vesicles destined for photodynamic therapy of cutaneous diseases: invasomes (Dragicevic-Curic et al., 2009a), flexosomes (Dragicevic-Curic et al., 2010), and liposomes-in-gel formulations (Dragicevic-Curic et al., 2009b).

48.8 SKIN PERFUSION MODELS

Although they can be considered *ex vivo* human skin models due to their specific features, we have categorized this separately. To more closely mimic the conditions within the living skin, skin perfusion models have been proposed as the closest to *in vivo* animal or human studies. Skin perfusion models are composed of a surgically prepared portion of skin panni, also called a flap, involving the active circulation of the dermis layer, skin metabolism, and the presence of subcutaneous fatty tissue (Patel et al., 2016). These models offer the advantage of perfusion with a tissue–culture medium by cannulization of one of the vessels in the skin panni. To confirm and monitor flap perfusion during skin penetration experiments, dermofluorimetry is the most used technique (Black et al., 2001). Miland and colleagues (2008) proposed a less invasive technique, dynamic infrared thermography (DIRT), which was also used to differentiate between well-perfused and less perfused areas (Miland et al., 2008). Additionally, methods used in *in vivo/ex vivo* investigations, e.g., mass balance, surface washings, tape stripping, are applicable to be utilized in the skin perfused model (Schaefer et al., 2008). Several animal specimens have been used to obtain skin perfusion models, such as pig, mouse, and rat. The first studies were performed with a perfused ear model, which showed high permeability and was later used only as a tool to predict penetration through premature neonate skin (Schaefer et al., 2008). The perfused cow udder (Kietzmann et al., 1993), pig forelimb (Wagner et al., 2003), and isolated perfused pig skin flap (obtained from pig abdomen) (Riviere et al., 1986) were also used as perfused models. The perfused bovine udder skin (BUS) model comprises the isolated udders continuously perfused via the left and right external pudendal arteries with an oxygenated nutrient solution (Pittermann et al., 2013). Comparison with *ex vivo* human and porcine skin has demonstrated that bovine udder skin is well-correlated, but also a less variable barrier against caffeine, benzoic acid, testosterone, and flufenamic acid penetration (Netzlaff et al., 2006b). This model enables the comparison of the dermal penetration, metabolism, and absorption of the substances after topical administration. A good correlation with *in vivo* studies has been obtained when testing dermal absorption of organophosphates, steroids, benzoic acid, and caffeine on the isolated perfused pig skin flap (Carver et al., 1989). Wester and collaborators (1998) also found similar dermal absorption of other compounds between the pig skin perfusion model and *in vivo* studies on humans. However, the limitations of using animal skin also applies for skin perfusion models.

The isolated perfused human skin flap (IPHSF) has been used to study (trans)dermal penetration (Ternullo et al., 2017a,b). The model was used to directly compare the skin penetration enhancement potential of three lipid-based nanocarriers (CLs, DLs, and SLNs) incorporating either calcein or rhodamine, markers of different lipophilicities. The confocal laser scanner microscopy (CLSM) technique was used to follow the penetration profiles. The penetration profiles were directly compared to established skin penetration models such as cellophane membrane and full-thickness pig/human skin in the Franz diffusion cell (Ternullo et al., 2017b). The model was proven to be a valuable tool in skin penetration studies, as well as in optimization of dosage forms/delivery systems for skin therapy.

With the development of microfluidics and organ-on-a-chip concept, skin equivalents emerged as a novel type of perfused skin model. They are not yet widely studied due to the limited access to technology; however, Mori et al. (2017) confirmed the feasibility of a skin equivalent containing vascular channels (perfusion conditions) as a model for studying vascular absorption. The results suggested that this skin equivalent can be used for skin-on-a-chip applications, including drug development, cosmetics testing, and studying skin biology.

In summary, the advantages and limitations of the most commonly used *in vitro/ex vivo* models are provided in Table 48.1.