

and the levels were maintained for up to 24 hours. Hence, the authors developed an efficient, noninvasive, and persistent transfollicular (as the transfollicular route was the main penetration route for liposomes) delivery system for insulin that used a combination of liposomes and iontophoresis.

In addition to dual penetration enhancement strategies, triple enhancement strategies can be used, i.e., nanocarriers can be used with two physical enhancement methods, and this approach was used to enhance the transdermal delivery of insulin. Chen et al. (2009b) showed *in vitro* in guinea pig skin that positively charged unilamellar insulin-loaded liposomes driven by iontophoresis through MNs-treated skin provided permeation rates of insulin 713.3 times higher than that during its passive diffusion. The positive surface charge and small diameters of liposomes were advantageous to the penetration of the insulin-loaded liposomes when combined with iontophoresis and MNs. *In vivo* studies in diabetic rats showed that the blood glucose levels in rats topically treated with insulin, as mentioned, were 33.3% and 28.3% of the initial levels at four and six hours after the application of insulin, which is comparable to those induced by subcutaneous injection of insulin. These results were encouraging, as they introduced a new, very efficient strategy for noninvasive delivery of peptides of large molecular weight using liposomes, which have been to date efficiently used mostly for intradermal delivery of drugs with low molecular weight.

By using the combination of DOTAP-EPC-Chol (2: 2: 1 molar ratio) cationic liposomes and iontophoresis, superoxide dismutase (SOD), representing a potent antioxidant agent protecting against UV-induced skin damage, can be delivered into the skin, besides its high molecular weight which normally prevents its efficient delivery into the skin (Kigasawa et al., 2012). Iontophoretic delivery of liposomes encapsulating SOD caused in rats *in vivo* a marked decrease in the production of oxidative products, such as malondialdehyde, hexanoyl lysine, and 8-hydroxi-2-deoxyguanosine, in UV-irradiated skin. Hence, SOD encapsulated in cationic liposomes and subjected to anodal iontophoresis represents an efficient intradermal SOD delivery approach, which could also be useful for delivering other macromolecules.

Mohammed et al. (2016) have investigated the transdermal delivery of vancomycin hydrochloride using the combination of ethosomes and iontophoresis *in vitro* and *in vivo*. They used cathodal iontophoresis for negatively charged ethosomes and anodal iontophoresis for the free drug solution and positively charged vesicles. The maximal transdermal flux was obtained with cathodal iontophoresis of negatively charged ethosomes (550 $\mu\text{g}/\text{cm}^2/\text{h}$) compared to the free drug solution and other ethosomes. The parameters of iontophoresis were varied, and the transdermal flux was reduced by altering the current mode from continuous to ON/OFF mode, reducing current density and by using normal saline as drug solvent, while the flux was increased by increasing the drug concentration. The performed *in vivo* study in rats revealed that there was no significant difference between the i.m. application of vancomycin and the iontophoretic delivery of vancomycin-loaded ethosomes, indicating a successful transdermal delivery of vancomycin by ethosomes and iontophoresis.

Bernardi et al. (2016) investigated the potential of employing iontophoresis for transcutaneous immunization using a formulation composed of OVA-loaded liposomes and silver nanoparticles (AgNPs). The *in vitro* cathodal iontophoresis of the OVA-liposomes associated with AgNPs increased OVA penetration into the viable epidermis by ninety-two-fold in comparison to passive delivery. *In vivo*, transcutaneous immunization with a suitable combination of liposomes and iontophoresis induced the production of antibodies, differentiation of immune-competent cells, and appeared to present an alternative strategy for needle-free vaccination.

Jose et al. (2017) investigated the liposomal delivery of curcumin and STAT3 siRNA when combined with iontophoresis in order to treat skin cancer. Curcumin was encapsulated in cationic liposomes and then complexed with STAT3 siRNA. Results showed that the co-delivery of curcumin and STAT3 siRNA using liposomes resulted in significantly greater ($P < 0.05$) cancer cell growth inhibition and apoptosis compared with neat curcumin and free STAT3 siRNA treatment. Penetration data obtained in excised porcine skin showed that iontophoresis enhanced skin penetration of the nanocomplex penetrating the viable epidermis (Jose et al., 2017). Hence, the authors concluded that cationic liposomes can be used with iontophoresis to deliver curcumin and siRNA to the skin to treat skin cancer.