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Investigation of Protein Binding in Skin with Dermal Open Flow Microperfusion (dOFM)

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Purpose

Quantification of the drug concentration at the site of action is of major importance during drug development. In dermatology, this site is located in the dermis, where the pharmacological effect of the drug is also influenced by protein binding. Dermal open flow microperfusion (dOFM) allows direct access to the dermal interstitial fluid (ISF) in skin and can be used to assess the rate and extent to which a topical drug becomes available in the dermis.
The aim of the study was to develop a new dOFM sampling strategy for in-vivo sampling of undiluted ISF in dermal tissue and subsequently quantify total, bound and unbound drug concentrations using diclofenac for proof of concept.

Conclusion

- Two new dOFM sampling strategies, namely OFM **recirculation** and OFM **suction**, were successfully used to sample undiluted ISF from the dermis of anesthetized pigs.
- Undiluted ISF had a significantly lower albumin concentration than lymph, indicating that lymph may not appropriately represent ISF.
- Both new strategies can be used to measure total drug concentrations and protein binding in the dermal compartment.
- In-vivo protein binding analysis revealed a three times higher free fraction in the dermis compared to plasma.

Material & Methods

Establishment of a dOFM method for undiluted ISF sampling

- Two dOFM sampling strategies, namely **recirculation** and **suction** were evaluated for their feasibility of sampling undiluted ISF from the skin of anesthetized pigs (n = 5).
- Albumin concentration was determined photometrically in undiluted ISF, lymph and plasma with Anthos ht II.

Total concentration and protein binding in dermal ISF

- Anesthetized pigs (n=2) received an intravenous infusion of diclofenac for 9 hours (bolus 75 mg in 2h, infusion 5 mg/h) paralleled by sampling of undiluted ISF, lymph and plasma.
- Equilibrium dialysis was performed with all body fluids. Total, bound and unbound concentrations were determined by HPLC in all samples.

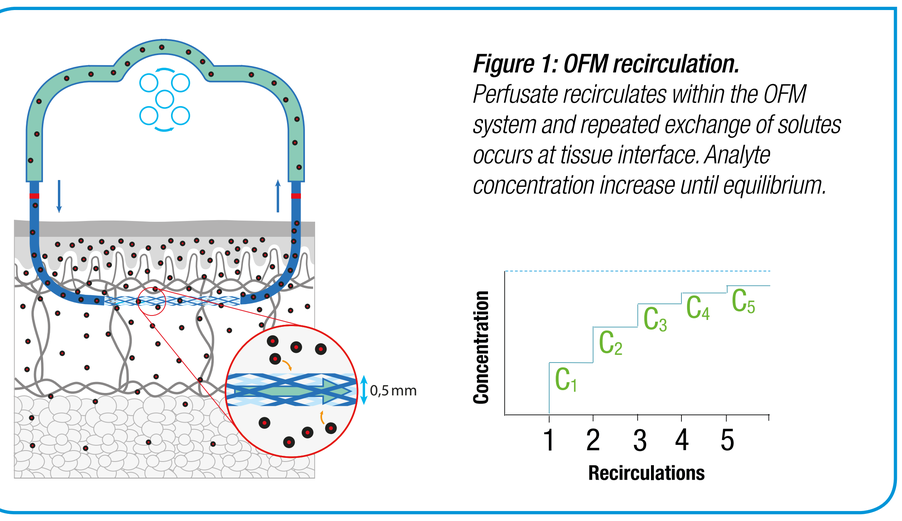

Figure 1: OFM recirculation.
Perfusate recirculates within the OFM system and repeated exchange of solutes occurs at tissue interface. Analyte concentration increase until equilibrium.

Results

- Increasing number of recirculations increased albumin concentration until saturation (Figure 2). The plateau indicates equilibrium between perfusate and ISF. Thus, OFM recirculation can sample undiluted ISF.
- ISF sampled by recirculation had an ISF-to-plasma ratio of 0.42 ± 0.12. ISF sampled by suction had an ISF-to-plasma ratio of 0.49 ± 0.11. Lymph showed a significantly higher albumin concentration than ISF with a lymph-to-plasma ratio of 0.67 ± 0.22 (p=0.003).

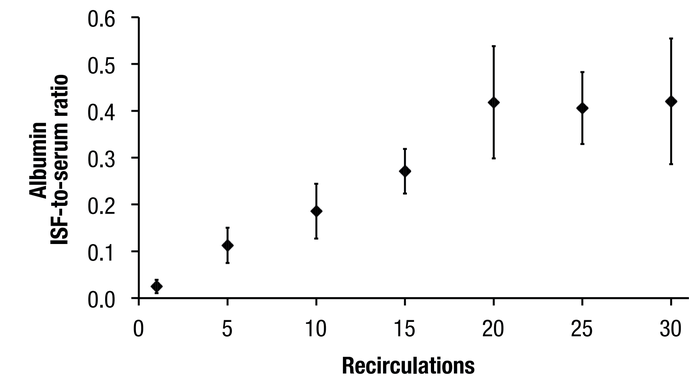

Figure 2: Albumin concentrations in ISF from OFM recirculation.

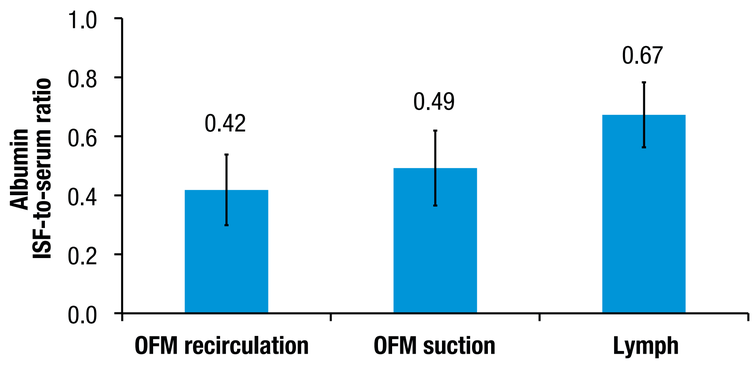

Figure 3: Comparison of albumin concentrations.

	Total diclofenac concentration at 9 h (ng/ml)	Protein bound (%)	Free fraction (%)
Plasma	4238 ± 226	99.5 ± 0.1	**0.5 ± 0.2**
Dermal ISF … from OFM recirculation … from OFM suction	 2166 ± 166 2247 ± 306	98.6 ± 0.5	**1.4 ± 0.5**
Lymph	2713 ± 370	98.8 ± 0.2	1.2 ± 0.2

Table 1: Total diclofenac concentration and protein binding.

- Total diclofenac concentrations in were 50% of the plasma levels at 9 h after start of infusion. Slightly higher concentrations were measured in lymph at the same time.
- Protein binding was lower in dermal ISF compared to plasma.
- The free fraction in the dermis (1.4 ± 0.5%) is three times higher compared to plasma (0.5 ± 0.2%).

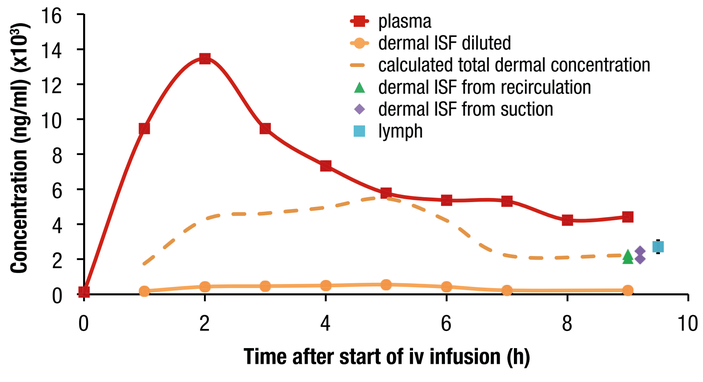

Figure 4: Pharmacokinetics of diclofenac in plasma, lymph and ISF after iv infusion.
ISF from OFM recirculation and OFM suction was sampled at the end of the infusion duration of 9 h.

Acknowledgement

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