Skin penetration and dexamethasone release from polymer nanoparticles in ex vivo human skin

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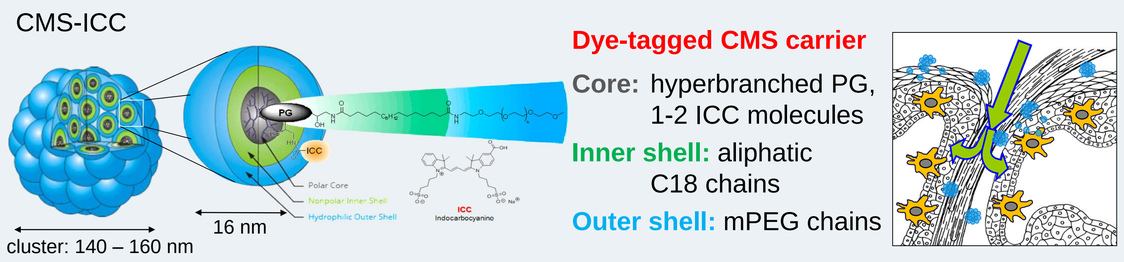
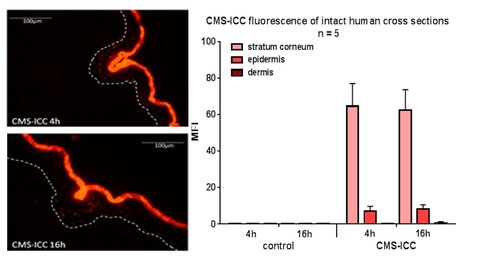
Abstract Encapsulation in nanocarriers could help to improve the selectivity of dermatotherapy and may even make novel drug classes available for topical applications. Here, we investigated the penetration of polymer-based dendritic core-multishell nanocarriers and the release of dexamethasone (DXM) as anti-inflammatory model drug in excised human skin.
Covalently labelled carrier were found to accumulate in the stratum corneum and hair follicles with very limited deeper penetration. Anti-DXM Enzyme-linked Immunosorbent Assay (ELISA) and Liquid Chromatography-Triple Quadrupole Mass Spectrometry (LC-MS) for DXM in extracts from the different skin layers revealed first evidence for retarded penetration in the dermis compared to application in solution. However, the penetration process of the nanomaterials and drug molecules in the outermost layers of the skin is poorly understood and encouraged us to develop protocols for selective probing of DXM in excised human skin using soft X-ray spectromicroscopy (SXR-SPEM). The technology allowed for label-free detection of DXM and provided quantitative concentration profiles as well as two dimensional drug distribution maps of DXM on human tissue sections. The combination of conventional skin penetration studies with such high resolution methods will improve our understanding of nanocarrier-skin interactions and drug delivery.

Most of topically applied dendritic core-multishell (CMS) nanocarriers remain in stratum corneum of intact skin but a small amount cross over to epidermis



MFI-analysis of particle fluorescence



IHC-staining of CD1a-positive cells

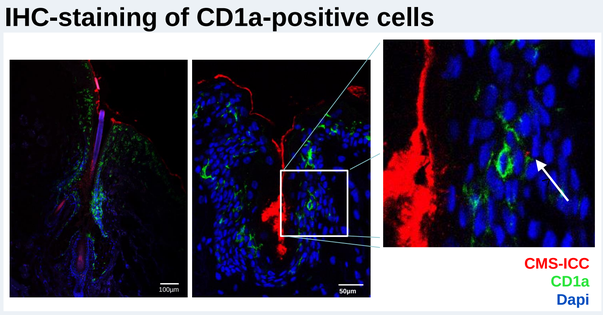
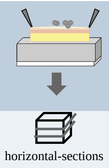


Fig.1: Fluorescence microscopy analysis of cross sections from intact human skin samples treated with CMS-ICC nanocarriers. The vast proportion of CMS carriers could be detected in stratum corneum (SC) or accumulated in hair follicles (HF), equally after 4h or 16h of incubation. However, mean fluorescence intensity analysis showed that a very small amount of the CMS particles translocated into epidermis and appeared as little spots. Laser scan microscopy allowed visualiziation of some spots next to CD1a-positive cells underneath the SC near HF, suggesting that HF could be relevant entry points for nanomaterials in human skin.

