In vitro screening of a human skin equivalent model - LabSkin™

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Introduction

The use of human skin models for the evaluation of percutaneous absorption offers an alternative to human or animal experimentation. LabSkin™ is a full thickness living human skin model, comprised of fully-differentiated epidermal and active dermal material which functionally mimics the skin. The purpose of the present investigation was to characterise LabSkin™ using trans-epidermal water loss (TEWL) measurements and *in vitro* permeation characteristics of ibuprofen (IBU).

Methodology

The LabSkin™ (Evocutis, UK) was cut with a scalpel from the plate and mounted in static glass Franz diffusion cells and left to equilibrate in the water bath at 32ºC ± 1ºC. Phosphate buffered saline (PBS) was used as the receptor phase. The surface of the tissue was gently wiped to remove excess water and TEWL measurements were subsequently performed using an AquaFlux™ instrument (AF200, Biox Systems Ltd., UK).

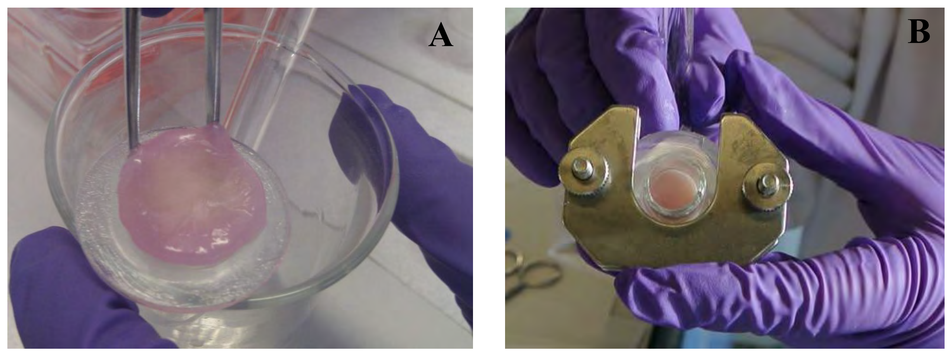

Fig. 1 Assembly of LabSkin™ in a Franz diffusion cell., **A**: Mounting the LabSkin™ in the receptor compartment of a Franz diffusion cell, **B**: Franz diffusion cell after assembly.

For **infinite dose** studies, 1 mL of a saturated solution of IBU in propylene glycol (PG) was tested. Donor and receptor compartments were covered to prevent evaporation of the solutions. Receptor solution was replaced at each sample collection time. For the **finite dose** studies, a solution of IBU (1.47% w/v) in 5% PG and 95% IPA (isopropyl alcohol) was used. At the beginning of the experiment 3.6 µL of the solution were added to the donor compartment. 200 µL samples were collected from the receptor compartment at determined time points and replaced by fresh PBS. All permeation experiments were conducted for 24h and samples were assayed by UV-HPLC.

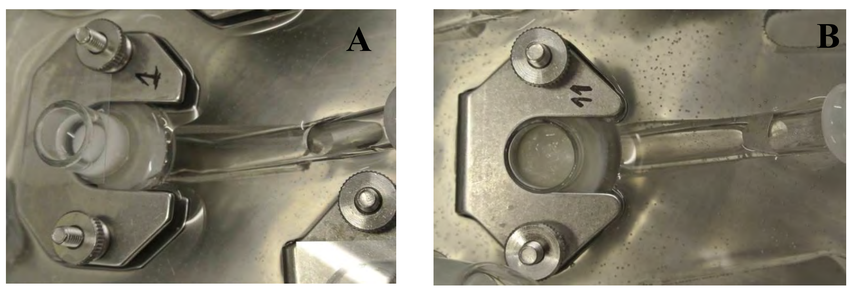

Fig. 2 Franz diffusion cell., **A**: Infinite dose study, **B**: Finite dose study.

Results

Figure 3 shows the TEWL values measured in one cell where no formulation was added, over the time-course of the experiment (24h) for infinite and finite dose study.

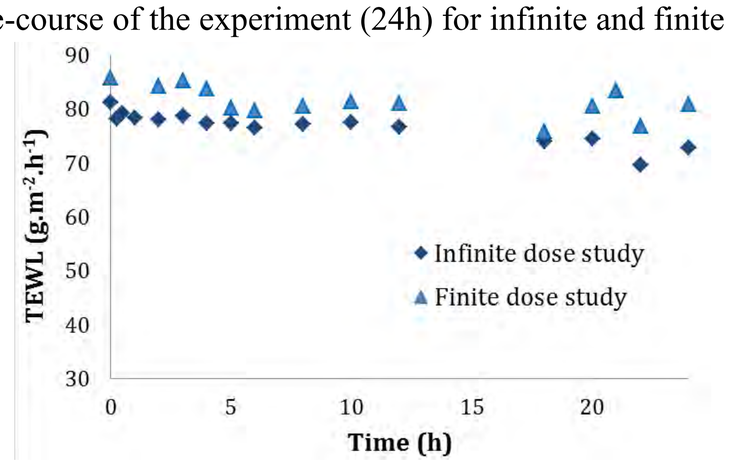

Fig. 3 TEWL measurements for 24h (n=1).

The TEWL values obtained for Labskin™ were higher than human skin *in vitro* (40g/m²/h - unpublished data from Lane/Hadgraft group), however they remained constant over the time-course of the experiments.

