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Research paper

A novel diffusion cell model for the *in vitro* assessment of transcutaneous breast cancer therapeutics: Effect of permeants on MCF-7 cells cultured within the receptor compartment

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ABSTRACT

A novel model is described for investigating the potential efficacy of topically delivered anti-breast cancer agents. Using all-glass Franz diffusion cells, the permeation of 4-hydroxytamoxifen, two EGFR inhibitors (PD98059 and LY294002) and eicosapentaenoic acid (EPA) were determined from a fish oil vehicle across Cyclopore track etched membrane (CTEM) alone, full-thickness porcine ear skin alone and CTEM plus full-thickness porcine ear skin. Finally, the effect of the simultaneous permeation of these compounds was determined on the breast cancer cell line, MCF-7, cultured directly into the diffusion cell receptor compartments. The CTEM was found to be not rate limiting, and all compounds permeated the skin, with a large excess of EPA. The applied combined dose reduced the growth of MCF-7 cells by 66% after 7 days. The following conclusions were obtained: (1) MCF-7 breast cancer cells can be successfully cultured within glass Franz diffusion cells. (2) A composite diffusion cell/cell culture model can indicate the potential efficacy of topically delivered anti-breast cancer therapeutic agents. (3) The levels of LY294002, PD98059, 4-hydroxytamoxifen and EPA delivered across full-thickness skin have a major inhibitory effect on the growth of MCF-7 breast cancer cells.

1. Introduction

It has been estimated that the number of deaths in 2008 from breast cancer in USA to be 40,480 (female) and 450 (male); and the number of new cases to be 182,460 (female) and 1990 (male) [1]. Although there have been numerous important advances recently, and breast cancer survival rates have been improving over the last 20 years, there remains an urgent need for new strategies to combat the disease. Nearly all currently available breast cancer drugs are orally or intravenously administered and are thus subject to a range of pharmacokinetic issues even before the site of action is reached, including extent of gastro-intestinal absorption (oral), first pass hepatic metabolism, wide distribution, etc. The rationale behind targeting downstream survival pathways as a means of reducing resistance to tamoxifen has been established [2], and the plausibility of the transcutaneous delivery of a combination of anti-breast cancer agents: PD98059, LY294002, 4-hydroxytamoxifen and eicosapentaenoic acid (Fig. 1) was recently demonstrated [3]. Franz diffusion cells (FDC) were used to determine permeation across full-thickness skin, and cell culture work found that the levels of agent permeating skin were sufficient to significantly inhibit the growth of the MCF-7 breast cancer cell line. Thus, the development of a topically applied patch is proposed, which could be of value particularly in prophylaxis of breast cancer.

The current work examined the plausibility of a composite Franz diffusion cell-based model that incorporates elements of both transcutaneous delivery and cell culture. The first part of the model involves a full-thickness skin membrane, mounted between the flanges of the diffusion cell. The second part consists of MCF-7 cells cultured at the bottom of a glass Franz diffusion cell (FDC) receptor compartment, as shown in Fig. 2. Drug solutions applied to the skin in the donor phase permeate the skin, enter the receptor phase and diffuse to the cells, where their activity against cell growth may be determined. We used fish oil as vehicle as it possesses anti-inflammatory properties by virtue, in particular, of its high proportion of the omega-3 fatty acid eicosapentaenoic acid (EPA). The final consideration of model development surrounds the necessity to exclude microbial contamination of the receptor phase/cell growth medium. To this end, we employed 0.22-μm Cyclopore track etched polycarbonate membranes that have defined pore sizes, high flow rates, and excellent chemical and thermal resistance [4]; they have previously been used for the isolation and determination of bacterial content [5]. Placed between the lower surface of the skin and receptor phase, it had previously been

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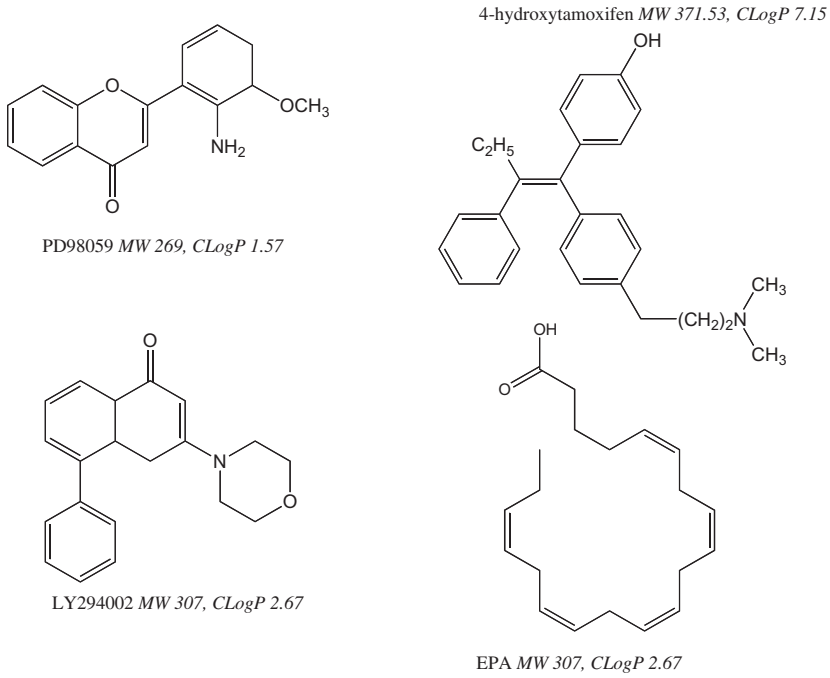

