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Characterization of TEWL and Skin Impedance in a Clinical Study Measuring Acyclovir Permeation by Dermal Open Flow Microperfusion

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Background and Aim

Skin penetration studies routinely demonstrate considerable intra- and inter-subject variability with respect to permeability results, with the greatest variability observed between subjects. This is conventionally attributed to differences in the barrier properties of the *stratum corneum*. Trans-Epidermal Water Loss (TEWL) and skin impedance have been suggested as useful methods to characterize the barrier integrity of human skin, both *in vitro* and *in vivo* in clinical studies. These measures of barrier integrity specifically assess the function of the skin as a barrier to water.

TEWL and skin impedance were assessed in a clinical study in order to investigate the role of skin barrier in modulating the permeation of acyclovir from topical acyclovir creams.

Figure 1: *In two test sites with three application areas each, 12 OFM probes were implanted to investigate the penetration profile of acyclovir after topical application over 36 hours.*

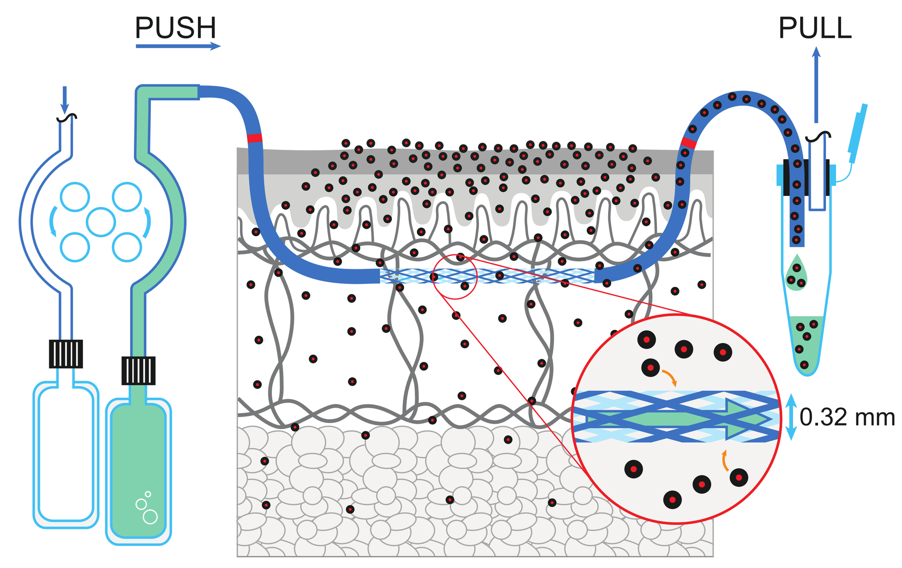

Figure 2: *OFM sampling method – The dOFM probe is inserted into the dermal layer of the skin, connected to the OFM pump and the sampling vial. Acyclovir is applied to the surface of the skin and the active agent penetrates into the skin. The dOFM probe is continuously perfused with perfusate. Through the large openings of the probe direct exchange between perfusate and dermal interstitial fluid occurs.*

Material and Methods

Topical acyclovir treatment and in vivo permeation testing

In six healthy volunteers, 12 dermal open flow microperfusion (dOFM) probes were implanted in two skin test sites (on the right and left upper leg) to assess the dermal interstitial fluid concentration of acyclovir after topical application (Figure 1). The dOFM method is a minimally invasive technique to continuously collect high-molecular weight or lipophilic substances directly from interstitial fluid in the dermis (Figure 2). On each test site, acyclovir formulations were applied on three application areas. Following the application of the formulations the penetration of acyclovir into the dermis was continuously profiled with dOFM for 36 hours to determine the area under the curve (AUC). dOFM samples were quantified by HPLC-MS (LLQ: 0.1 ng/mL)

TEWL and Impedance measurements

Before product application, TEWL measurements were performed in duplicate close to each skin test site using an Aquaflux 200 device (Biox Ldt, London, UK) (Figure 3).