Quantitative depth profiles: detection of DXM in tissue lysates of the different skin layers by ELISA and LC-MS



Separation of skin layers

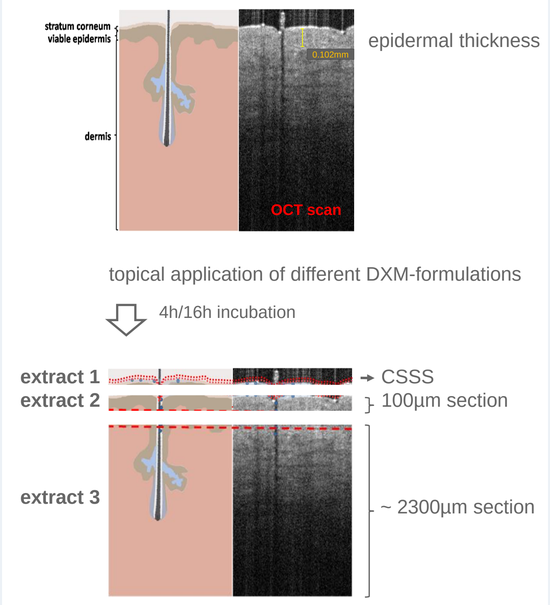
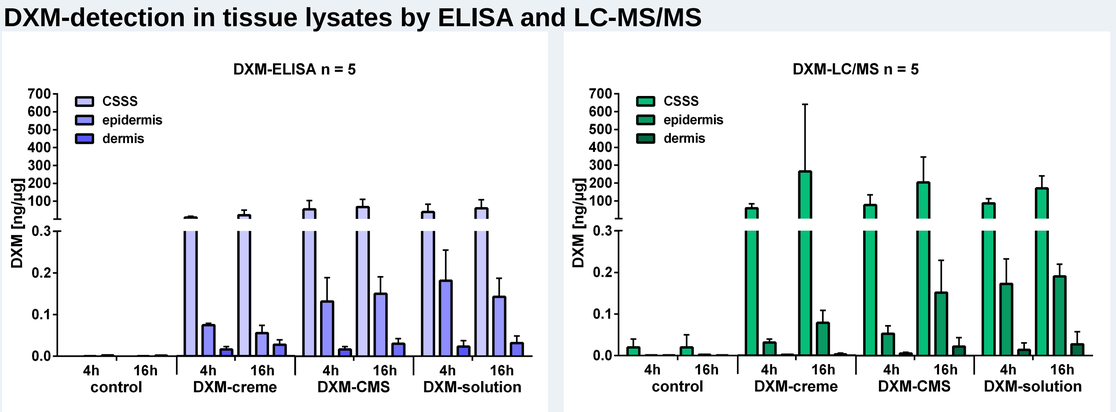


Fig. 4: Quantification of DXM in tissue extracts. A competitive ELISA as well as LC-MS were used to analyze DXM distribution in the skin (limit of detection: 0.2 ng/ml DXM). For best accuracy of depth profiles, the epidermal thickness of *ex vivo* human skin was identified by optical coherence tomography (OCT) scans. After topical treatment with different DXM formulation, as described in Figure 3 skin was separated horizontally in three layers: superficial SC (CSSS: cyano-acrylate skin surface strip), epidermis and dermis. Skin layers were lyzed, and the DXM concentrations were normalized to the overall protein level of each lysate.
Results of both analytical methods confirm the semiquantitative MFI-analysis of DXM-stained cryosections. CMS-delivered DXM penetrate deeper than when applied in creme but delayed compared to DXM solution.

DXM-detection in tissue lysates by ELISA and LC-MS/MS



IHC: Dexamethasone (DXM) release from CMS-carrriers into stratum corneum, epidermis and dermis

IHC-staining of DXM

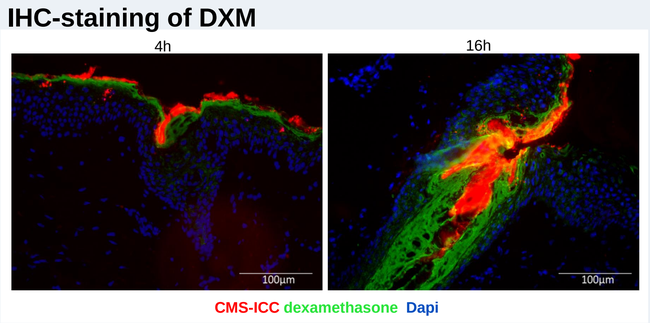
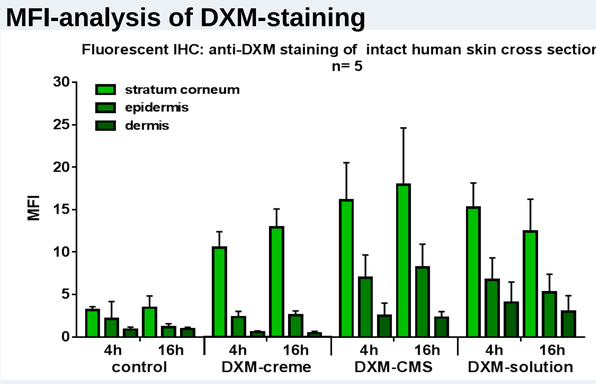


