the protein (purple) and lipid (red). We see that retinol has penetrated via the hair shaft into the sebaceous gland, which was one of three hypothesized penetration routes into the skin. In addition, they were able to visualize with SRS that drug penetration of the topically applied trans-retinol (Figure 56.10c) occurred along the hair shaft. This study has shown the possibility of using high-speed imaging SRS in in vivo settings. However, there was no quantification from the images in this study. Label-free optical imaging is likely to play an increasingly important role in drug penetration assessment in humans.

56.7 ELECTRON MICROSCOPY

Transmission and scanning electron microscopy can provide ultrastructural evidence on the penetration profile of electron-dense nanoparticles. Transmission electron microscopy (TEM) uses a beam of electrons that is transmitted through a specimen to form an image. The contrast comes from differences in electron density with the sample, e.g., gold is black due to absorbed electrons. The specimen is usually an ultrathin section less than 100 nm thick or a suspension on a grid. The most important difference between a transmission and scanning microscope is that a scanning electron microscopy (SEM) beam is focused to a fine point and scans line by line over the surface of the sample, whereas the TEM images are focused through a thin sample. TEM and SEM approaches in topical drug delivery research are generally focused on tracing electron-dense nanoparticles applied to skin. These technologies can be used to characterize nanoparticles made of gold, silver, iron oxide nanoparticles, and silica-based nanoparticles in skin tissue.

In 2015, a study done by Colombo et al. described the use of SEM to investigate the delivery of Fe_3O_4 nanoparticles in cream formulations to ex vivo human skin [22]. Freshly excised human skin was mounted in a Franz cell, and nanoparticle-containing cream was applied. The skin was collected at three time points (1 to 3 hours, 5 to 7 hours, and 24 hours) and then fixed in formalin solution for paraffin sectioning. The cross-sections of the treated skin were subsequently imaged by SEM (Figure 56.11). The skin architecture was viewed at high resolution. The nanoparticles were distinguished in all skin layers based on the differences between the electron density of the skin, which is relatively low, compared to the relatively high electron density of the nanoparticles. The images showed that a small number of nanoparticles penetrated into viable epidermis, and some of them reached the deeper dermis. There seemed to be no difference in enhancement of penetration between solution or cream formulations. This study shows that the SEM system can detect nanoparticles and trace them in fixed human skin sections. One of the limitations of this type of analysis is the limited field of view of the skin section due to the super high resolution.

The effects of preparation for sectioning on skin morphology have also been the subject of concern in the field. In 2014, a clinically relevant drug penetration study was done by Sjovall et al. revealing substantial cutting artifacts from the sample preparation [23]. Their SEM images and analysis from these images by time-of-flight secondary ion mass spectrometry (TOF-SIMS) showed asymmetric and uneven tissue distribution on either side of the central cartilage and morphological distortions caused by the drying of the tissue (Figure 56.12). SEM images can identify the architecture of the skin strata, and TOF-SIMS can analyze the presence of a variety of lipids in the mouse ear cross-sections, including phospholipids, cholesterol, fatty acids, and triglycerides. The antiinflammatory drug roflumilast was applied topically to mouse ear daily for one week. At the end of the treatment, the ear tissue was collected for frozen sections. The sections were imaged by SEM and analyzed by TOF-SIMS. The spatial distribution of roflumilast in the mouse ear cross-section are shown in SEM images (Figure 56.13) confirmed by TOF-SIMS. Roflumilast was homogenously distributed through the stratum corneum but did not penetrate any farther into the skin. TOF-SIMS could identify lipid and ceramide from the cell membrane and locate them accurately on the skin tissue section. A drug penetration profile can be confounded by the distortion of skin while being prepared for imaging unless the study is done in vivo. In addition, they pointed out that there was a strong signal from TOF-SIMS analysis, indicating there was a contamination possibly due to the