Fig. 1. Chemical structures and physicochemical information for LY294002, PD98059 and 4-hydroxytamoxifen and EPA.

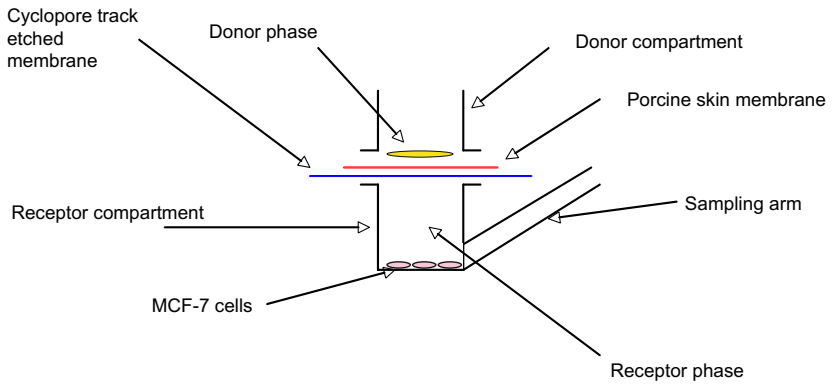

Fig. 2. Illustration of experimental set-up of the composite transcutaneous breast cancer model: MCF-7 breast cancer cells cultured inside glass Franz-type diffusion cell receptor compartments. Drug delivery is determined from doses applied to full-thickness skin separated from the receptor phase by a Cyclopore track etched membrane. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

found that microorganisms could largely be excluded from receptor phases [6]. However, fungal and bacterial contamination of the receptor phase over the full 48-h experiment was only eradicated by the combination of the CTEM and inclusion of penicillin/streptomycin and 2.5 μg mL^{-1} fungizone within the receptor phase.

A successful composite system based on the above would provide a more realistic model of a topical drug delivery system than two disparate experiments. In this work, the individual elements of the proposed model were validated and used to determine the effects of the aforementioned drug combination on the cells cultured directly within the FDC receptor compartment.

2. Materials and methods

2.1. Materials

LY294002 and PD98059 were purchased from Promega, Southampton, UK. Fish oil capsules (Boots super-strength, ~40% EPA) were purchased from a local Boots store. In addition, Cyclopore track etched membrane (CTEM; 47 mm diameter, 0.22 m), ethanol and Dow Corning high vacuum grease were purchased from Fisher Scientific, Loughborough, UK. Hanks balanced buffered salt solution (HBBSS), gentamycin sulphate, bovine serum albumen (BSA), DMSO, foetal calf serum (FCS) and 4-hydroxytamoxifen were purchased from Sigma–Aldrich Ltd., Poole, UK. Fungizone and streptomycin were purchased from Bio-Rad Laboratories Ltd. (Hemel Hempstead, UK). Cell culture medium RPMI (1640) was obtained from Invitrogen (Paisley, UK). Porcine ears were supplied by a local abattoir and kept on ice during transportation to the laboratory. The ears were then washed under running water, patted dry and stored at 2–4 °C overnight, before the full-thickness dorsal membranes were liberated by blunt dissection and cut into 2-cm^2 sections. Fungizone and streptomycin were purchased from Bio-Rad Laboratories Ltd. (Hemel Hempstead, UK).

2.2. Culture of MCF-7 cells directly within FDC receptor compartments

Glass FDCs (nominal diffusion area 0.8 cm^2 and receptor phase volume 3 mL) were thoroughly cleaned and autoclaved (121 °C), then stored in a sterile environment until required. MCF-7 cells were

grown in plastic flasks in RPMI plus 5% FCS until 70% confluent. At this point, cells were passaged, using the protocol outlined previously [3] and 1 million cells, predetermined by counting total cell using the Coulter Multisizer II counter, were seeded per FDC in 1 mL of RPMI. The medium was supplemented with 5% FCS, 10 U mL^{-1} and 10 μg mL^{-1} penicillin/streptomycin and 2.5 μg mL^{-1} fungizone. The diffusional area of the receptor compartment was sealed with a sterile cover slip, while the sampling arm was sealed with sterile tin foil. This time point was day 0; on day 4, the medium was removed and replenished with fresh solution, on this instance the whole receptor compartment was filled. On day 7, cells were counted using the Coulter Multisizer II counter and corrected for the area of the receptor compartment.