Impedance measurements were also carried out in duplicate in a three-electrode setting, using self-adhesive hydrogel foam electrodes (Figure 4). Impedance components (resistance Zreal, reactance Zim and phase angles) in the low frequency range from 1 to 100 Hz were recorded by a PalmSens^3 potentiostat.

AUC, TEWL and impedance components for each of the two test sites from 6 subjects were statistically analysed (SAP, ProcReg).

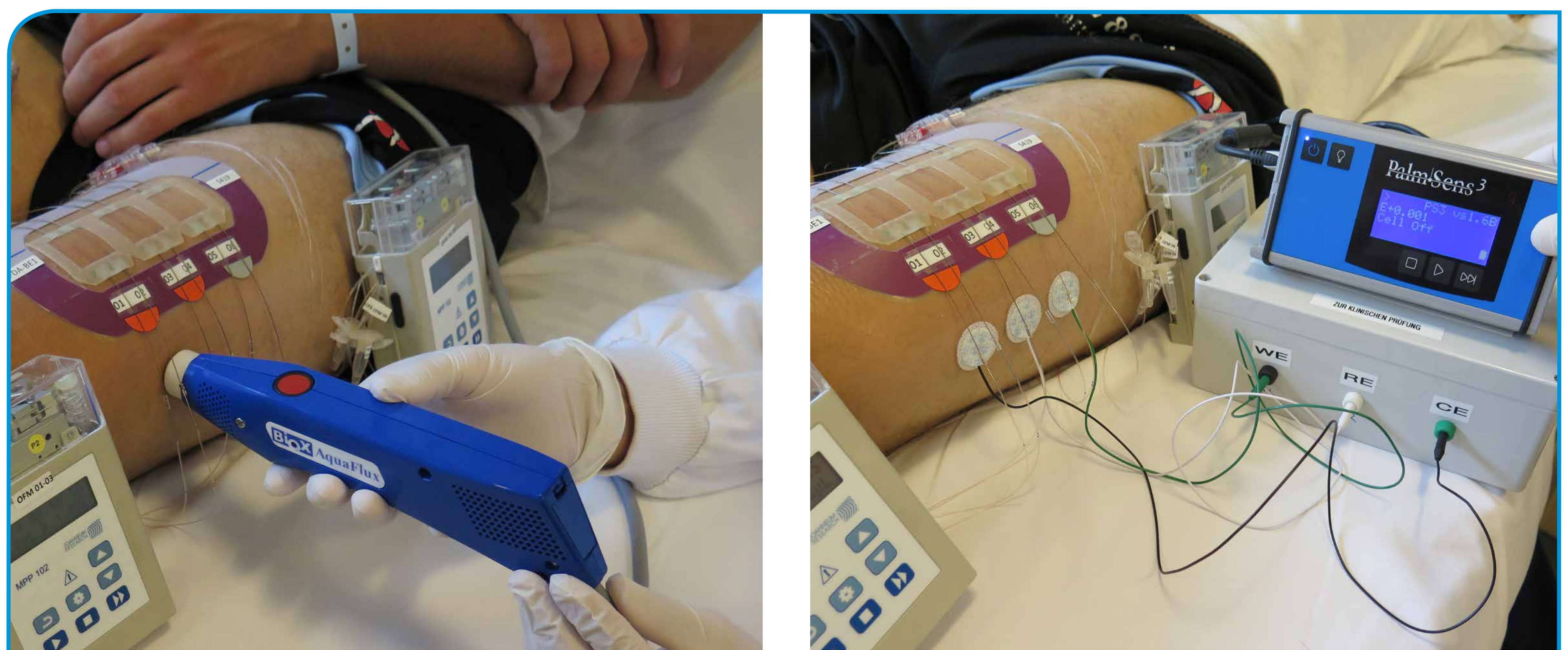

Figure 3 and 4: *TEWL and impedance measurements*

Results

The ability of TEWL and impedance measurements to predict the permeation of acyclovir into the skin was evaluated by comparing the TEWL and impedance results with the measured AUCs (Figure 5). TEWL results ranged from 6.78 to 13.7 g cm^{-2} h^{-1}) and correlated well with AUC (R^2=0.69). At different frequencies, impedance components were examined and the results imply that the reactance at a frequency of 10 Hz showed a good correlation with AUC (R^2=0.49).

