A PERTINENT SCREENING TOOL TO MEASURE PERMEABILITY COEFFICIENT: EPISKIN® RECONSTRUCTED HUMAN SKIN MODEL

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INTRODUCTION

Risk assessment from topical exposure to chemical in human requires reliable models and test procedures. For such study, *ex vivo* human skin is the tool recommended by regulators. However, its use is time consuming and requires numerous replicates due to the variability of the donors. According to their similarities to native human tissue in terms of morphology, lipid composition and biochemical markers, reconstructed human epidermis (RhE) have been identified as useful tools for the in vitro testing of phototoxicity, corrosivity and irritancy. These last years, some papers claim that RhE are appropriate alternatives to human skin for the assessment of skin permeation and penetration in vitro[1,2]. Such studies were conducted under infinite-dose conditions to measure permeability coefficient.
Among all RhE models commercially available, Episkin® from SkinEthic (www.skinethic.com) are particularly adapted for testing. Indeed, its design allows to measure penetration directly in the insert without mounting the tissue in a diffusion cell[3].

MATERIALS AND METHODS

The experiments were performed on Episkin® model cultured for 13 days, supplied by Episkin® SNC (Lyon, France). Two experimental setup were used to measure permeability coefficient (Kp) on reconstructed skin.

1 Dynamic set-up with flow through PermeGear cell system:
For such purpose a peristaltic pump is used to deliver a constant flow. Sampling is done with fraction collector.

2 Semi-dynamic set-up using directly inserts:
For such purpose total or partial replacement of a given volume of receptor fluid is done at given time gaps. The chosen solution was to remove 200 µl of receptor fluid every hour and replace them with fresh one. This solution allows to maximize amount in the receptor fluid and thus prevent problem of analytical sensibility.

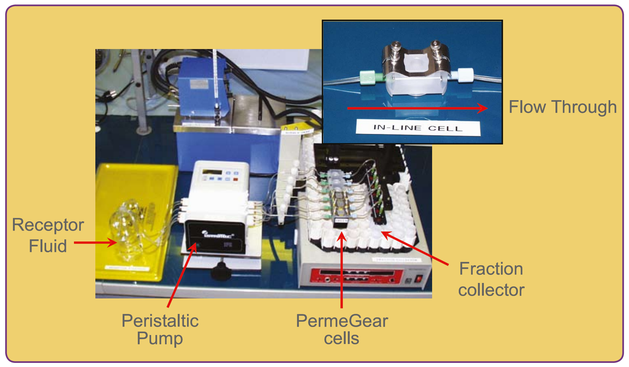

Figure 1A: Flow through diffusion cell (as PermeGear cell)

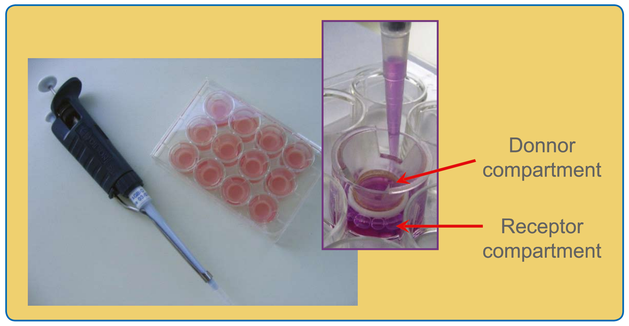

Figure 1B: Insert with partial replacement of a given volume of receptor fluid at given time gap

Caffeine, Aminopyrine and Antipyrine were used as test substances. These chemicals were solubilised in Phosphate Buffer at 1/10th of their saturated concentration.
500 µl/cm² of these aqueous solutions were applied on Episkin for 7 hours for Inserts and up to 12 hours for PermeGear cells. Receptor fluid was in both cases PBS.

FICK'S LAW OF DIFFUSION UNDER INFINITE-DOSE CONDITION

For infinite-dose condition, the cumulative amount of chemical permeating the skin, Q, as a function of time, may be modeled using the appropriate version of Fick's second law; where Kp is the chemical's permeability coefficient, t_{lag} its lag time and C_v° its concentration in the solution :

$$Q(t) = C_v^\circ Kp\left[t - t_{lag} - \frac{12t_{lag}}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-\frac{n^2\pi^2 t}{6t_{lag}}\right)\right] \quad (1)$$

At steady state, the exponential term is negligible. This equation is simplified as follow:

$$Q_{SS}(t) = C_v^\circ Kp\left[t - t_{lag}\right] \quad (2)$$

Thus, cumulative amount of chemical is a linear function of time. The derivative as a function of time, defines the maximum flux, which is constant at steady state:

$$\frac{dQ_{SS}(t)}{dt} = J_{SS} = C_v^\circ Kp \quad (3)$$

If all assumptions are respected, constant donor concentration (i.e. infinite-dose) receptor sink condition and stratum corneum is rate limiting, then flux is constant at steady state.

COMPARISON BETWEEN PERMEGEAR CELLS AND INSERTS

Caffeine was used to compare the two experimental set ups: PermeGear cells and Inserts. Figure 2 reports cumulated permeated amount as a function of time. For both systems, this amount seems to follow a linear relationship as a function of time from 3 to 7 hours.

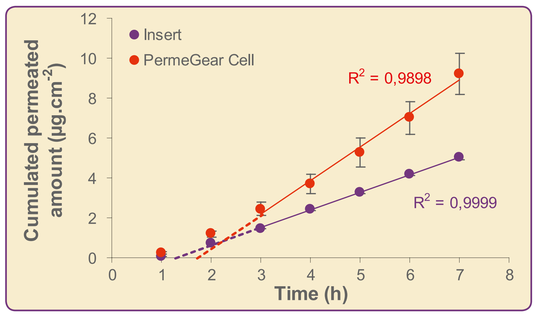

Figure 2: Cumulative permeated amount of Caffeine with Episkin as a function of time (4 replicates, batch 09-Epis-012) using Insert and PermeGear cell

Derivatives of these curves as a function of time lead to another conclusion. Using PermeGear cells, steady state was not achieved, since flux was not constant over that period of time, even at longer exposure time.
Moreover, flux is higher using PermeGear cells than using Inserts. Similar behavior was observed with the two other tested references.

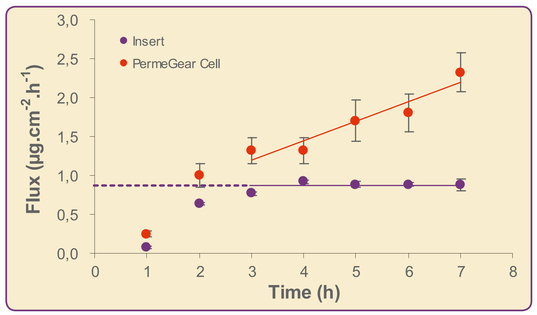

Figure 3: Flux of Caffeine with Episkin as a function of time (4 replicates, batch 09-Epis-012) using Insert and PermeGear cell

Sink condition seems to be not respected for Episkin mounted on PermeGear cells.
According to equation 3, two possible explanations can be considered :

- Such design could induce a mechanical stress on the RhE model, leading to a degradation of the barrier function of the model. Thus, Kp value is affected.
- The applied aqueous solution could evaporate leading to an increasing concentration of the applied solution C_v°.

INTRA AND INTER-BATCH REPRODUCIBILITY

12 different batches were evaluated over two years. Each batch was evaluated at least on 3 samples with the same protocol on Insert.

		Kp (cm/h)			Lag time (h)		
	Batch Number	Mean	SD	CV%	Mean	SD	CV%
March 2009	09-Epis-011	5,7E-03	2E-04	3,2	1,1	0,2	15
	09-Epis-012	4,5E-03	1E-04	2,2	1,3	0,03	2,4
	09-Epis-013	6,5E-03	6E-04	9,5	1,5	0,3	22
	09-Epis-014	6,6E-03	7E-04	11	1,6	0,1	6,8
July 2009	09-Epis-026	5,4E-03	5E-04	9,3	1,3	0,02	1,9
	09-Epis-027	5,0E-03	4E-04	7,6	0,77	0,07	8,5
Februar 2010	10-Epis-007	5,4E-03	4E-04	7,9	0,61	0,3	55
	10-Epis-008	5,8E-03	4E-04	7,1	0,32	0,1	41
	10-Epis-009	5,3E-03	3E-04	5,7	0,33	0,2	76
	10-Epis-010	6,2E-03	2E-04	2,5	1,0	0,06	5,6
June 2011	11-Epis-021	9,6E-03	1E-04	1,1	1,06	0,12	12
	11-Epis-024	6,5E-03	1E-03	19	1,65	0,13	8,0
Mean		6,0E-03			1,05		
SD		1E-03			0,46		
CV%		21			44		

Table 1: Intra and Inter-batch reproducibility of Kp and Lag time measurement of Caffeine evaluated on Episkin using Insert

Intra-batch as well as inter-batch reproducibility was good. CV% is not greater than 20% for Kp values, and higher for lag time values. Moreover, Kp values remain almost constant, whitin a factor of two, from week to week until year to year.

COMPARISON WITH HUMAN SKIN DATA

A simple protocol was set to measure Permeability coefficient on Episkin model without using diffusion cell. In order to demonstrate benefits of the Episkin model, Kp measurement was enlarged in a first step to three different chemicals: Caffeine, Aminopyrine and Antipyrine. These chemicals were chosen in the Flynn Database.

	Antipyrine	Caffeine	Aminopyrine	Coefficient of correlation between Human skin and Episkin
Human skin	-4,18 [5]	-3,41*	-2,99 [5]	
09-Epis-026	-2,69	-2,27	-2,20	0,9731
09-Epis-027	-2,67	-2,31	-2,21	0,9874

Table 2: Comparison of Kp measurements on Episkin vs. Human skin

As previously observed[1], Episkin model has a lower barrier function than human skin with an average of a factor 15 (from a factor 32 for Antipyrine to a factor 6 for Aminopyrine). Despite this defective barrier function, the ranking of substances permeation through Episkin are similar to those through human skin.

CONCLUSION

Episkin design allows to measure permeability coefficient without using diffusion cells, especially as PermeGear cells do not allow to achieve steady state. Automation could be considered for sampling, providing Episkin as a screening tool for permeation studies.

Furthermore, a preliminary comparison on three chemicals with human skin data reinforced previous studies conclusion on Episkin model. It is then a relevant alternative to human skin for *in vitro* penetration studies.

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