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Note

A simple diffusion device to study transport across cells cultured on microporous membranes

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Summary

Microporous membranes are used extensively to culture epithelial and endothelial cells. A simple diffusion device to study the transport of a model lipophilic compound, testosterone, across Caco-2 cells cultured on Millicell™ is described. The device which requires minimal manipulation of cells, decreases the unstirred water layer.

Cultured cell systems have been used extensively to study drug transport and metabolism in specific biological barriers (Audus et al., 1990). For example, Caco-2 cells have been used as an in vitro model to study intestinal absorption (Hidalgo et al., 1989) and cultured keratinocytes have been used to study peptide metabolism in skin (Shah and Borchardt, 1988; 1989). These cells have been cultured on microporous polycarbonate (Transwell™, Costar Corp.) and cellulose esters (Millicell™, Millipore Corp.) membranes. One problem with transport studies on these membranes has been the presence of a large unstirred water layer (UWL). This UWL has been shown to distort the results from transport experiments (Dietschy and Westergaard, 1975; Thompson, 1979). Here we describe a simple diffusion device which incorporates stirring of the media in the apical and basolateral chambers to study transport across monolayers of polarized Caco-2 cells.

Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD) and seeded at a density of 63000 cells/cm^2 on Millicell-CM inserts (1.13 cm^2 surface area and 0.4 μm pore size) that were coated with a thin layer of rat tail collagen (type I) purchased from Collaborative Research (Bedford, MA). The culture media consisted of Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, 1% non-essential amino acids and antibiotics. All media components were obtained from Hazleton Research (Lenexa, KS).

The diffusion experiments were carried out on 20–25 day old Caco-2 cultures. Previously, Hidalgo et al. (1989) had shown that cells cultured for at least 15 days were impermeable to solutes such

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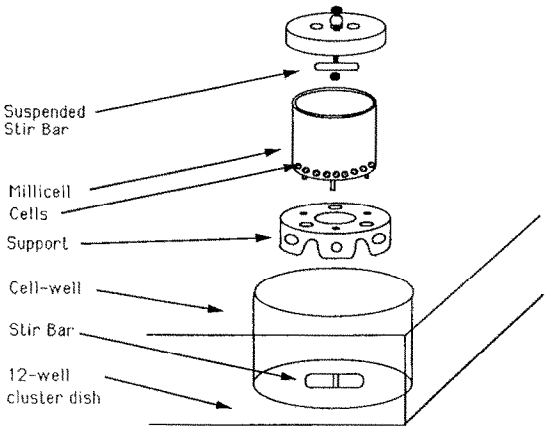

Fig. 1. Diffusion device designed to study transport across cells cultured on Millicells. The Millicell cultured with Caco-2 cells fits snugly on the support which is placed in a 12-well cluster dish. A magnetic stirrer mixes the buffer in the receiver compartment and inside the insert with the suspended stir bar.

as mannitol. Mannitol, a highly polar solute (log partition coefficient = −3.10), does not penetrate the cell membrane and is used as a 'leakage' marker. Testosterone, a highly non-polar solute (log partition coefficient = 3.13), is used to study transcellular diffusion across the monolayers.

Transport experiments were performed using the diffusion apparatus shown in Fig. 1. The 'legs' of the Millicell fit snugly into the holes of the support. The support, which has several additional holes and slits for easy mixing (Fig. 1), was designed to fit into the well of a readily available 12-well cluster dish or a 10 ml beaker. Mixing was achieved by one magnetic stirring bar in each chamber (i.e., apical and basolateral) set at a rate of 500 rpm. Hank's balanced salt solution (350 μl, Sigma, St. Louis, MO) containing [^{3}H]testosterone (spec. act. 55.2 Ci/mmol, NEN Research Products, Boston, MA) and D-[1-^{14}C]mannitol (spec. act. 50–60 mCi/mmol, American Radiolabeled Chemicals, St. Louis, MO) was added inside the insert (i.e., the apical domain of the monolayer). The receiver compartment (basolateral side) contained 3.5 ml Hank's buffer (pH 7.4). Aliquots (200 μl) were withdrawn at 5–15 min intervals from the basolateral side and replenished with fresh buffer. Since the purpose of the study was to determine the validity of the device, the experiments were conducted at ambient temperature.

Table 1 shows the transport of testosterone and mannitol across Millicells with or without the cultured cells. Equal amounts of testosterone (75.5%/cm^2 per h) and mannitol (77.8%/cm^2 per h) were transported across Millicells without cells, suggesting that the microporous membrane does not constitute a barrier to the diffusion of the polar compound mannitol or the non-polar compound testosterone. In the presence of stirring, the transport of mannitol and testosterone across cell monolayers was 0.32 and 48.6%/cm^2 per h, respectively. These data indicate that the diffusion of testosterone across Caco-2 cells was mainly transcellular and not paracellular since the transport of the leakage marker was minimal. In the absence of stirring, the transport of testosterone is only 3.0%/cm^2 per h. Stirring is believed to have a significant impact on the transport of testosterone because of a substantial decrease in UWL. The values of the UWL thickness, calculated according to Komiya et al. (1980), were 2740 and 109 μm in the absence and presence of stirring, respectively. These data agree with a related study by Hidalgo et al. (1990) who found that an increase in stirring rate increased the permeability of testosterone across Caco-2 cell monolayers mounted on a newly developed side-by-side diffusion cell where mixing is achieved by gas lift. The somewhat higher values of testosterone permeability found in this study

TABLE 1

Transport of [^{3}H]testosterone and [^{14}C]mannitol across Caco-2 cell monolayers cultured on Millicells for 20–25 days [a]

	Stirring [c]	Transport (%/cm^2 per h) [b]	
		Testosterone [d]	Mannitol [d]
No cells [e]	+	75.5 ± 2.25	77.8 ± 3.02
With cells	−	3.0 ± 1.95	0.37 ± 0.26
With cells	+	48.6 ± 3.09	0.32 ± 0.04

[a] Cells were cultured on the collagen-coated Millicell-CM and refed with the DMEM media as described.
[b] Percent of radioactivity from the donor (apical) side transported to the received (basolateral) side.
[c] Stirring speed was 500 rpm.
[d] Values are mean ± SD (n = 3–5).
[e] Millicells were coated with collagen.

suggest that this diffusion apparatus provides a more vigorous stirring of the transport buffer than that achieved by the diffusion device described by Hidalgo et al. (1990). Furthermore, the virtually identical diffusion of the leakage marker, mannitol, in both devices indicates that the higher permeability of testosterone obtained with this diffusion cell is a true reflection of the magnitude of the effect of stirring on the UWL thickness, and not the result of an increase in leakage due to partial deterioration of monolayer integrity in the present diffusion device. Two consecutive experiments on the same cell monolayers gave similar transport results (data not shown), indicating that monolayer integrity did not decrease throughout the duration of the experiments. Light microscopy did not show any damage to the monolayer from stirring.

There are two main reasons for the lack of significant difference in the transport of mannitol across Caco-2 cells in the presence or absence of stirring. Firstly, the transport of highly polar molecules like mannitol occurs only through the transjunctional route (aqueous pores) of the cell monolayers, which represents a negligible area relative to the total monolayer surface area. Secondly, the transport of mannitol is not susceptible to the presence of an UWL since this compound is very hydrophilic and thus its permeability is determined by the sealing capacity of the occluding junctions.

In conclusion, the device described involves minimal potentially damaging manipulation of the cell monolayers and decreases substantially the UWL adjacent to cell monolayers grown on permeable supports. The device is easy to use and maintains the monolayer integrity.

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