cell number (Fig. 3B).

As mentioned above, it is important to note that cells co-cultivated in the lower compartment may be grown not only on the surface of the multiwell plate, but also on the underside of the membrane. Therefore, the distance between the two cell populations can be quite close (in the range of the diameter of a single cell). Both sides of the membrane are tissue culture treated to encourage this possibility.

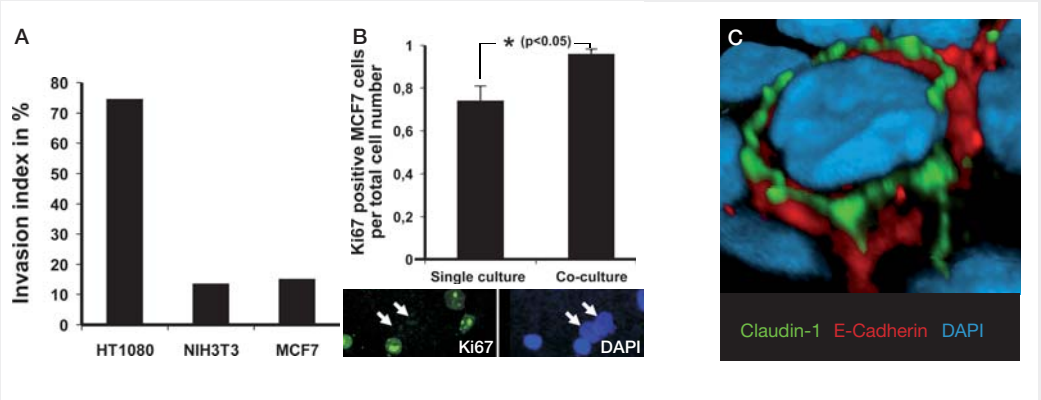

Figure 3: Overview on applications of ThinCert™ cell culture inserts
A: In ECM coated inserts invasive HT1080 cells reveal a higher invasion index towards an FCS source in the lower compartment than non-invasive MCF7 and NIH3T3 cells. **B:** MCF7 cells were grown on a 0.4 µm pore membrane and obtained a proliferative stimulus from human fibroblasts co-cultivated in the lower compartment. Non-proliferative cells were identified by lack of Ki67 immunofluorescence (arrows). **C:** For transport studies epithelia may be generated from MDCK II cells on 0.4 µm ThinCert™ cell culture inserts. Immunofluorescence indicates the polarised localisation of Claudin-1 in the tight junctions and E-Cadherin basolaterally.

2.3 Epithelia, immunocytochemistry and transport studies

Transport studies are among the most frequent applications of ThinCert™ cell culture inserts. Here, the goal is to reconstruct a functional epithelium from individual cells and to study the active transport of substances from one compartment through the epithelium to the other compartment. It has been shown that polarised epithelia with tight junctions and basolateral and apical membrane compartments can be generated in cell culture inserts with porous membrane supports. The interaction of two phenomena seems to account for this effect: (1) the insert membrane, which usually carries an ECM treatment, can mimic the basement membrane; and (2) the pores of the membrane allow the cells to take up nutrients from the basolateral side (Chambard et al., 1983; Guguen-Guillouzo and Guillouzo, 1986; Saunders et al., 1993). Again, it is the similarity between the insert system and the *in vivo* situation that maintains the native cell function *in vitro*.

One prerequisite for a successful transport study is the establishment of a tight epithelium without trans-cellular leakage. Several techniques may be used to verify how tight the cultivated epithelium is, such as the measurement of transepithelial electric resistance (TEER) and the determination of transcellular leakage with non-transportable markers (^{3}[H]inulin; Kopplow et al., 2005). Additionally, some of the prepared cultures may be sacrificed and analysed for tight junction formation using immunofluorescence (Mettlen et al., 2006).

Fig. 3C represents a microphotograph of a MDCK II cell layer cultivated on translucent ThinCert™ cell culture inserts with 0.4 µm pores. The insert membrane has been cut out and subjected to immunocytochemistry. Confocal fluorescence images have been acquired and combined to a three dimensional image with red fluorescence indicating immunoreactivity against the basolateral marker E-Cadherin and green fluorescence showing the localisation of the tight junction protein Claudin-1. Nuclei were counterstained with DAPI. Polarisation is evident from the localised expression of E-Cadherin and Caudin-1.

In general, ThinCert™ cell culture inserts with translucent membranes are recommended for transport assays. Additional hints on the appropriate membrane quality and the experimental design are given elsewhere (Letschert et al., 2005 and continuative literature resources below).