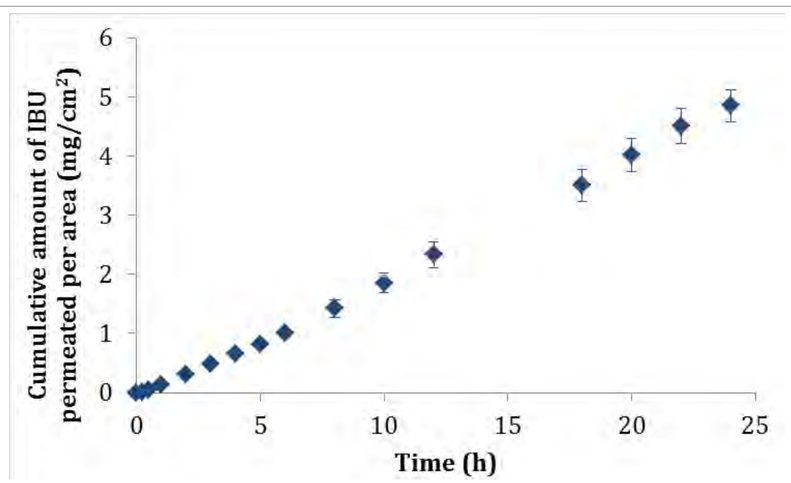

Fig. 4 Cumulative amount of ibuprofen permeated from a saturated solution of ibuprofen in PG for 24h across LabSkin™ at 32ºC (n=3, Mean ± SD).

The variability in Figure 4 is much less than typically observed for human or porcine skin as reported for other human skin equivalent tissue culture models [1].

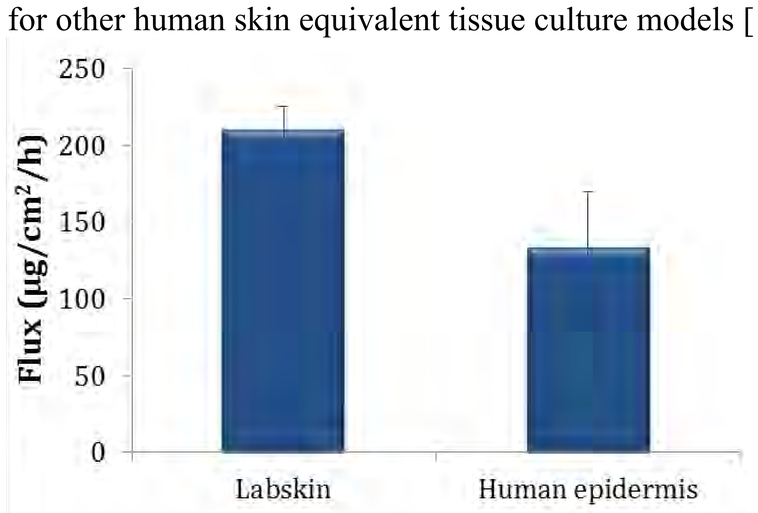

Fig. 5 Steady-state fluxes of ibuprofen through different membranes from saturated solutions in PG. (3≤ n ≤5, Mean ± SD).

The flux values observed for the infinite dose study were approximately 1.5 times higher for LabSkin™ compared with human epidermis [2].
The cumulative amount of ibuprofen permeated during a finite dose study through LabSkin™ and human epidermis was plotted against time and is shown in Figure 6.

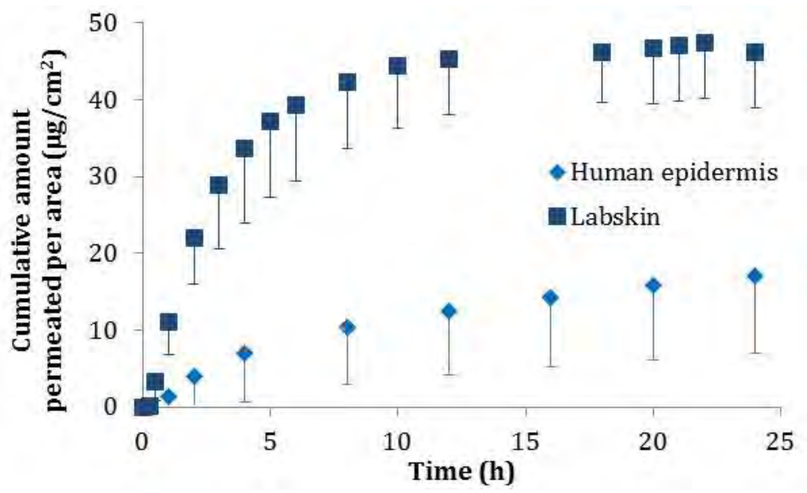

Fig. 6 Cumulative amount of ibuprofen permeated from 95:5:1.47 (IPA:PG:IBU) solution over 24h across LabSkin™ and human epidermis at 32ºC (n=5, Mean ± SD).

The total amount of ibuprofen permeated across LabSkin™ after application of 3.6 µL of the formulation was 46.2 µg/cm² and approximately 17 µg/cm² across human epidermis [3].

Conclusions

The amount of IBU permeated through LabSkin™ was higher than in human skin. The results are encouraging as the flux values indicate that LabSkin™ permeability is comparable to porcine ear tissue - currently the closest animal model to human skin. Further investigations of the applications of LabSkin™ as a surrogate for human skin and in the development and evaluation of formulation screening are ongoing.

References

[1] Netzlaff, F., *et al., European Journal of Pharmaceutics and Biopharmaceutics,* 2005. 60: p. 167-178.
[2] Watkinson, R.M., *et al*., *Skin Pharmacology and Physiology*, 2009. 22: p. 225-230.
[3] Vieira, R., Volatile formulations for (Trans) dermal drug delivery. 2012, UCL - School of Pharmacy.