2.3. Permeation of PD98059, LY294002, 4-hydroxytamoxifen and EPA across a 0.22-μm CTEM

A 0.22-μm CTEM was employed to inhibit microbial contamination of the receptor phase containing the cultured breast cancer cells. It was envisaged that the 0.22-μm pores would be sufficient to prevent microorganisms from entering the receptor phase from the skin, based upon simple size exclusion, but not provide a rate-limiting barrier to the permeants. This section was aimed at validating this latter point. The CTEM was immersed in receptor phase overnight to ensure saturation, then gently patted dry with tissue paper. The FDC was then assembled with a CTEM mounted between the pre-greased flanges, and the two halves held together using a stainless steel clamp. Receptor phase (de-gassed cetrimide 30 mg mL^{-1}) was added. In a significant departure from conventional skin permeation experiments, no stirring of the receptor phase was employed, as this would perturb the cultured cells at the bottom of the receptor compartment. After 15 min, the donor phase was added: 200 μL of 1 mg mL^{-1} 4-hydroxytamoxifen, PD98059 and LY294002 in fish oil. The donor and receptor compartments were occluded with laboratory film and ground glass stoppers, respectively. At 1, 2 and 3 h, the receptor phase was emptied and sampled, after being subjected to 5× in–out pipetting actions to make the solution homogeneous, before being replaced with fresh solution. At 24 h, the final sample was taken and the FDC dismantled. Replication was $n = 5$.

2.4. Permeation of PD98059, LY294002, 4-hydroxytamoxifen and EPA across both excised porcine skin and 0.22-μm CTEM

The CTEM was soaked in receptor phase overnight, as above, prior to assembly of the FDC. First, a CTEM placed on the pre-greased receptor phase flange, then a porcine ear skin membrane was carefully placed on top, stratum corneum uppermost, and the donor compartment placed in position. A stainless steel clamp was used to hold the device together. Other experimental details were as above, except the sampling times were extended to 3, 6, 12, 24 and 48 h in line with increased barrier due to the presence of the skin barrier.

2.5. Effect of PD98059, LY294002, 4-hydroxytamoxifen and EPA on MCF-7 cells growth cultured within FDCs, having permeated the CTEM

MCF-7 cells were prepared within the FDC receptor compartment as described earlier. On day 1, the medium was removed from the receptor compartment and a 0.22-μm CTEM placed on the pre-greased receptor phase flange, prior to fitting the donor chamber. The receptor chamber was then filled. After 15 min, a donor phase of 1 mg mL^{-1} 4-hydroxytamoxifen, PD98059 and LY294002 in fish oil or fish oil alone (control) was applied to the surface of the CTEM. On day 7, the FDC was dismantled and the MCF-7 cells trypsinized and suspended before being counted in a Beckman Coulter counter.

2.6. Effect of PD98059, LY294002, 4-hydroxytamoxifen and EPA on MCF-7 cells cultured within FDCs, having permeated the CTEM and full-thickness porcine ear skin

The experimental protocol was as described in this section. However, in this case a porcine skin membrane was placed, stratum corneum facing upwards, between the CTEM and donor compartment. It was ensured that the area of the CTEM was greater than that of the porcine skin membrane to restrict passage of microorganisms under the CTEM.

2.7. HPLC analysis

Samples were analysed by reverse phase liquid chromatography using an Agilent 1100 series automated system with Chemstation software. PD98059 and LY294004 were separated using Luna C18 5 m ODS 150 × 4.6 mm column (Phenomenex, Macclesfield, UK), with gradient elution of 80:20 to 70:30 methanol/water over 20 min at a flow rate of 0.5 mL min^{-1}, 20 μL injection volume, detection was at 254 nm. Under these conditions, baseline resolution of PD98059 and LY294004 was obtained, with retention times of 12.5 and 14.5 min, respectively. For the analysis of 4-hydroxytamoxifen, a mobile phase of 80:20 methanol/water with 0.02% v/v TFA was used and detection at 240 nm. Run time was 10 min, and detection was at 240 nm. Under these conditions, 4-hydroxytamoxifen had a retention time of 4.7 min. For the analysis of EPA, isocratic elution of 20:80 methanol/water with 2.5% acetic acid was employed, and detection was at 210 nm. Under these conditions, EPA had a retention time of 6.5 min. For each analyte, the amounts permeated were determined against the appropriate standard calibration curves prepared in receptor phase, all of which demonstrated good linearity, with $R^2 = 1.000$.

