Visualizing the Enhanced Penetration of Active Ingredients into Skin via Liposomal Delivery using Two-Photon Microscopy

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Introduction:

Percutaneous delivery of topical administration of active ingredients and a controlled release into the underlying epidermal and dermal tissue faces a unique set of hurdles. A fundamental understanding of their penetration ability, which includes to understand the role of liposomes used to enhance the tissue penetration, is mandatory to enable a functionality [1].

The aim of this study was to characterize the permeability of target specific active ingredients into the skin, to expose the cosmetic ingredients to the living cell population underneath the stratum corneum. Furthermore, we aimed to determine influences of a topical administered liposome formulation on the stratum corneum lipid packing. Therefore, a Franz Diffusion Cell setup was used to understand the percutaneous penetration in superficial and deep tissue layers of three potent hydrophilic active ingredients within a liposome-based formulation (RonaCare® VTA) [2]. The uptake kinetics were monitored using LC-MS/MS to quantify the depth depended penetration of the target specific active ingredient. The tissue morphology and a precise determination of the penetration paired with its efficacy was monitored using histological tissue sections and supported via Two-Photon microscopy. To examine this, specific labeled (Laurdan/DiI) and unlabeled liposomes were characterized and influences on the penetration of the active ingredients and the skin lipid packing were identified [3].

This work highlights technological advancements that can be used in further studies to better understand the penetration of active ingredients into the skin. Furthermore, the mechanistic role of liposomes in the delivery of molecules beyond the stratum corneum to achieve its biological function is better understood and serves as a good basis for similar future work. Based on a model system combining lipophilic characteristics due to a liposomal vector and hydrophilic active ingredients as targeted cargo, RonaCare® VTA is ideal for a comprehensive dermal penetration study.

Materials & Methods:

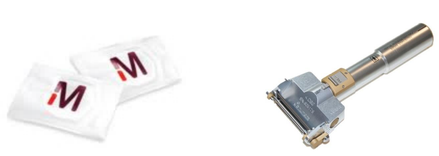
Materials

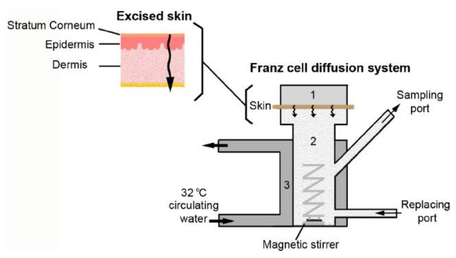
Phosphate buffered saline (Receptor fluid), RonaCare® VTA, propylene glycol, oleic acid (3 x active ingredients within the formulation) were purchased from Merck KGaA (Darmstadt, Germany).

The size and membrane thickness of the liposomes was determined via cryo transmission electron microscopy (Cryo TEM).

Methods & Penetration Setup

A Franz Diffusion Cell setup, was used to compare the percutaneous penetration in superficial and deep tissue layers. Thereby the penetration ability of the three active ingredients was tested on artificial membranes and dermatomed skin.

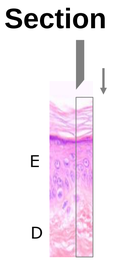




Penetration Analytics and Skin Integrity

The skin morphology and a precise determination of the penetration depth paired with its efficacy was monitored using tissue sections and supported via Two-Photon microscopy. To examine this, specific labeled (Laurdan/DiI) and unlabeled liposome were characterized and influences on penetration ability/depth of the active ingredients and the stratum corneum lipid packing were identified.

Cryo Tissue Section



Two-Photon Microscopy

Penetration Analytics

The active ingredient uptake kinetics were analyzed using liquid chromatography–mass spectrometry (LC-MS/MS) to identify and quantify the depth depended penetration of the target specific ingredient.

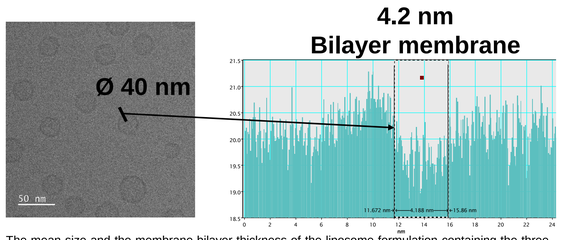
LC-MS/MS

Results & Discussion:

Liposome
via Cryo TEM

The liposome formulation was confirmed using Cryo TEM.