Regression analysis showed a significant relationship between TEWL and electrical reactance at 10 Hz (p = 0.0061) as well as between reactance and AUC (p = 0.0107) and TEWL and AUC (p = 0.0009).

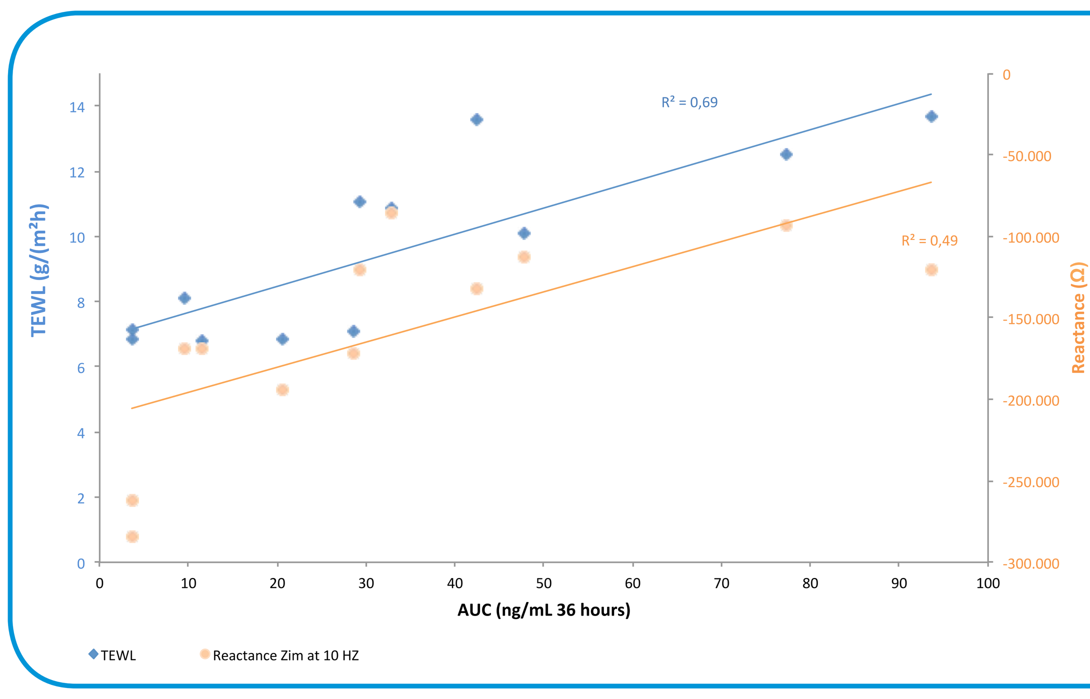

Figure 5: *AUC/TEWL and AUC/reactance (10 Hz) correlations for 6 six subjects with two test sites (n = 12)*

Discussion and Conclusion

As *stratum corneum* is the main barrier for drug permeation, skin impedance was measured at low frequencies (1-100Hz) where impedance is mostly influenced by the *stratum corneum* composition. This clinical study showed a relationship between skin impedance, TEWL and acyclovir permeation, confirming prior impedance results from an *ex vivo* study with clobetasol-17-propionate.

In conclusion, TEWL and impedance measurements are promising methods to detect differences in the skin barrier that influence acyclovir permeation. This clinical study is ongoing and an additional 20 subjects will be involved. Furthermore, an additional *ex vivo* study will be performed. Final results from these measurements may be used to normalize AUC results with the objective to minimize the intra- and inter-subject differences as well as the amount of subjects needed in clinical studies.

Acknowledgment

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