2.8. Data processing

Cumulative permeation (μg cm^{-2} and μmol cm^{-2}) was determined and, where appropriate plotted against time to provide a steady state flux (J_{ss}) or maximum flux (J_{max}) from the linear portion. Statistical analysis was performed by a two-way non-parametric Kruskal Wallis ANOVA test followed by a Dunnet's post hoc test (Instat 3 for Macintosh GraphPad Software, Inc. Hercules, California, US). A p-value of <0.05 was considered significant, and a p-value of <0.0001 was considered extremely significant.

3. Results

3.1. Culture of MCF-7 cells directly within Franz diffusion cell (FDC) receptor compartments

Fig. 3 shows cell counts after 7 days of culture within the glass FDC receptor compartments. Five diffusion cells were investigated for each run, each of which demonstrated a high degree of consistency in MCF-7 cell counts ($p > 0.05$). Reproducible cell counts meant that any effects on cell growth occurring in treated diffusion cells could confidently be attributed to the effects of the compounds. The presence of cetrimide appeared to have no deleterious effect.

3.2. Permeation of LY294002, PD98059, 4-hydroxytamoxifen and EPA across a 0.22-μm CTEM

Cumulative permeation data are provided in Table 1. Fig. 4 shows that the delivery of PD98059, LY294002 and 4-hydroxytamoxifen was rapid, with equilibrium generally being approached by 10 h. The amounts of PD98059 and 4-hydroxytamoxifen delivered

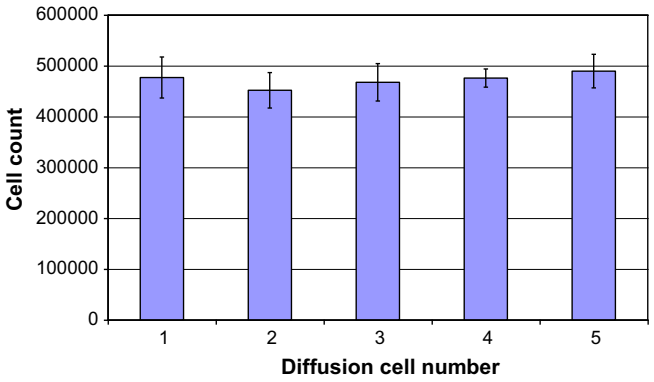

Fig. 3. Histogram showing the counts of MCF-7 breast cancer cells following incubation for 1 week in the receptor compartments of glass Franz-type diffusion cells ($n = 5 \pm SD$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were very similar, approximately 15 μg cm^{-2} after 24 h ($p > 0.05$), indicating the delivery through the membrane was in proportion to their relative concentrations in the applied dose (i.e. 1 mg mL^{-1} of each). The amount of EPA delivered was ~3 orders of magnitude greater than the other three permeants, reflecting its large excess in the donor phase; however, the delivery of EPA appeared to approach equilibrium within 24 h. Rapid drug delivery was expected due to microconduits within the CTEM barrier. Less important than the numerical data obtained in this section is the provision of benchmark data against which to establish the CTEM provided no rate-limiting steps when skin was included in the model.

3.3. Permeation of LY294002, PD98059, 4-hydroxytamoxifen and EPA across composite porcine ear skin and 0.22-μm CTEM barriers

Cumulative permeation data are provided in Table 1. Fig. 5 is a histogram comparing the amounts (in terms of mass) of the four analytes permeated across the CTEM alongside skin and CTEM after 24 h. The main purpose of this plot is to illustrate the fact the presence of the CTEM was not rate limiting, as permeation through both skin and CTEM was much lower than CTEM alone in each case with very little lag phase.

Fig. 6 shows permeation profiles for masses of LY294002, PD98059, 4-hydroxytamoxifen (left) and EPA (right), across composite excised porcine skin and CTEM. Lag phases for each perme-

Table 1
Cumulative permeation of LY294002, PD980059, 4-hydroxytamoxifen and EPA across Cyclopore track etched membrane (CTEM) and CTEM plus full-thickness porcine ear skin.