Liposome size: ~40 nm
Membrane thickness: 4.2 nm

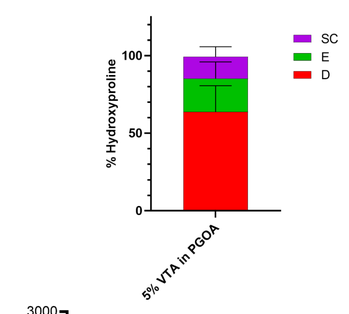


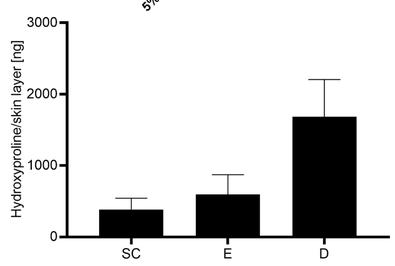
The mean size and the membrane bilayer thickness of the liposome formulation containing the three active ingredients was determined via Cryo TEM analysis.

Penetration Depth
via LC-MS/MS

Hydroxyproline was determined as lead active ingredient of the liposome formulation.

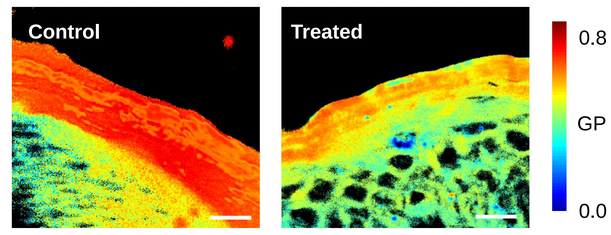
Hydroxyproline penetrated through every skin layer with the highest concentration in the dermis.





The mean distribution of penetrated hydroxyproline in the stratum corneum (SC), epidermis (E) and dermis (D) on 500 µm porcine split-skin after 4 h at 32°C was determined. 500 µL of a 5% VTA in PGOA formulation was topically applied. The penetration for six replicates (n = 6) from a single donor. Values represent mean ± SD.

Skin Integrity
via Fluorophore



Gel Phase (GP) images of RonaCare® VTA treated (right) and untreated control skin (left). A clear lowering of the GP values (lipid packing) in the SC of the treated sample is seen. Scale bar is 20 µm.

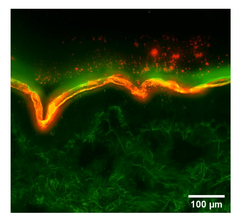
Laurdan is used to investigate membrane qualities of the phospholipid bilayers of cell membranes.

It is sensitive to membrane phase transitions as well as the alteration to membrane fluidity.

GP values: 0.4 – 0.7 ordered lipids
< 0.1 fluid phase

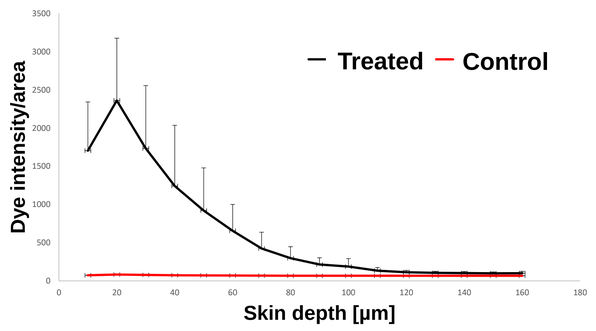
→ The lipids of the treated skin are less ordered

Penetration Depth
via Fluorophore



The fluorophore (DiI) penetration measurements show that the RonaCare® VTA liposomes transport the DiI inside the skin:

→ ~ 100 µm deep



Epifluorescent images of skin treated with RonaCare® VTA with DiI. Labeled liposomes on the surface of the SC can be seen. Penetration of the DiI into the skin as a function of depth.

Conclusion:

RonaCare® VTA provides an ideal system for a comprehensive study of dermal penetration. The liposomal vector is used to envelope the three active ingredients that enables penetration. Here we put forward qualitative, quantitative, and visual evidence that liposomal cargo reaches the targeted dermal tissue with the use of this system. It is shown that Hydroxyproline, a prominent active ingredient within the formulation, penetrates into the different skin layers. The liposomal penetration to at least to a depth of 100 µm within the skin which enables efficient penetration was visualized. Additionally, the liposomal formulation with the active ingredients changes the uniformity of the stratum corneum lipid packing. Overall, the dermal penetration of liposome-based formulation exceeds that of the non-liposomal formulations and increases targeted delivery of active ingredients enabling an enhancement of its effect brought about by cellular modulation.

Acknowledgments:

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References:

1) A. Fahr et al. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur J Pharm Biopharm., 2003

2) https://www.merckgroup.com/business-specifics/performance-materials/Cosmetics/brochures/Cosmetics-That-Shape-Your-Brands-EN.pdf

3) John D. Bell et al. Use of Laurdan fluorescence intensity and polarization to distinguish between changes in membrane fluidity and phospholipid order. Biochimica et Biophysica Acta, 2002.