encapsulated in PLGA nanoparticles in full-thickness porcine skin *in vitro*. They reported a 5.4-fold higher ($P < 0.005$) skin permeation of flux of encapsulated Rh B compared to the solution of free Rh B. Nanoparticles were preferentially deposited in the hair follicles (diameter of approximately 200 μm in porcine skin), which has already been found by other groups (Lademann et al., 2007; Wang et al., 2008; Zhang et al., 2010). The authors proposed that Rh B was released from the nanoparticles localized in the follicles and that it laterally diffused into the viable epidermis, which explained the enhanced transdermal drug delivery (as it was found in the receptor solution), while nanoparticles were not detected in the receptor solution. Skin *pretreatment with MNs* enhanced the transdermal flux of free Rh B 3.7-fold compared to free drug without MNs, and significantly more of Rh B loaded in nanoparticles, i.e. 13.76-fold compared to free drug without MNs (2.5-fold higher compared to nanoparticles without MNs), as nanoparticles were preferentially deposited in the microchannels formed by MNs, creating dye reservoirs, releasing Rh B which freely diffused into the skin layers below the SC. This resulted in accelerated drug permeation. However, no particles were detected in the receptor solution, which was in accordance with findings of Zhang et al. (2010). As to the epidermis and deeper skin layers, the highest drug deposition was also obtained by the combined use of nanoparticles and MNs. In conclusion, the combination of Rh B nanoencapsulation and skin treatment with MN produced the greatest apparent dye infiltration into deeper skin layers.

In the same study, use of *different needle lengths* showed that the insertion of 600-μm-long needles yielded a significantly higher permeation enhancement of encapsulated Rh B compared to the insertion of 400-μm-long MNs, as well as compared to the untreated skin. However, the further increase of needle length, i.e. use of 1000-μm-long needles did not lead to further significant permeation increase, which can be explained by the increased frictional resistance characteristic for the longest needles; different threshold needle lengths have been reported by different groups (Gomaa et al., 2012). Kumar et al. (2011) reported that the use of a MN roller with larger MNs (1000 μm long, base diameter 80 μm) allowed more extensive permeation of SLN with conjugated ovalbumin (OVA) than the pretreatment with a roller containing smaller MNs (200 μm long, base diameter 20 μm).

Regarding the *MN density*, the highest density was less effective than the lower densities, i.e. the 361 MNs/array provided a smaller steady-state flux of encapsulated Rh B (5.08 ± 0.34 μg/cm^2/hour, $P = 0.008$) than lower densities, such as the 121 MNs/array (6.19 ± 0.77 μg/cm^2/hour), while the cumulative amount of dye permeated (Q_{48} of 5.44 ± 0.16 mg/cm^2) was almost the same as with lower densities (121 MNs/array, Q_{48} of 5.40 ± 0.39 μg/cm^2)(Gomaa et al., 2012). The authors explained it by the "bed of nails" effect, where the pressure exerted by each needle tip can be decreased to a potentially insufficient level to penetrate as deeply into the skin as an array of lower density when the same force is spread over a very large number of needles (Yan et al., 2010; Gomaa et al., 2012).

As to the *number of array insertions* (one, five or nine insertions), the more insertions were applied the higher was the permeation of encapsulated Rh B, as more microchannels were created in the skin. Values obtained at one and nine insertions differed statistically significantly. The same group (Gomaa et al., 2010) showed in another study that this phenomenon may be due to the finding that when skin poration, i.e. number of pores, is high, pore re-closure may be suppressed to an extent since in that case the skin cannot effectively contract and reduce the pore size. Among different used *durations of MN insertion* (2 second, 3 minutes and 5 minutes), the highest permeation of Rh B was provided by the shortest insertion duration, indicating that the duration of MNs insertion in the skin should be short (Gomaa et al., 2012). This was in accordance with their previous study (Gomaa et al., 2010). The authors explained this by the possibility that accelerated elastic contractions caused by prolonged embedding of MNs in the skin result in partial closing of many microconduits formed by MNs, which inhibits the decrease of the skin barrier function (Gomaa et al. 2012).

64.1.2.3 Transcutaneous Immunization and Other Indications

Transcutaneous immunization (TCI) with needle-free formulations is a noninvasive approach which overcomes the drawbacks of the parenteral vaccination methods. The skin represents an