In general, phospholipids are assumed to be among the most eudermic options in formulation development even for sensitive target areas such as mucosae or skin. *In vivo* application studies have nonetheless indicated certain negative effects on the state of the skin barrier during regular use, at least for lysophosphatidylcholine mixtures [6]. Although these effects were reversible, our aim was to evaluate the biocompatibility of different commercially available phospholipid mixtures in aqueous and multiphase systems. To this end, *in vitro* 2D cell culture methods were established in our lab. Despite modern alternatives to these classic techniques, they have their rightful spot to evaluate effects of individual formulation compounds or entire formulations on human tissues. An obvious advantage is a more time- and cost-efficient workflow when compared to the more complex 3D culture models [7, 8].

Evaluation of biocompatibility using cell culture systems is a vital step when developing new formulations. A wide variety of established cell lines are available for research purposes. A common example are different murine cell lines, which are easily available and very robust; they can be passaged without limitation [9, 10]. These advantages render them useful for standard biocompatibility tests in preliminary studies. However, results obtained with immortalized cell lines may not reflect the actual *in vivo* situation. It is the general opinion that cytotoxicity tests *in vitro* are more convincing when performed with cells homologous to human tissue. Thus, appropriate cell lines for cytotoxicity and tolerance tests concerning human skin are human dermal fibroblasts and human epidermal keratinocytes [11, 12]. The present chapter summarizes our work and recent findings in context with the use of human primary skin cells—epidermal keratinocytes and dermal fibroblasts—for evaluation of the cytotoxic potential of formulation compounds. In particular, the effect of surfactants on skin was evaluated and set into context with recent skin penetration and permeation studies using these surfactants.

49.3 METHODOLOGICAL ASPECTS AND OUTCOMES

For our experiments, the following approach was taken, which is discussed in the next sections.

49.3.1 Isolation of Primary Skin Cells

For the isolation of primary keratinocytes and fibroblasts, skin of healthy female and male donors aged between 20 and 65 years is used as obtained during plastic surgery. Approval of the internal ethics committee of the involved medical facility is required; in our case, this is accounted for by the Medical University of Vienna.

Skin used for the isolation of cells stems from abdomen, back, or breast of surplus skin. There is also the possibility to isolate keratinocytes from neonatal foreskin; however, these cells are more prone to expression of psoriasis-associated markers, which might cause problems, especially in organotypic cultures [13]. Furthermore, neonatal skin cells exhibit significantly faster healing rates in experimentally induced wounds *in vitro* when compared with skin cells derived from adult skin [14]. Thus, the use of skin cells derived from adult skin is recommended for all regular experiments.

Superficial skin strips from adult skin are incubated in dispase II in phosphate buffered saline (PBS) overnight at 4°C. The epidermis is separated mechanically and then digested with trypsin and DNase I for 30 minutes at 37°C. Keratinocytes are cultured in a serum-free keratinocyte growth medium (KGM-2); for details see [15, 16].

As for the isolation of fibroblasts, dermal specimens are incubated in a fibroblast isolation enzyme working solution diluted in distilled water for approximately 4 hours while shaking at 37°C. Afterwards, the suspension is filtered and washed with PBS, then filtered again. The cell suspension is spun down, and cells are washed with Dulbecco's Modified Eagle's Medium (DMEM), then spun down again. The supernatant is removed, and the cells are resuspended in medium (DMEM supplemented with 10% fetal bovine serum [FBS] and 1%