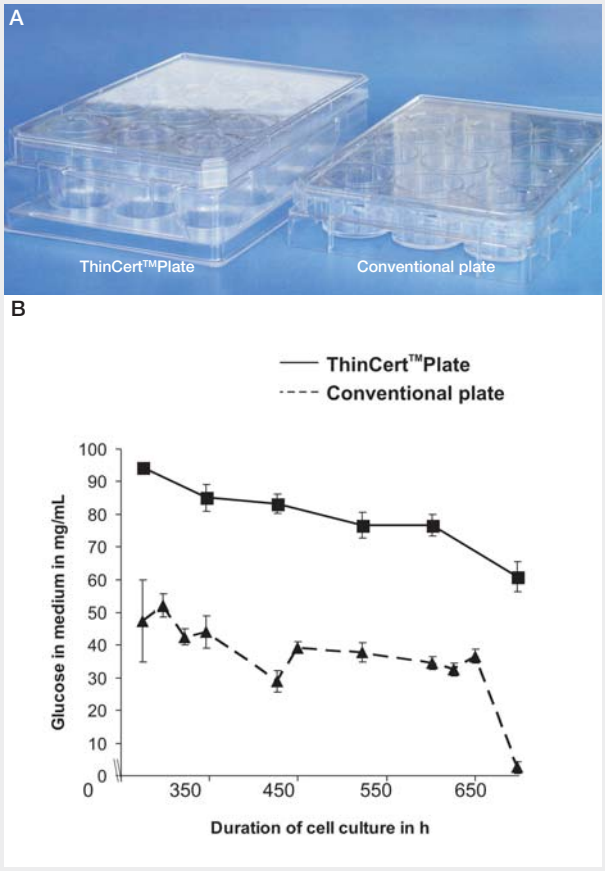

Figure 4: Tissue cultivation in the ThinCert™Plate
A, B: The ThinCert™Plate comprises extra deep wells and allows larger medium volumes to be applied during tissue culture at the air-liquid-interface. B: Cultivation of a gingival epithelium in ThinCert™ cell culture inserts in the ThinCert™Plate and a conventional multiwell plate. With the ThinCert™Plate a higher glucose content could be maintained in the medium.

2.4 Organotypic culture and air-lift-culture

Besides the above mentioned applications, ThinCert™ cell culture inserts are widely used to reconstruct and/or maintain tissue *in vitro*. In organotypic culture a tissue, previously isolated from an anesthetised animal, can be kept alive for prolonged periods (up to several months). In contrast the term 'tissue reconstruction' refers to the *de novo* generation of a tissue from single cells utilising cell culture techniques. Both procedures use cell culture inserts and entail tissue growth at the air-liquid-interface. The latter allows the cultivated tissue to reach or maintain the required high cell density without limitations from gas exchange. Furthermore, for some tissue types, the direct exposure of the cultivated cells to the surrounding atmosphere serves as an indispensable differentiation stimulus. For example, keratinocytes only form a horny layer (*stratum corneum*) when exposed to the surrounding air.

Due to the hanging geometry of most cell culture inserts, only a little space remains between the insert membrane and the bottom of the multiwell plate. Therefore, only a small amount of medium is available to the cultures at the air-liquid-interface. This limited medium reservoir conflicts strongly with the increased nutrient consumption of three-dimensional tissues, which reach cell densities far greater than those of two-dimensional cell layers. In order to solve this disparity, Greiner Bio-One developed the ThinCert™Plate – a novel cell culture plate with extra deep wells (**Fig. 4A**). The ThinCert™Plate permits the application of large medium volumes to cultures at the air-liquid-interface. Therefore, the frequency of medium changes is severely reduced.

In the provided application example (Fig. 4 und 5), gingival epithelia were generated using 12 well ThinCert™ cell culture inserts with 0.4 µm pores in combination with either conventional 12 well plates or the novel ThinCert™Plate. The enlarged medium reservoir of the ThinCert™Plate reduced the medium changes from every day, as required by the conventional plate, to every 4th day. In addition to reducing the number of medium changes, a significantly higher glucose concentration was maintained in the ThinCert™Plate compared to the standard conditions. (Fig. 4B). Moreover, tissue generated in the ThinCert™Plate appeared thicker and had more cell layers (Fig. 5).

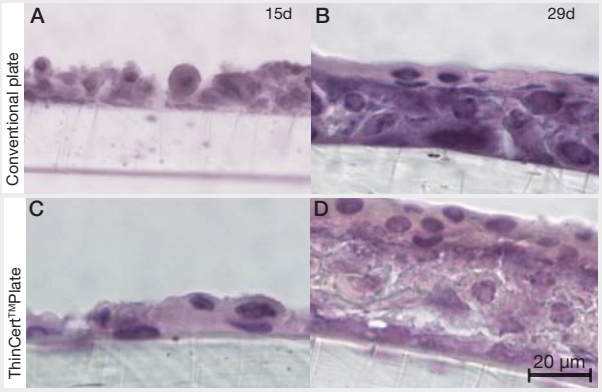

Figure 5: Tissue cultivation in the ThinCert™Plate
Cultivation in the ThinCert™Plate yields an improved tissue quality with more cell layers (15d/29d: 15/29 days in culture).