Fig. 2: Colocalisation of drug and carrier via fluorescence microscopy. Immunhistochemical staining of DXM in longitudinal cryosections of intact excised human skin after topical application of DXM-loaded CMS-ICC carriers revealed DXM release into SC, epidermis and dermis.

MFI-analysis of DXM-staining



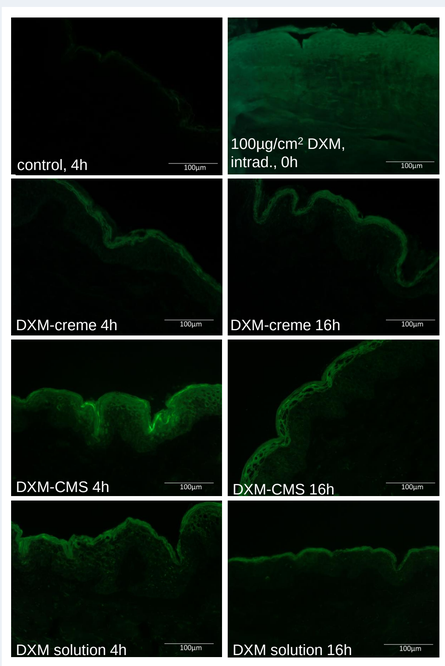


Fig. 3: Semiquantitative MFI-Analysis of DXM-stained cryosections. Equal amounts of DXM were topically applied in different formulations and excised skin was incubated for 4h and 16h at 37°C. MFI analysis of DXM-stained cryosections indicated deeper DXM penetration when delivered by CMS carrier than by 0.05% LAW- creme. Compared to DXM penetration in aqueous solution with 10% ethanol the carrier delivered DXM similary into epidermis but slower into dermis.

Selective probing of DXM penetration by soft X-ray spectromicroscopy (SXR-SPEM)

Depth profiles of differential absorption of DXM

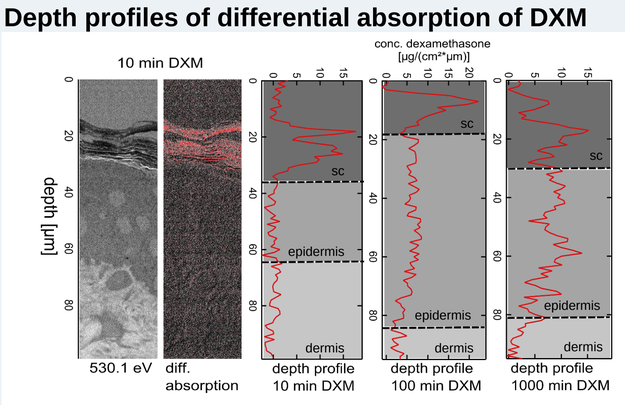


Fig. 5: DXM penetration into the skin with a lateral resolution of 1 µm. Label-free DXM detection on cryosections of human skin is feasible using SXR-SPEM and yielded spatially resolved absorption maps which can be quantified. Following DXM exposure for 10,100,1000 min most of DXM was found in SC between corneocytes, but over time a considerable increase in DXM concentration was also detected in the viable epidermis.

Conclusion & Outlook

- Using the corticosteroid DMX as model molecule and the polymer-based CMS-carrier we demonstrate a comprehensive analysis of carrier and DXM penetration in excised human skin.
- While carrier material largely remains in superficial SC compartments, there is evidence for higher DXM penetration rates of loaded CMS compared to creme and slower penetration into dermis compared to solution.
- Both characteristics could be further developed to maximize local concentrations in epidermal compartments with reduced side effects (e.g. dermal atrophy).
- SXR-SPEM cold become a valuable tool for label-free drug detection.

Overall, this type of carrier systems appears to be a promising tool for the improvement of topical dermatotherapies and present new treatment approaches for novel drug classes.

Acknowledgements: