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Continuous Sampling of Immune Cells Using Open Flow Microperfusion

M. Bodenlenz[1], S. Kainz[1], B. Prietl[2], E. Lechner[2], G. Rauter[2], T.R. Pieber[2], T. Birngruber[1], F. Sinner [1,2]
[1] Joanneum Research Forschungsgesellschaft m.b.H, Graz, Austria
[2] Medical University of Graz, Graz, Austria

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PURPOSE

BACKGROUND

- Inflammatory skin diseases involve pronounced immune-cell infiltration and local cytokine release.
- Dermal Open-Flow Microperfusion (dOFM) has proven to enable continuous sampling of different drugs and cytokines in inflamed and healthy skin in humans *in vivo* in PK-PD and topical bioequivalence trials[1-3].

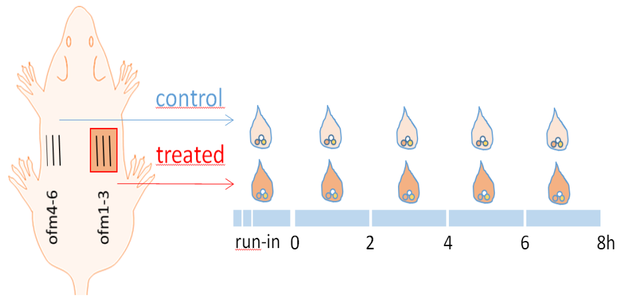
AIM

- To investigate whether dOFM enables continuous sampling of immune cells from inflamed and healthy skin.

METHOD

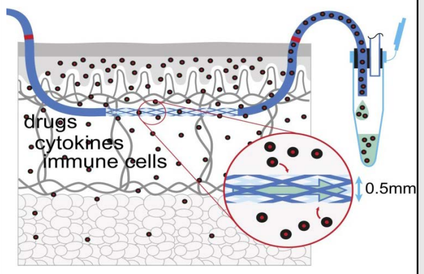
- Sprague-Dawley rats (n=6)
- Day 1-8: Introduction of a psoriasis-like skin lesion by daily topical dosing of imiquimod (IMQ) cream
- Day 8: Dermal interstitial fluid sampling by dOFM
  - from IMQ-treated skin (inflamed, right back)
  - from control skin (non-inflamed, left back)
  - Fig. 1 shows protocol, Fig. 2 dOFM principle
- FACS analysis:
  - Non-quantitative analysis of immune cells by size vs. granularity plots (Fig. 3)
  - Quantitative analysis of immune cells (Fig. 4) using BD TruCOUNT Tubes [cells/µL]

Figure 1: Daily treatment with imiquimod and sampling in the skin on day 8



Rats were treated with imiquimod for 8 days on their right back to induce skin inflammation. dOFM probes (3 in treated skin, 3 in untreated skin) deliver interstitial fluid for FACS-analysis.

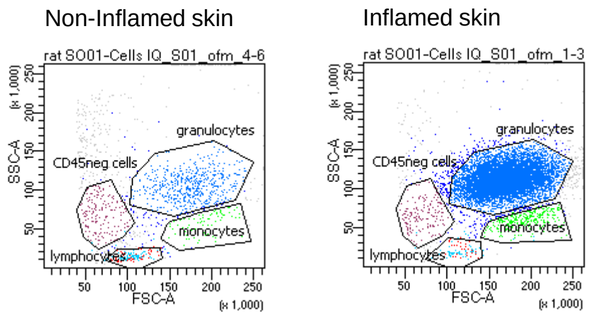
Figure 2: dOFM sampling working principle



dOFM continuously delivers unfiltered dermal interstitial fluid for analysis of biomarkers, drugs, and immune cells.

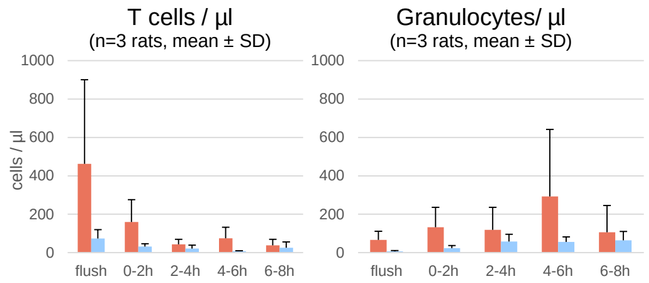
RESULTS

Figure 3: Size vs granularity plots of leukocytes
(example for one rat for period 6-8h on day 4)



Size vs. granularity plots show different leukocyte types:
Granulocytes: Basophils, eosinophils, neutrophils, mast cells
Lymphocytes: T-cells, B-cells >> mem.cells, nat. killer cells
Monocytes >> macrophages, dentritic cells, foam cells
Staining and true count analysis deliver absolute count (Fig.4).

Figure 4: Absolute counts from dOFM samples
(example: T-cells and granulocytes in 3 rats on day 8)



Absolute count confirms a significant number of cells in all dOFM samples. Inflamed skin delivers higher cell number. Lymphocytes/T-cells decrease over time (left panel). Granulocytes are stable or slightly increasing (right panel) Monocytes decrease over time on day 8 (data not shown)

CONCLUSION

- dOFM enables continuous sampling of immune competent cells from inflamed tissue.
- Significant differences seen between treated (inflamed) and non-inflamed skin.
- The method of OFM has the potential to enable a combined investigation of locally released cytokines and the involved immune competent cells in clinical and preclinical in vivo studies of inflammatory diseases.

REFERENCES

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