Application properties of materials used for porous membranes in cell culture inserts

Abstract

Cell culture inserts with porous membrane growth surfaces have a multitude of uses and are available in several different materials. Each of these materials has advantages and disadvantages, and the choice of material is dependent on the application of the insert. The priority of three characteristics in particular—cell attachment potential, fluorescence-imaging quality, and cell-barrier assay suitability—can determine the material of the insert selected. Researchers must determine which of these characteristics is the most important for their application. Here we show direct comparisons in cell attachment, fluorescence imaging, and barrier assay capabilities of several different porous membrane materials, to aid researchers in decision-making.

Introduction

The applications of monolayer cell culture on standard cultureware are limited due to the nature of cell growth and attachment on an impermeable surface. This is especially evident when studying certain polarized cell types that are normally exposed to different environments on opposite surfaces *in vivo*. Cell culture inserts permit the area of a cell that is attached to the culture surface to be exposed to the medium. Different media conditions can be used for the upper and lower surfaces of the porous membrane where cells attach. A confluent monolayer of cells with tight junctions between the cells can be established on inserts with small pores, providing a barrier that blocks diffusion through the pores. Such conditions promote the polarization of certain cell types and provide a model for testing compound transportation across the monolayer of cells. Furthermore, membranes with larger pore sizes allow cells to migrate through the growth surfaces into the lower compartment of the dish. Such a system is useful in studying cell migration in response to chemoattractants and tumor invasion through an extracellular matrix. In addition, the mobile nature of the insert allows an established monolayer to be easily moved and exposed to different culture conditions, or even lifted to the surface of the medium at the air–liquid interface.

The use of cell culture inserts with porous membrane materials considerably expands the applications of monolayer cell culture, providing a more biologically relevant and versatile platform for cell biology research.

With so many different applications for cell culture inserts, there are several materials that are commonly used to make the porous membrane in a variety of pore sizes. Different applications require different pore sizes, so the first step is to determine the pore size needed. The type of experiment will also determine the optimal membrane type to use, as materials such as polycarbonate (PC), polyethylene terephthalate (PET), and polytetrafluoroethylene (PTFE) each have strengths and weaknesses and are made for use under specific conditions. For example, PC membranes, used in Thermo Scientific™ Nunc™ cell culture inserts, are normally treated to promote cell attachment and are made to have high pore density to allow more exchange of cell culture media through the membrane. Thus, PC inserts are best suited for transport studies and other applications where optimal cell growth is desired. PET membranes with lower pore density allow greater transparency for microscopy and imaging. Low–pore density PET, therefore, is the membrane of choice when microscopic examination and/or imaging is necessary. PTFE is also highly transparent for microscopy and has low fluorescence background for immunofluorescence studies. However, the low binding properties of the PTFE material require coating with extracellular matrix proteins prior to seeding to enable the cells to properly attach.

Here we test each type of insert membrane material for a variety of applications. We examine their strengths and weaknesses through experiments that allow direct comparison between the materials. These direct comparisons provide valuable information for researchers to evaluate their priorities in membrane material selection.

Results and discussion

PC inserts have the best cell attachment property among the materials tested

The most important characteristic of any culture substrate is its ability to promote cell adhesion and growth.

To determine the cell attachment properties of the insert membrane materials, HEK 293 cells were seeded at the same density onto cell culture inserts of several different materials. All inserts were incubated at 37°C for 24 hours. Since cell density was not quantifiable through microscopic visualization, the inserts were stained with fluorescein diacetate (FDA, Cat. No. F1303). A spectrophotometer was used to determine fluorescence intensity, which is proportional to the number of cells present on the inserts. PC inserts showed the highest average cell density among the materials tested, followed by PET and PTFE. The fluorescence intensity of the PC membranes was almost twice that of the PET and PTFE membranes, despite the fact that all inserts were seeded with the same cell density. Pre-coating PTFE membranes with collagen improved the cell density, although it was still significantly lower than that of the PC inserts (Figure 1).

It is important to note that the FDA staining required a wash step prior to reading. The cells were washed carefully to minimize disturbance, but the possibility remains that cells may have been dislodged from surface materials with less than ideal binding. This is unlikely to account for the large cell growth advantage that the PC surface had over PTFE, since the collagen-coated PTFE surface also showed substantially lower cell growth than PC. Control inserts without cells were analyzed with the spectrophotometer to determine the levels of membrane autofluorescence; autofluorescence was negligible for all membrane types.