Barrier, time/permeant		LY294002		PD980059		4-Hydroxytamoxifen		EPA	
		μg cm^{-2}	$\times 10^{-3}$ μmol cm^{-2}	μg cm^{-2}	$\times 10^{-3}$ μmol cm^{-2}	μg cm^{-2}	$\times 10^{-3}$ μmol cm^{-2}	μg cm^{-2}	μmol cm^{-2}
CTEM	1	0.087 ± 0.02	0.283 ± 0.066	0.094 ± 0.05	0.535 ± 0.186	0.144 ± 0.05	0.243 ± 0.129	345 ± 35.0	1.124 ± 1.114
	2	1.400 ± 0.67	4.56 ± 2.18	2.63 ± 0.41	11.49 ± 1.49	3.09 ± 0.40	6.805 ± 1.04	889 ± 217	2.896 ± 0.707
	3	7.76 ± 1.68	25.3 ± 5.47	11.6 ± 1.72	37.55 ± 11.90	10.10 ± 3.10	30.01 ± 4.398	2780 ± 601	9.055 ± 1.954
	24	14.90 ± 2.58	48.53 ± 8.40	15.9 ± 2.61	61.71 ± 10.04	16.60 ± 2.70	41.14 ± 6.73	5440 ± 978	17.72 ± 3.19
CTEM and skin	3	0.027 ± 0.006	0.089 ± 0.02	0.014 ± 0.002	0.051 ± 0.008	0.017 ± 0.008	0.044 ± 0.002	27.2 ± 2.40	0.089 ± 0.008
	6	0.982 ± 0.298	3.20 ± 0.97	0.436 ± 0.094	1.622 ± 0.35	0.259 ± 0.061	0.671 ± 0.158	158.3 ± 34.6	0.516 ± 0.113
	12	4.20 ± 0.545	13.68 ± 1.78	1.173 ± 0.109	4.36 ± 0.41	1.555 ± 0.175	4.027 ± 0.455	327.7 ± 69.1	1.066 ± 0.225
	24	6.409 ± 0.910	20.88 ± 2.93	3.655 ± 1.035	13.59 ± 3.85	4.132 ± 0.205	10.70 ± 0.53	692.6 ± 94	2.256 ± 0.31
	48	7.827 ± 0.518	25.50 ± 1.69	5.046 ± 0.763	18.76 ± 2.84	6.240 ± 0.278	16.17 ± 0.72	1115.5 ± 143	3.634 ± 0.467
	Mean Flux (μmol cm^{-2} h^{-1})		1.544×10^{-3}[a]		5.88×10^{-4}[a]		5.57×10^{-4}[a]		0.101[b]

[a] J_{max}.
[b] J_{ss}.

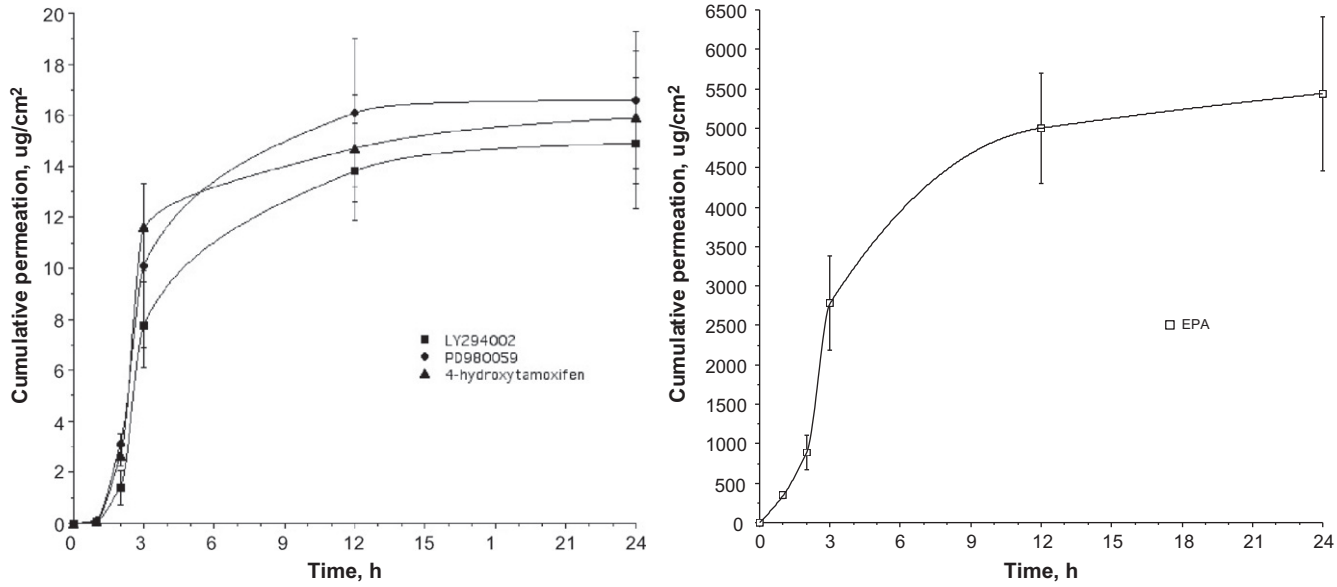

Fig. 4. Permeation profiles for PD98059, LY294002, 4-hydroxytamoxifen (left) and EPA (right) through a 0.22-μm Cyclopore track etched membrane ($n = 5 \pm SD$).

