mice, but only when the skin was pretreated with MNs. Co-administration of cholera toxin further augmented the immune responses. The highest transcutaneous immunization was achieved by the use of SPC-HBsAg vesicles, being superior to L595-HBsAg vesicles due to the higher degree of HBsAg association, adjuvanted with cholera toxin, on MN-pretreated skin. This transcutaneous immunization achieved in mice was comparable to immunization by conventional intramuscular (i.m.) vaccination.

DeMuth et al. (2012) coated poly(lactide-co-glycolide) MN arrays with multilayer films via layer-by-layer (LbL) assembly of a biodegradable cationic poly(β-amino ester) (PBAE) and negatively charged interbilayer-cross-linked multilamellar lipid vesicles (ICMVs) as a potential device for vaccine delivery. This approach enhances the stability of lipid vesicles, as covalent crosslinks are introduced between adjacent phospholipid bilayers in the walls of multilamellar vesicles to create a robust lipid nanocapsule. Without such stabilizing measures, LbL deposition would result in spontaneous vesicle disruption into lipid bilayers on the target substrate. Obtained ICMVs were loaded with a protein antigen and the molecular adjuvant monophosphoryl lipid A. The study revealed that MNs with ICMV-carrying multilayers promoted in mice robust antigen-specific humoral immune responses, while bolus delivery of soluble or vesicle-loaded antigen via intradermal injection or transcutaneous vaccination with MNs encapsulating soluble protein elicited a weak humoral immune response, indicating the potential of nanocarriers delivered by MNs as a promising approach for noninvasive vaccine delivery applications (DeMuth et al., 2012).

Guo et al. (2013) used dissolving microneedle arrays (DMAs) for achieving transcutaneous immunization polyvinylpyrrolidone (PVP), where the tips were loaded with an antigen and adjuvant encapsulated in cationic phospholipid liposomes. Ovalbumin (OVA) was used as a model antigen, CpG oligodeoxynucleotide (CpG OND) as an adjuvant, and cationic liposomes to deliver the antigen and adjuvant. It was shown that mice were indeed transcutaneously immunized with DMAs containing OVA, OVA-CpG OND, OVA encapsulated in liposomes, OVA-CpG OND encapsulated in liposomes, and conventional i.m. injection with OVA solution. The anti-OVA IgG antibody level was highest in the group immunized with the DMAs containing OVA-CpG OND encapsulated in liposomes. Thus, the described device could effectively deliver the liposomes encapsulating CpG OND-OVA into the skin, enhancing the immune response and changing the immune type.

Chen et al. (2015) investigated in a rat model of rheumatoid arthritis a triptolide-loaded transdermal delivery system, termed the triptolide-loaded liposome hydrogel patch (TP-LHP), applying it together with a microneedle array. The pharmacokinetic results showed that MNs together with TP-LHP yielded plasma drug levels which fit a one-compartment open model. Further, TP-LHP treatment mitigated the degree of joint swelling and suppressed the expressions of fetal liver kinase-1, fetal liver tyrosine kinase-4, and hypoxia-inducible factor-1α in synovium. Hyperfunction of the immune system was also observed. The authors proposed that the therapeutic mechanism of TP-LHP might rely on the regulation of the balance between Th1 and Th2, as well as inhibition of the expression and biological effects of vascular endothelial growth factor (Chen et al., 2015).

Qiu et al. (2016) developed dissolving microneedle arrays (DMAs) loaded with cationic phospholipid liposomes encapsulating hepatitis B DNA vaccine and adjuvant CpG ODN and applied them *in vivo* in mice to investigate their capability to induce an immune response after DNA delivery. The results showed that pGFP could be delivered into skin by DMAs and expressed in skin, and the amount of expressed GFP was likely to peak at day 4. DMAs-based DNA vaccination could induce an effective immune response, while CpG ODN significantly improved the immune response and achieved the shift of immune type from predominate Th2 type to a balance Th1/Th2 type, while cationic liposomes further improved the immunogenicity of the DNA vaccine. In conclusion, the authors showed that this novel system based on microneedles and liposomes can effectively deliver hepatitis B DNA vaccine into skin, inducing an effective immune response and changing the immune type by adjuvant CpG ODN.

Zhao et al. (2017) combined the model subunit vaccine ovalbumin (OVA) with platycodin (PD), a saponin adjuvant, in order to enhance its immunogenicity and loaded them together into liposomes. Afterwards liposomes were incorporated into a dissolving microneedle array. The authors showed