A recent study *in vivo* in ovariectomized rats revealed that propranolol encapsulated at low doses in liposomes applied together with iontophoresis onto the skin enhanced the bone microarchitecture volumes and hence exhibited optimal effects against bone loss to a higher extent compared to propranolol used at higher doses in liposomes and applied by s.c. injection (Teong et al., 2017). Namely, liposomes with propranolol at low doses (0.05 mg/kg) elevated via iontophoresis over twofold the ratio between bone volume and total tissue volume (BV/TV) in proximal tibia to 9.0%, whereas treatment with liposomes containing propranolol at low and high (0.5 mg/kg) doses via s.c. injection resulted in smaller increases in BV/TV. The authors also reported a significant improvement of BV/TV and bone mineral density (BMD) in the fourth lumbar spine when low-dose liposomal propranolol was iontophoretically administered. In addition, iontophoretic low-dose liposomal propranolol also elevated trabecular numbers in tibia and trabecular thickness in spine (Teong et al., 2017).

38.2.5.1 Combined Use of Liposomes and Ultrasound

Liposomes may be used together with ultrasound. Percutaneous penetration enhancement induced by ultrasound is termed sonophoresis, indicating that the enhanced transport of molecules is under the influence of ultrasound. Various frequencies of ultrasound (in the range of 20 kHz to 16 MHz) have been used to enhance skin permeability. However, transdermal drug delivery induced by low-frequency ultrasound (f <100 kHz) has been found to be more efficient than that induced by high-frequency ultrasound. This method has been used to enhance transdermal transport of various drugs, including macromolecules (Mitragotri, 2017). For more information on ultrasound, the reader should refer to Escobar-Chávez et al. (2009), Lee et al. (2017), and Mitragotri (2017).

Elastic liposomes containing hydrogenated phosphatidylcholine and cholesterol as well as Tween 80 were used together with low-frequency ultrasound in order to enhance the transepidermal delivery of the hydrophilic and high-molecular-weight hyaluronic acid (Kasetvatin et al., 2015). The *in vitro* permeation studies demonstrated that hyaluronic acid in solution cannot permeate through the porcine epidermis. However, when elastic liposomes were applied with ultrasound, the skin permeation of hyaluronic acid was higher than those obtained with passive delivery of elastic liposomes and ultrasound-mediated delivery from the hyaluronic solution, 2.1 times and 6.4 times, respectively. In addition, no skin damage was observed at the optimized one-minute exposure time. Thus, the study demonstrated *in vitro* that the combination of elastic liposomes and ultrasound provided an efficacious transcutaneous delivery of hyaluronic acid.

Limonene-containing PEGylated liposomes (PL) and low-frequency sonophoresis were used to achieve transdermal delivery of galantamine HBr (GLT). It was shown that sonophoresis might improve drug permeation through the intracellular pathway, while limonene-containing liposomes play an important role in delivering GLT through an intercellular pathway by increasing the fluidity of intercellular lipids in the SC (Rangsimawong et al., 2018). As the authors showed that liposomes with the highest limonene content (2%) were superior to other liposomes, they concluded that small vesicle size and high membrane fluidity due to high limonene content might enhance the transportation of intact vesicles through the skin.

38.2.5.2 Combined Use of Liposomes and Electroporation

Electroporation is a physical technique that involves the application of very short duration (microsecond to millisecond) high-voltage electric pulses to reversibly enhance cell or tissue permeability for bioactive molecules such as drugs, dyes, vitamins, peptides, proteins, DNA, RNA, etc. (Medi et al., 2017). In comparison to other percutaneous penetration enhancement methods, it may not show significant difference in the penetration enhancement of small ions/molecules; however, compared to them it induces significantly higher fluxes of macromolecules. The major advantage of this technique is that the macromolecules, such as peptide and gene-based drugs, could be used for transdermal delivery, while for more detail on electroporation, the reader should refer to Angamuthu and Murthy (2017) and Medi et al. (2017).

Essa et al. (2003) investigated the influence of electroporation on the skin delivery of estradiol from ultra-deformable liposomes containing sodium cholate as an edge activator. They found that