the ability of achieving TCI with protein antigens carried by nanoparticles that are applied onto skin pretreated with MNs (without this pretreatment there is no sufficient particle penetration into the skin) will be discussed (Bal et al., 2010, 2011; Kumar et al., 2011, 2012).

As to the choice whether to incorporate the drug inside the nanoparticles or to conjugate it onto the nanoparticle's surface when applying the nanoparticles onto the MNs-treated skin, Bal et al. (2010) reported that incorporation of protein antigens into nanoparticles does not lead to a stronger immune response compared to free antigens. The authors showed that diphtheria toxoid (DT) incorporated into N-trimethyl chitosan (TMC) nanoparticles and applied onto MN-treated skin did not induce a stronger antibody response than DT alone. However, when DT was conjugated with TMC nanoparticles and applied on MN-pretreated skin, it induced a stronger immune response than DT alone, i.e. the immunoglobulin G (IgG) titers after the second boost were eightfold higher compared to application of a solution of DT ($p<0.001$) and comparable to those elicited by subcutaneously applied DT-alum. This result indicated that conjugation of antigen with nanoparticles instead of incorporation of antigens inside nanoparticles was a better solution, which is in agreement with Kumar et al. (2011).

Bal et al. (2011) investigated the effects of formulations of OVA with TMC on the TCI conducted in the skin pretreated with MNs. They prepared three formulations of OVA: TMC + OVA mixtures, TMC–OVA conjugates and TMC/OVA nanoparticles.

After the prime vaccination, TMC–OVA conjugates induced significantly higher IgG titers than the other two formulations. Also after the boost, TMC–OVA conjugates proved to be significantly better than plain OVA, although the TMC/OVA nanoparticles also significantly elevated the IgG titers compared to plain OVA. A physical mixture of TMC + OVA elicited IgG levels that were not significantly higher than plain OVA. In conclusion, results revealed that after transcutaneous administration, TMC–OVA conjugates were most immunogenic, probably because they penetrated through the skin more easily than nanoparticles and consequently were better delivered to dendritic cells (DCs), while they show higher uptake by DCs than TMC + OVA mixtures (Bal et al., 2011).

Kumar et al. (2011) reported that the pretreatment of mice skin with MNs enabled the permeation of SLN, 230 nm in diameter, with OVA conjugated on their surface (not incorporated in SLN) through the skin. Further, this TCI induced a significantly stronger anti-OVA antibody response than OVA alone after MN pretreatment. Without the MN pretreatment, neither only protein in solution nor protein conjugated onto nanoparticles was able to induce an immune response. Upon the use of MNs, even of smallest size, permeation of both OVA alone and OVA-nanoparticles was achieved, and it increased with increasing needle size and the highest permeation and IgG response was induced after the pretreatment with largest needles (1000 μm long, base diameter 80 μm) compared to medium and small needles. The IgG response was significantly higher when OVA-nanoparticles were applied, compared to OVA alone, indicating that incorporating a protein antigen into nanoparticles can enhance its immunogenicity. As to permeation, a minimum amount of OVA-nanoparticles permeated through the skin pretreated with small MN (200 μm long, base diameter 20 μm), whereas 13.6 ± 2.4% of the OVA-nanoparticles permeated through the skin treated with the largest MN. Pretreatment with largest MN induced a significantly higher permeation of OVA in solution (28.3± 6.5%) compared to the permeation of OVA-nanoparticles, which was explained by the larger size of OVA-nanoparticles compared to OVA molecules. In addition, regarding subcutaneous injection of OVA-nanoparticles, the antigen dose determined whether MN treatment followed by application of OVA-nanoparticles was more effective than the subcutaneous injection of OVA-nanoparticles. The finding that OVA-SLN (230 nm) permeated through the skin and were detected in the receiver solution was in agreement with findings of Coulman et al. (2009), but was in disagreement with the reports by Zhang et al. (2010) and Bal et al. (2010) who used PLGA nanoparticles (166, 206, or 288 nm) and DT-N-TMC nanoparticles (211 ± 4 nm), respectively.

Kumar et al. (2012) showed that MN-mediated TCI with plasmid DNA coated on the surface of cationic (PLGA:DOTAP [1,2-dioleoyl-3-trimethylammonium-propane]) nanoparticles induced a stronger immune response than the plasmid DNA alone. Further, the study showed that the surface