Regarding formulation properties, it is evident that only formulations with low viscosity that can be rinsed off easily are suitable for the envisioned experiments. Formulations with high viscosity such as thick oil-rich emulsion systems would exhibit increased stickiness to the cell layer; trying to wash them off with medium would be futile, and the growth area could be damaged in the attempt.

In regard to experimental parameters of the described cell culture experiments, human primary skin cells are generally more sensitive to external influences than cells derived from immortalized cell lines. Immortalized cell lines such as HaCaT keratinocyte cell lines or mouse fibroblast cell lines 3T3 or L929 are more robust in nature; they are a useful model to answer basic research questions [9]. However, more meaningful results are obtained by using primary cells homologous to human tissue for final studies. Cell density should be closely monitored, and experiment durations should be confined to a few hours.

49.5 COMPARISON TO OTHER METHODS

During the course of our studies, other techniques were deployed to assess the effect of different phospholipid-based nanoemulsions on skin barrier function. Thus, results of the *in vitro* cell culture experiments were confirmed in regard to their general implications.

Diffusion cell studies with porcine skin as a model membrane and a hydrophilic model drug were performed to compare nanoemulsions with different kinds of surfactants [16]. Results showed that formulations based on anionic surfactants were able to deliver the drug more efficiently through the model than skin formulations containing the amphiphilic lecithins or nonionic surfactants [16]. These findings are in line with the cytotoxicity results, as anionic surfactants exerted a stronger effect on skin cells *in vitro* and can thus be expected to also have a stronger impact on skin penetration of co-applied substances.

Furthermore, we compared the skin penetration of a lecithin mixture and the anionic SDS as basic aqueous formulations (liposomal/aqueous dispersions) [17]. *Ex vivo* skin penetration studies were conducted using attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy in combination with tape stripping [25–27]. It was of interest to compare the penetration behavior of the two surfactants to obtain an idea of their skin irritation potential, since the latter is most likely linked to the spatial distribution of the surfactant in the stratum corneum [28]. The experiments showed a rapid decrease of the relative lecithin concentration with increasing skin depth, with traceable amounts only until about 15% of stratum corneum depth. In contrast, the more aggressive SDS penetrated into the stratum corneum in larger relative amounts and reached deeper stratum corneum layers [17]. This is in line with previous findings [29, 30]. Regarding the penetration of other formulation compounds such as cosmetic oils, these were only detectable in the outermost cell layers of the stratum corneum. The observed penetration behavior of the surfactants lecithin and SDS corresponds well with the diffusion cell and cell culture data. In summary, it can be concluded that SDS affects skin barrier function and the individual epidermal and dermal cells to a much stronger extent than lecithin-based surfactants. To investigate effects on a molecular level, analysis of human skin cells is currently being performed via atomic force microscopy. Thus, changes in cell morphology caused by the surfactants can be observed directly [31, 32].

49.6 CONCLUSION

The effect of phospholipid-based nanoemulsions on cell viability *in vitro* was investigated using human primary keratinocytes and fibroblasts. Cell viability was confirmed to be high for all investigated nanoemulsions stabilized by lecithin mixtures with both EZ4U and BrdU assays. In contrast, survival rates around 0% were observed for control formulations based on sodium dodecyl sulfate. Ongoing scratch assays using human primary fibroblasts aim to explore the potential of phospholipid-based nanoemulsions with and without additives for wound healing purposes.