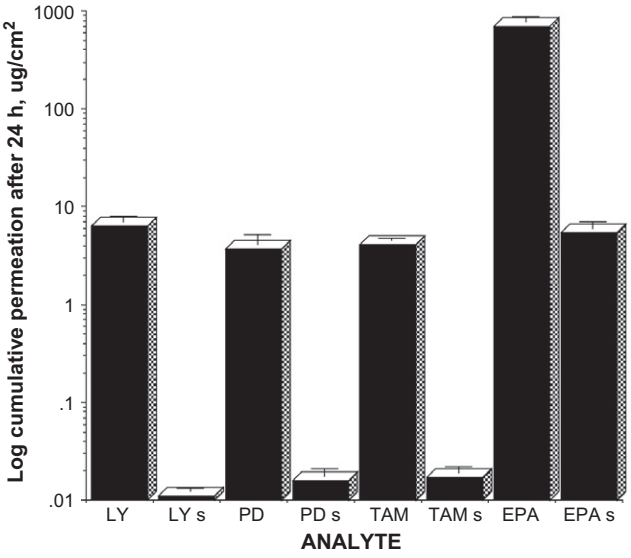

Fig. 5. Histogram of log cumulative permeation of the four analytes after 24 h across Cyclopore track etched membrane (CTEM) *versus* CTEM plus full-thickness porcine ear skin (denoted by s), demonstrating that the presence of the CTEM is not rate limiting in the composite model.

ant were similar at approximately 3 h. Short-duration steady states were apparent for LY294002, PD98059 and 4-hydroxytamoxifen, estimated at 1.544×10^{-3}, 5.88×10^{-4} and 5.57×10^{-4} μmol cm^{-2} h^{-1}, respectively, before rates declined. As cetrimide provides good sink conditions for these permeants, this was probably due to depletion, e.g. following binding to skin tissues. Interestingly, the delivery of LY294002 was significantly ($p < 0.05$) greater than that of both PD98059 and 4-hydroxytamoxifen, which were similar ($p > 0.05$). A similar result was noted in an earlier paper [3], where cumulative permeation after 48 h was $58.0 \pm 9 \times 10^{-3}$ μmol cm^{-2} for LY294002 compared to $34.0 \pm 8 \times 10^{-3}$ μmol cm^{-2} for PD98059; in the current work, the corresponding figures were $25.5 \pm 1.69 \times 10^{-3}$ and $18.76 \pm 2.84 \times 10^{-3}$ μmol cm^{-2}. The numerical differences between the current and previous work may be attributable to the inclusion of penetration enhancers in the latter.

In terms of EPA, delivery was again much greater than the other analytes, with steady state flux maintained for longer duration at a rate of 0.101 μmol cm^{-2} h^{-1}, prior to depletion.

Fig. 7 compares the percentage of applied dose permeated of each active formulation after 24 h for both CTEM and CTEM + skin. Across CTEM + skin, the levels were below 5%, whereas for CTEM, between 20% and 35% of LY294002, PD98059 and 4-hydroxytamoxifen were delivered. The plot thus serves to reaffirm that permeation through the CTEM was significantly greater than through the porcine skin membranes for each active constituent of the formulation. The percentage of EPA was smallest, as it had the highest initial concentration.

3.4. Effect of combined dose of LY294002, PD98059 and 4-hydroxytamoxifen on the growth of MCF-7 cells cultured within FDCs, having permeated the CTEM

This experiment was intended to establish whether the permeant compounds could penetrate the CTEM and exert their growth inhibitory effects on the MCF-7 cells seeded below. Fig. 8 shows cell counts of MCF-7 cells after 7 days, treated with either fish oil

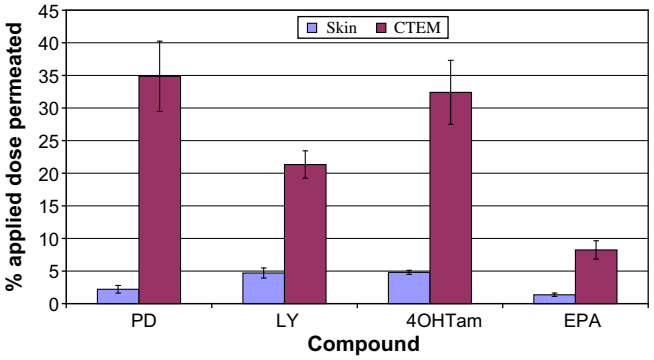

Fig. 7. Histogram comparing the percentage of the applied dose permeating across both skin and the Cyclopore track etched membrane after 24 h ($n = 5 \pm$ SD). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

