IN VIVO MOLECULAR CUTANEOUS ABSORPTION IN HUMAN SKIN USING COHERENT ANTI-STOKES RAMAN SCATTERING (CARS) MICROSCOPY

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Percutaneous penetration of small molecules is a major issue for safety and efficacy evaluation in cosmetics and pharmaceutics research. So far, to quantify active compounds in human skin following topical application, *ex vivo* skin samples mounted on Franz cell diffusion set-up together with appropriate analytical methods is mostly used [1]. CARS microscopy approaches have also been applied on *ex vivo* skin [2], but few examples have been described *in vivo*.

A novel framework for imaging and quantifying active molecular penetration in human skin in vivo is introduced and validated in this work. Our approach combines nonlinear imaging microscopy modalities: CARS is used to monitor deuterated molecular penetration, whereas two-photon excited auto-fluorescence (TPEF) is used to observe skin morphology. The 'λ-switch' imaging scheme is implemented to detect C-D resonant and non-resonant CARS signal at the same time, for increasing detection sensitivity and correcting unavoidable photon loss in skin, since non-resonant CARS signal is only sensitive to the scattering and attenuation of the excitation beams with skin depth.

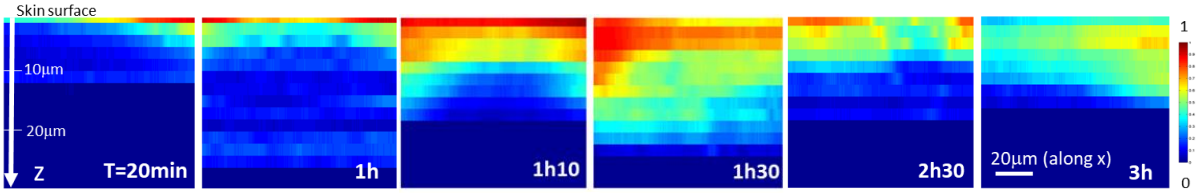

Figure 1: *In vivo* kinetic glycerol percutaneous penetration. Deuterated glycerol/Xanthan gel vehicle = 40% (mass). Topical application at T=0 on forearm with a dose of 5 mL/cm^2.

With this method, we demonstrate *in vivo* glycerol quantitative percutaneous penetration over time (see Figure 1) and compared two vehicles, water and xanthan gel. We found that contrary to water, xanthan gel vehicle retains glycerol on the skin surface over time enabling its constant release for much longer time. More generally, the proposed imaging framework provides an enabling platform for establishing functional activity of topically applied products in vivo.

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