human skin from abdominoplasty was mounted on a Franz cell system, and two AuNP formulations (aqueous and toluene) were applied to the skin. After 24 hours at 32°C treatment, en face skin was visualized using RCM with dermoscopy. 3D reconstructed images from RCM z-stacks confirmed the aggregates were present within the furrows in the AuNP-aqueous group but were absent in the toluene-containing groups. The limitation of RCM is the resolution of images, especially greater than 100 um deep in the skin. It is challenging to accurately identify small particles in these images. Several imaging techniques were employed in this study to confirm the findings both qualitatively and quantitatively. This study is a good example showing that nanoparticle skin penetration is a multifactorial and multistep process that can be confirmed by complementary imaging techniques that each have strengths and weaknesses.

56.10 MATRIX-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY IMAGING

MALDI mass spectrometry imaging technology is an emerging label-free imaging technology that can provide information about drug distribution profiles across the tissue. The quantification of drug molecules on the skin is the unique strength of this technology. The major limitation is the cost of equipment and extensive training needed to apply this powerful technology to topical drug delivery characterization. Equipment access and funding currently limit the uptake of this approach in the field of drug delivery. But most of the large pharmaceutical companies have a mass spectrometry imaging group to look at drug distribution and quantification in tissue.

Bonnel et al. (2018) [29] used MALDI-MSI to conduct a feasibility study to show (1) if this technique could be useful for pharmacokinetics studies; (2) if the results can be reproducible; (3) if this method could be used to compare two different formulations; (4) if the results could be quantified; and (5) if the high spatial resolution data could be varied. Excised human skin from abdominoplasty surgery was mounted in a Franz cell system. The skin was treated topically with three different drugs (ruxolitinib, tofacitinib, and LEO29102 [undisclosed drug]) in two different formulations (transcutol solution and cream) and incubated at 37°C for three hours. An 8-mm punch biopsy was collected for each sample and embedded for frozen sections at the end of the experiment. For each tissue section, three individual sections spaced more than 100 µm apart were analyzed by MALDI-MSI. Histology staining (H&E) was done on the same sections after the MALDI-MSI imaging. Image analysis was done based on molecular distributions in skin and signal intensity per pixel of each drug molecule extracted from the image data set. Figure 56.16 shows drug penetration from the stratum corneum to the fat layers. Tofacitinib in solution remained at the surface of the skin but was not distributed homogenously, whereas in a cream formulation, tofacitinib was distributed evenly on the skin surface and penetrated deeper in some areas. There was a significant contamination signal from punch biopsy (Figure 56.16, red circles), which needed to be subtracted from the total signal. Overall, MALDI-MSI technology can provide pharmacokinetic data on the skin. Biopsy preparation or sample handling contribute to higher variability of the data, which needs to be taken into consideration when the data are analyzed. Images were able to show the different penetration profiles between the two formulations. In skin, there is high spatial variation due to the individual skin layers, hair follicles, and sweat glands. Some limitations of MALDI-MSI are that the sample preparation can introduce artifacts, increasing the resolution decreases sensitivity, and the mass of the active needs to be sufficiently different from the matrix (n.b. OCT, commonly used to embed skin for cryosectioning, is problematic for MALDI-MSI and must be avoided). These need to be taken into consideration when developing experiments to detect drug levels at pharmacologically relevant concentrations.

Hendel et al. (2019) published a study where they quantified fractional laser-assisted delivery of a topical drug, bleomycin, by a liquid chromatography-mass spectrometry (LC-MS), and its skin distribution was visualized by MALDI-MSI [30]. Bleomycin is a hydrophilic molecule, and their hypothesis was that ablative fractional laser-assisted delivery could enhance topical drug delivery of large and hydrophilic molecules in skin. Thawed excised pig skin was treated with a fractional