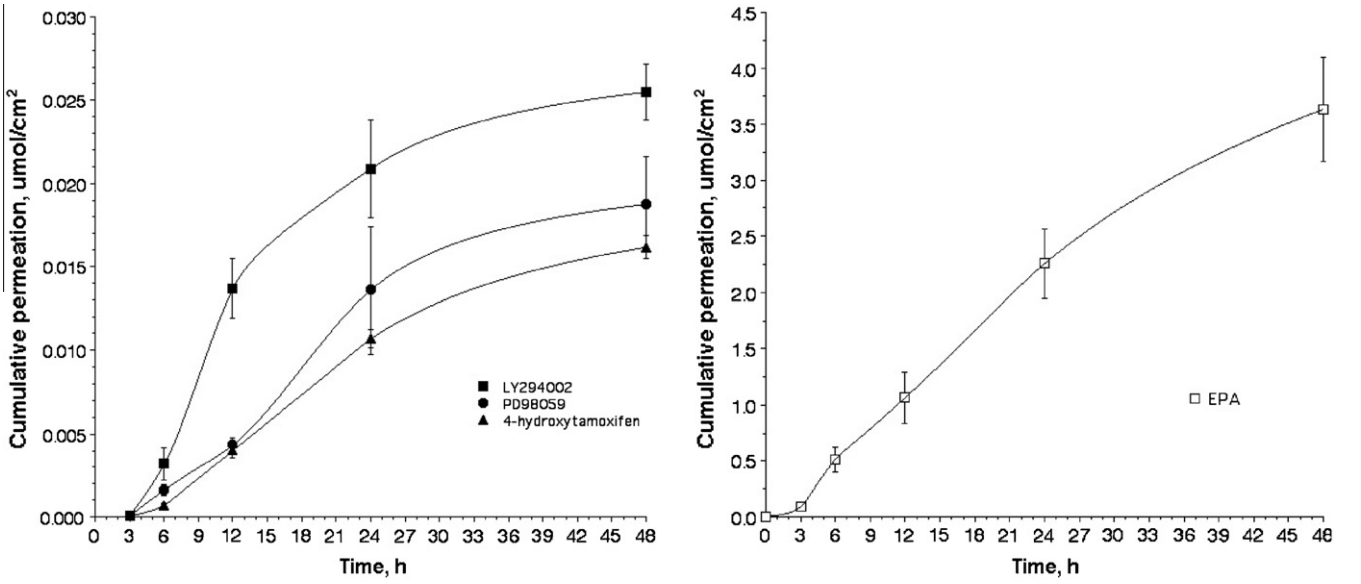

Fig. 6. Permeation profiles for LY294002, PD98059, 4-hydroxytamoxifen (left) and EPA (right) across both full-thickness excised porcine skin and a 0.22-μm Cyclopore track etched membrane ($n = 5 \pm$ SD).

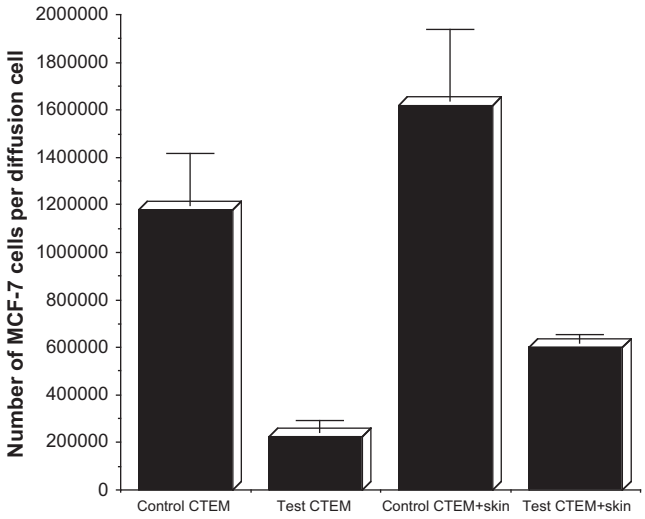

Fig. 8. Histogram showing the effect of the formulation after 7 days on MCF-7 cells seeded in glass diffusion cells after permeating the Cyclopore track etched membrane alone or both porcine skin and Cyclopore track etched membrane. The control formulation consisted of fish oil ($n = 6$ for control and 12 for test ± SD).

alone (control) or with the final formulation, demonstrating that the three compounds permeated the CTEM and reduced cell growth to 20.8 ± 5.1% of control cell growth. This inhibition in cell growth was significant compared to control cell growth (p 0.01); however, it was significantly less (p 0.022) than the inhibition in growth of MCF-7 cells when treated with IC_{50} concentrations of compounds which reduced cell growth to 2.6 ± 0.5% of control.

