41.6.4 Histological Examination

Histological examination of MN-treated skin allows observations of MNs' ability to breach the stratum corneum barrier [66] and the depth of created channels [120]. However, these techniques are cumbersome; they involve taking a biopsy of the MN-treated skin followed by traditional histological sectioning of MN-treated skin and staining and then searching for skin sites where a MN has penetrated [115, 118, 123].

41.6.5 Scanning Electron Microscopy

Creation of pores in MN-treated skin can be confirmed using scanning electron microscopy (SEM) [74]. This technique has limitations, as it involves lengthy dehydration processes that can result in altered skin structure and dimensional distortions. Most importantly, this technique cannot be utilized in an *in vivo* scenario. Generally, in cases where the technique requires MNs to be removed prior to application of the dye/marker, the inherent elasticity of the skin could cause a partial retraction of the tissue and provide an unreliable indication of the microchannel dimensions.

41.6.6 Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (CLSM) is a well-established technique for obtaining high-resolution images from biological and other specimens [124]. It offers a noninvasive means for visualization of MN-created channels both *in vitro* and *in vivo* [125]. Furthermore, it allows development of depth penetration profiles of fluorescent permeants or microparticles by visualization of images parallel to the surface of the sample, at multiple depths, without the need for mechanical sectioning of the sample [126]. CLSM has been used to detect the dimensions of created microchannels by following the penetration depth profile of fluorescent microparticles through excised hairless rat skin samples treated with maltose or metallic MNs [66]. However, this method is severely limited by the fact that its maximum optical penetration depth is currently <0.25 mm into skin; it also relies on diffusion of a fluorescent dye [127]. As such, it is unable to provide information on MNs that may penetrate deeper than the epidermis or superficial dermis.

41.6.7 Optical Coherence Tomography

Optical coherence tomography (OCT) is a noninvasive optical imaging technique currently capable of penetrating to tissue depths of approximately 2 mm. Therefore, OCT is a useful optical method for cross-sectional imaging of the epidermis and upper dermis *in vivo* [127]. No prior sample preparation is needed, thus removing the need for addition of a dye or radiolabeled marker to the system. OCT has been successfully used to visualize, in real time, polymeric MNPs while inserted into human skin *in vivo* [65], thus allowing for measurement of the exact depth of MN penetration. OCT has been investigated for studying the effect of varying MN geometric parameters—such as needle length and density, as well as application force—on resultant penetration characteristics (depth of MN penetration, width of pore created in the skin) of dissolving polymeric MNs *in vitro* [61]. Importantly, OCT could be successfully applied to follow *in situ* dissolution of dissolving MNs in real time [61].

41.7 COMBINING MICRONEEDLES WITH OTHER SKIN PERMEATION-ENHANCING STRATEGIES

A range of other enhancing techniques such as iontophoresis, electroporation, and use of drug carriers in combination with MNs to enhance drug delivery through MN-induced micropores have been investigated.