48.5 TISSUE CULTURE–DERIVED SKIN EQUIVALENTS

Tissue culture–derived skin equivalents have become increasingly important as an alternative to animal and human skin for the testing of percutaneous absorption, phototoxicity, corrosivity, and irritancy of dermal and cosmetic formulations. The equivalences are composed of several layers of human cells in a culture spread over a polymeric matrix. Such a design allows incorporation of the various cell types to form a structure of targeted composition and complexity (Abd et al., 2016; Capallere et al., 2018; Godin and Touitou, 2007). These reconstructed skin equivalents are intended to mimic the epidermis (reconstructed human skin epidermis models) or full human skin (living skin equivalents). Reconstructed human skin epidermis models are commercially available as SkinEthic and EpiDerm®, while EpiSkin®, GraftSkin®, EpiDermFT®, and Pheninon® represent living skin equivalents (Abd et al., 2016; Netzlaff et al., 2005; Van Gele et al., 2011).

Numerous studies have compared the efficacy of the tissue culture–derived skin models with *ex vivo* human and animal skin models. Overall, the reconstructed epidermis equivalents have been proven to be more permeable than *ex vivo* human skin. However, they are more consistent in permeability and responsiveness than human skin, which is highly variable. The relatively weak barrier function of the tissue-cultured models is considered to be their major limitation and is a consequence of the impaired desquamation and the presence of unkeratinized microscopic foci (Netzlaff et al., 2005). Therefore, these models are not recommended for studying the permeation of lipophilic drugs. For instance, the permeation of terbinafine and clotrimazole (hydrophobic drugs) through the GraftSkin® and SkinEthic® was more than 800-fold higher than through the human skin, while the flux of salicylic acid (hydrophilic drug) was similar to that obtained with the human skin (Schmook et al., 2001). The increased permeability of the tissue-cultured models was also demonstrated by Schäfer-Korting et al. (2008). In a study by Zghoul et al. (2001), a five times higher flux for flufenamic acid (hydrophobic drug) was found with the EpiDerm® in comparison to *ex vivo* human skin. However, the model was shown able to distinguish the drug permeation profiles from the different topical formulations, i.e., ointment and solution. Similarly, EpiDerm® was found to be suitable for evaluation of liposomes differing in size and bilayer elasticity (Babu et al., 2009). Comparison of EpiSkin® with the PVPA artificial skin model in the evaluation of topical nanoformulations demonstrated that the reconstructed human epidermis was able to detect only minor differences between the examined liposomal formulations encapsulating drugs of different molecular weights and lipophilicity in comparison to the PVPA model (Engesland et al., 2015).

Currently, the use of reconstructed skin models is approved by guidelines of Organisation for Economic Co-operation and Development (OECD) for skin corrosion, acute skin irritation, and phototoxicity testing (Küchler et al., 2013; Lin et al., 2019). However, none of these models is yet approved for skin absorption testing. Moreover, additional work is required to validate the various models, especially the living skin equivalents, although they may be useful for *in vitro* screening (Abd et al., 2016). The relatively high cost and data reproducibility also limit their use in assessment of dermal formulations (Flaten et al., 2015).

Attempts have been made to utilize skin-on-chip devices as a state-of-the-art platform to study skin penetration. The organotypic cultures are cultivated in the chips to mimic human skin, and by measuring the transepithelial electrical resistance, information on the diffusion properties of tested substances is gained (Lukács et al., 2019; Mohammadi et al., 2016).

48.6 *EX VIVO* ANIMAL SKIN MODELS

Most of the permeability studies are performed using a static Franz diffusion cell method. It consists of donor and receptor chambers between which the animal model membrane is positioned so that the SC is facing the donor compartment where the examined formulation is applied, while the dermis (full-thickness skin) is touching the receptor compartment. For determining the skin