3.5. Effect of combined dose of LY294002, PD98059 and 4-hydroxytamoxifen on the growth of MCF-7 cells cultured within FDCs, having permeated the CTEM and full-thickness skin

The definitive experiment in this paper involved combining all the preliminary elements discussed above into the composite model (Fig. 2). Fig. 8 shows that the test formulation was capable of permeating the skin, passively diffusing through the receptor phase and inhibiting MCF-7 cell growth by 66.68 ± 13.41% compared to control growth (p 0.044). This was a significantly smaller inhibition in cell growth than that seen with IC_{50} concentrations of the three compounds (p 0.023) and that seen in the absence of the skin membrane (p 0.036).

4. Discussion

This investigation into the development of a composite model (Fig. 2), incorporating elements of both transcutaneous drug permeation and cell culture, necessitated the surmounting of a number of significant challenges. It has been widely held that glass is not an appropriate substrate for the growth of cultured cells, where treated plastic, e.g. Dow Corning tissue culture treated polystyrene, are conventionally used. In spite of this, MCF-7 cells were found to successfully adhere to glass interiors of diffusion cells and undergo normal proliferation, as demonstrated in Fig. 3.

Previous investigations had shown that cultured cells do not grow well on glass cells and require a coating for adequate seeding and growth. The ability of cells to adhere to glass is also cell specific. Bovine aortic endothelial cells show good attachment to glass, enabling normal growth of this cell line; however, human umbilical vein epithelial cells attached and grew poorly when the same methodology was followed. These two cell lines both attached and proliferated on plastic surfaces equally well [7]. It has been suggested that the mechanism of cell attachment to glass is not a result of interactions between substrate and charged regions of the cell surface, but as a result of interactions with non-polar regions of the cell surface; however, in the presence of serum, electrostatic forces may be responsible for cellular attachment [8]. Another study suggested that red blood cell attachment to glass was a result of long-range electrostatic repulsive and electrodynamic attractive forces [9]. However, we believe the most likely explanation for the unexpected cell attachment in the current work lies in the standard cleaning process used in this laboratory which involves treatment with Decon 90, which may have rendered the inner surfaces of the FDC in an etched and uneven state. It has previously been shown that grit blasted glass leads to better cell attachment than smooth glass surfaces [10].

Although providing a diffusional barrier (Fig. 4), the presence of the CTEM was not rate limiting to the diffusion of permeant drug into the receptor phase (Fig. 5). The flux of the permeants through the 0.22-μm CTEM was significantly greater than across the skin and short lag phases were seen. Conventionally, receptor phases are stirred continuously using micro magnetic stirrers to ensure homogeneity; however, this was not possible in the current work due to the presence of the cultured MCF-7 cells. In terms of sampling for drug analysis, the repeated in–out pipetting action ensured homogeneous sampling – an approach validated by applying a small aliquot of methylene blue solution to the top of a filled receptor compartment (data not shown). In the absence of such stirring, a stagnant diffusion layer would have existed at the membrane/receptor solution interface, and it would be necessary for the permeants to diffuse through this medium prior to interacting with the MCF-7 cells. To a certain extent, this mimics permeation through the tissues in an *in vivo* situation, although post-skin targets would need to be reached via passive diffusion through fatty tissue, which would vary between individuals. Furthermore, it remains to be determined whether the drugs, in the amounts delivered, could effectively diffuse through such tissue.

In the current work, a single dose was applied, and the effects on MCF-7 cells were determined after 7 days; permeation data were obtained over a 48-h period, at which point there was evidence of depletion. It is acknowledged that dermal clearance will remove some of the permeating drug, although a recent paper involving the transcutaneous delivery of ketoprofen in the Mexican hairless pig proves that the majority of the dose is *not* cleared from the skin into the systemic circulation [11]. However, dermal clearance was mimicked to a limited extent in the current work by replenishment of media on day 4 and by daily agitation of the receptor compartment. It is possible that no further growth inhibitory effects would have occurred after this time. In spite of this media removal and agitation, sufficient concentrations of compounds from the single dose application were capable of reaching the under-laying cells and inhibiting their growth significantly (p 0.044).

In summary, there are three major conclusions from this work. First, MCF-7 breast cancer cells can be successfully cultured directly within glass Franz diffusion cells, contrary to accepted wisdom. Second, a composite diffusion cell/cell culture model can be used to indicate the potential efficacy of topically delivered anti-breast cancer therapeutic agents – the model may be considered as an intermediate between *in vitro* and *in vivo* experiments. Third, by combining two separate and time-consuming experiments, such a model may be used as a high throughput screen in other transcutaneous drug delivery systems. Overall, the data support the development of a transcutaneous patch-based drug delivery system for these and potentially other compounds.

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