3. Continuative literature

Greiner Bio-One offers several application protocols for the practical integration of ThinCert™ cell culture inserts. Each application protocol addresses a specific question and provides detailed laboratory instructions. Application protocols and a list of primary literature implicating ThinCert™ cell culture inserts may be found on the homepage of Greiner Bio-One under www.gbo.com/bioscience/thincert.

Catalogue number	657640 665640 662640	657641 665641 662641	657610 665610 662610	657630 665630 662630	657631 665631 662631	657638 665638 662638
Pore size [µm]	0.4	0.4	1.0	3.0	3.0	8.0
Pore density [cm-2]	1×10^8	2×10^6	2×10^6	0.6×10^6	2×10^6	0.15×10^6
Membrane opacity	translucent	transparent	transparent	transparent	translucent	translucent
Bright field microscopy	-	+	+	+	-	-
Co-Culture	+	+	+	-	-	-
Culture at the air-liquid-interface	+	+	+	(+)	(+)	-
Electron microscopy	+	+	+	+	+	+
Fluorescence microscopy	+	+	+	+	+	+
Immunocytochemistry	+	+	+	+	+	+
Migration/Invasion	-	-	-	+	+	+
Organotypic culture	+	+	+	(+)	(+)	-
TEER	+	+	+	+	+	+
Transport studies	+	-	-	-	-	-

Table 1: Suitability of ThinCert™ membranes for different applications.
+ well suited,
- not suited

References

Albini A., Iwamoto Y., Kleinman H.K., Martin G.R., Aaronson S.A., Kozlowski J.M., McEwan R.N. (1987) A rapid in vitro assay for quantitating the invasive potential of tumor cells. Cancer Res. Jun 15;47(12):3239-45.

Boyden S. (1962) The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J Exp Med. Mar 1;115:453-66.

Chambard M, Verrier B, Gabrion J, Mauchamp J. (1983) Polarisation of thyroid cells in culture: evidence for the baso-lateral localisation of the iodide “pump” and of the thyroid-stimulating hormone receptor-adenyl cyclase complex. J Cell Biol. Apr;96(4):1172-7.

Guguen-Guillouzo C, Guillouzo A. (1986) Isolated and cultured hepatocytes. Paris, Les Éditions INSERM, John Libbey Eurotext: 1-12

Hiscox S, Morgan L, Green TP, Barrow D, Gee J, Nicholson RI. (2006) Elevated Src activity promotes cellular invasion and motility in tamoxifen resistant breast cancer cells. Breast Cancer Res Treat. 2006 Jun;97(3):263-74.

Kopplow K, Letschert K, Konig J, Walter B, Keppler D. (2005) Human hepatobiliary transport of organic anions analyzed by quadruple-transfected cells. Mol Pharmacol. Oct;68(4): 1031-8.

Letschert K, Komatsu M, Hummel-Eisenbeiss J, Keppler D. (2005) Vectorial transport of the peptide CCK-8 by double-transfected MDCK II cells stably expressing the organic anion transporter OATP1B3 (OATP8) and the export pump ABCC2. J Pharmacol Exp Ther. May;313(2):549-56.

Mettlen M, Platek A, Van Der Smissen P, Carpentier S, Amyere M, Lanzetti L, de Diesbach P, Tyteca D, Courtoy PJ. (2006) Src triggers circular ruffling and macropinocytosis at the apical surface of polarized MDCK cells. Traffic. May;7(5):589-603.

Saunders NA, Bernacki SH, Vollberg TM, Jetten AM. (1993) Regulation of transglutaminase type I expression in squamous differentiating rabbit tracheal epithelial cells and human epidermal keratinocytes: effects of retinoic acid and phorbolesters. Mol Endocrinol. Mar;7(3):387-98.

Revision: September 2007 - 073 017

Germany (Main office): Greiner Bio-One GmbH · (+49) 7022 948-0 · info@de.gbo.com, Belgium: Greiner Bio-One N.V. · (+32) 2 461 09 10 · info@be.gbo.com,
Brazil: Greiner Bio-One Brazil · (+55) 1934 68 9600 · office@br.gbo.com, China: Greiner Bio-One GmbH · (+86) 21 6272 7058 2051 · info@cn.gbo.com,
France: Greiner Bio-One SAS · (+33) 169 86 25 50 · infos@fr.gbo.com, Japan: Greiner Bio-One Co. Ltd. · (+81) 3 3505 8875 · info@jp.gbo.com,
Netherlands: Greiner Bio-One B.V. · (+31) 172 42 09 00 · info@nl.gbo.com, UK: Greiner Bio-One Ltd. · (+44) 1453 82 52 55 · info@uk.gbo.com,
USA: Greiner Bio-One North America Inc. · (+1) 704 261 7800 · info@us.gbo.com

greiner bio-one