In 2015, Witting et al. investigated the skin penetration of macromolecules using MPM-FLIM [4]. They hypothesised that HA would enhance the transdermal delivery of bovine serum albumin (BSA). HA was labeled with fluorescent N-methylanthraniloyl (MANT)-guanine nucleotide, and BSA was tagged with rhodamine B (RhB). They used pig skin as a model and tape-stripped 30 times to establish a barrier-deficient model. The tissue was placed in Franz cells. The skin was first treated with HA with different molecular weights (5 kDa, 100 kDa, and 1 MDa) and the penetration profile observed using FLIM. Then they selected the most efficient HA (5 kDa) and applied it together with BSA. All the samples were incubated at 32°C for 24 hours before being prepared for frozen sections. The resulting cross-sections of treated skin were imaged by MPM-FLIM. Bright-field images were also taken to show skin orientation. MPM-FLIM images were analyzed using fluorescence decay curves based on photon counts. MPM-FLIM images show that higher-molecular-weight HA stayed as a cluster on the surface of the skin in normal skin and penetrated to the dermis when the skin barrier was disrupted. The lowest-molecular-weight HA was found in clusters that were mostly on the stratum corneum in both normal and tape-stripped skin, but some signal was detected in the dermis. These observations were confirmed by FLIM photon counts. BSA penetrated to the dermis when it was delivered with 5 kDa HA. This study demonstrated that MPM-FLIM images and analysis were able to show the topical drug delivery enhancement deep into pig skin. The limitation was the overlap between HA emission and RhB absorption, which could result in false-positive signals in the FLIM images.

In 2018, Alex et al. used FLIM in a phase 1 clinical trial to assess topical antiinflammatory treatment in healthy volunteers [18]. Their aims were to investigate the distribution and residency of two topical drugs within the epidermis and dermis in vivo using FLIM. Seven healthy men (Fitzpatrick skin I to IV) were recruited for this study. Each participant received two topical creams (1% concentration of active ingredient) (A and B). Cream A was applied on one volar arm and cream B was applied to the other volar forearm once daily for seven days. FLIM images were taken every day for seven days following treatment. Prior to imaging, double-sided tape was used to place a a glass coverslip on a magnetic coupling ring, which was then attached to the volar forearm imaging site. The articulated arm of the system was attached to the magnetic coupling ring. FLIM images were taken in 5-µm steps from the skin surface down to a depth of 200 µm. Most of the drug signal was detected on the skin surface on treatment days 2 to 7. Accumulation of the topical formulation along skin ridges was also visible in images obtained on days 2, 4, and 9. By day 10, there was no detectable fluorescence from formulation residing in the skin. Semiquantitative analysis from the FLIM images was performed by the readout counts from the drug fluorescence signal against the depth of penetration and residency in one study participant for cream A and B. There was significant variation in the day-to-day observation of drug fluorescence from both formulations shown by FLIM. The signal reached the lower limit of detection after nine days. Accurate quantification of topical drug penetrating was challenging. Overall, this small pilot study of investigational topical drugs described the in vivo distribution and semiquantified measurement of drugs in the skin by using MPM-FLIM.

56.6 COHERENT ANTI-STOKES RAMAN SPECTROSCOPY

Raman scattering microscopy has been developed as a label-free chemical imaging tool that is used to acquired high-resolution images of multiple chemical components of a topical formulation penetrating mammalian skin. Coherent anti-Stokes Raman spectroscopy (CARS) has been used for fast imaging of biological samples, primarily with lipid contrast.

In 2018, we combined two transdermal technologies to investigate the enhancement of topical drug delivery [19]. In Yamada et al. (2018), we coated elongated microparticles with nanoemulsion formulations for topical application on ex vivo human skin. Our initial experiment was to use lipophilic dye, DiI in the core of a nanoemulsion to visualize the delivery under confocal microscopy. We proceeded to use a dye-free imaging system, CARS microscopy, to confirm the location of the lipid components of the nanoemulsion. Medical-grade glycerol (as a surrogate for a hydrophobic drug) was encapsulated into the core of nanoemulsion to enhance CARS imaging.