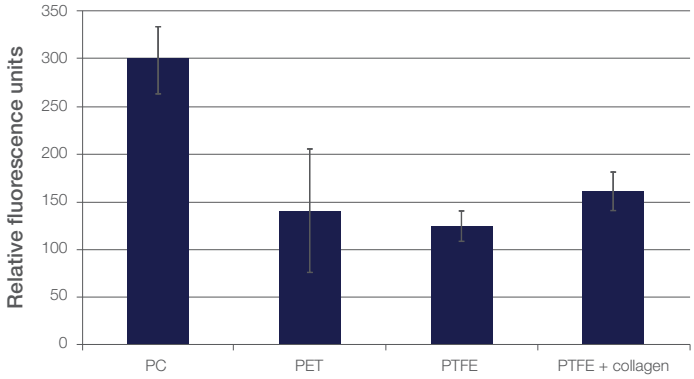

Figure 1. Relative fluorescence intensity of FDA-stained HEK 293 cells on PC, PET, PTFE, and collagen-coated PTFE inserts following 24-hour incubation. One-way ANOVA and post hoc least significant difference (LSD) tests indicate that cell density on PC inserts is significantly higher than on all other materials ($P < 0.05$), and that cell density is also significantly higher on PTFE with collagen than on PTFE alone.

PET inserts have the best fluorescence imaging quality among the materials tested

To determine the fluorescence-imaging quality of insert materials, cells grown on different types of inserts were stained and signals were acquired in multiple fluorescence channels. The expression of β-actin protein was detected using an anti–β-actin primary antibody (Cat. No. MA5-11866), followed by a secondary antibody conjugated to either Thermo Scientific™ DyLight™ 488 green (Cat. No. 21832) or a DyLight™ 550 red (Cat. No. 84542) fluorescent dye.

All cells were counterstained with the Invitrogen™ NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606) which, combined with the green and the red secondary antibodies, covered a wide spectrum of light emission. This allowed us to inspect the fluorescence-imaging quality of a variety of insert materials for cellular analysis. Cells were photographed in gray scale using fluorescence filters matching the emission characteristics of FITC, Cy®3 dye, and DAPI; data were then pseudocolored and merged to create the final images (Figure 2).

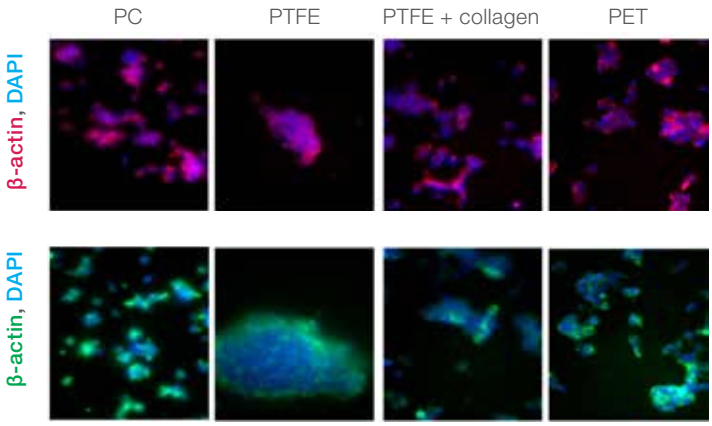

Figure 2. Microscopy images of HEK 293 cells immunostained for β-actin with either a DyLight 488 (green) or a DyLight 550 (red) fluorescent antibody conjugate. All cells were counterstained with DAPI (blue).

Images on the PC membranes appeared to have a slight haze, although cells were still visible and DAPI-stained nuclei vs. cellular staining were distinguishable. With images such as these, detection of gross morphological changes and some distinguishing cellular characteristics would be possible, while more sensitive measures such as colocalization of probes and detection of weak subcellular signals would likely not be accomplished. PET membranes, on the other hand, provided better-quality fluorescence images than the translucent PC membranes. Images on transparent PET membranes were nearly equivalent to what is normally seen on solid polystyrene culture surfaces. Interestingly, image quality on PTFE membranes is affected by two factors. On uncoated PTFE, the lack of cell attachment led to cell aggregation, which produced a three-dimensional cellular structure that prevented focusing in a single plane for imaging. The lack of cell attachment also led to most of the cells being washed away during the staining process, leaving very few cells to be imaged. Collagen coating significantly improved cell attachment on the PTFE membranes, resulting in adequate imaging and better focusing of imaged cells. However, image quality was sacrificed somewhat due to the coating material on the PTFE membrane. Overall, PET is the best insert membrane material for use in fluorescence-imaging applications.

PC, PET, and collagen-coated PTFE inserts are all suitable for barrier assays

Another common application for cell culture inserts is the barrier assay. A confluent cell monolayer is grown on top of the porous membrane. If the cells are capable of forming tight junctions to prevent passive diffusion, then any compound that crosses the insert membrane must be transported by the cells. This gives a means to measure the efficacy of transport of compounds by certain epithelial cells. To test the quality of the cell barrier on the porous membrane, electrical resistance across the membrane is measured. This trans-epithelial electrical resistance (TEER) is determined by the resistance against the flow of ions between the two compartments. Since ions flow easily through the porous membrane but not through cells, high TEER values indicate successful establishment of a cell barrier for compound transport studies. The formation of a cell barrier can take a significant amount of time, as the cells must grow to confluence and form tight junctions.

In this study, Caco-2 human colorectal epithelial adenocarcinoma cells were seeded (1.0 x 10^5 cells/cm^2) on different types of inserts with 0.4 µm pore size, maintained for 21 days, and fed with fresh medium every 2–3 days.

TEER values of the HEK 293 cells were low and were comparable to those across bare inserts. Caco-2 cells grown on collagen-coated PTFE membranes formed barrier layers with relatively low TEER values, PC membranes showed intermediate resistance, and cells grown on PET membranes created a barrier layer with the highest resistance among the material types (Figure 3). All three materials were able to form effective Caco-2 barriers that had substantially higher TEER values than those of the HEK 293 negative controls. During the 4 days of measurement, both PET and PTFE membranes showed a trend of decreasing resistance, while PC membranes maintained the resistance level. This may indicate that PC membranes are able to sustain a consistent Caco-2 barrier for longer periods of time, providing some advantages for an assay that normally must be conducted in a short window of time.

Conclusions

- PC insert membranes provide an excellent substrate for cell attachment and growth; they may be advantageous in barrier assays, as demonstrated by maintaining consistent TEER values over a longer period of time.

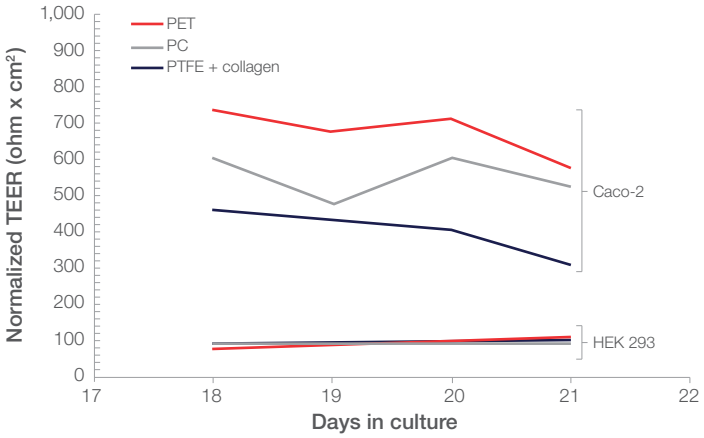

Figure 3. TEER values of Caco-2 cells grown on PC, PET, and collagen-coated PTFE inserts indicate successful formation of epithelial barriers. Non–barrier-forming HEK 293 cells were used as negative controls.

- PET membranes offset their suboptimal cell growth capabilities with better image quality.
- PTFE membranes generate good-quality images but must be coated with extracellular matrix proteins for adequate cell attachment.
- PC, PET, and collagen-coated PTFE inserts with a small pore size (0.4 µm) are all suitable for barrier assays with specific epithelial cell lines.

Ordering information

Product	Pore size (µm)	Pore density (pores/cm^2)	Culture area (cm^2)	No. of inserts/ carrier plate	No. of carrier plates/case	Cat. No.
Nunc PC cell culture insert and 24-well carrier plate systems	0.4	<0.85 x 10^8	0.47	24	4	141002
	3.0	<1.70 x 10^6				141004
	8.0	<0.85 x 10^5				141006
Nunc PC cell culture insert and 12-well carrier plate systems	0.4	<0.85 x 10^8	1.13	12	4	141078
	3.0	<1.70 x 10^6				141080
	8.0	<0.85 x 10^5				141082

Find out more at **thermofisher.com/cellcultureinserts**

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