49.3.3 Cytotoxicity Assays: Effect of Nanoemulsions on Skin Cell Viability

The goal of our current studies is to investigate the skin compatibility of individual additives or to evaluate new dermal formulations. For aqueous formulations with low viscosity, such as phospholipid-based submicron emulsions, or *nanoemulsions*, evaluation in cell viability assays is possible. To this end, we deployed two established cell viability assays: the EZ4U assay and BrdU assay.

The combination of these two cytotoxicity assays delivers important information about cell viability, but also about the cells' ability to proliferate. This is of particular interest when targeting wound healing properties.

The Biomedica EZ4U cell proliferation kit assay is a modification of the MTT test based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The assay scans for mitochondrial damage in cells and therefore correlates directly with cell viability [20].

The BrdU assay is a tool for the quantitative measurement of DNA replication. It measures the amount of the pyrimidine analogon BrdU being incorporated into DNA instead of thymidine [21]. Cellular incorporation of BrdU can be detected by anti-BrdU–specific antibodies following membrane permeabilization via enzyme-linked immunosorbent assay (ELISA).

For both assays, primary cells are suspended in a colorless culture medium at 10^4 cells per well for fibroblasts and 1.5×10^4 cells per well for keratinocytes. Then the cells are seeded on 96-well flat-bottom plates. After incubation for 24 hours at 37°C in a 5% CO_2 humidified environment, the formulation of interest is added to the culture at the desired concentration, e.g., 1 + 1 with sterile saline solution. After a specified incubation time, the formulation is removed from the culture plates. Plates are washed at least three times with colorless culture medium before adding the EZ4U substrate or BrdU labeling solution, respectively. Colorimetric staining of the cells and the supernatant are evaluated, e.g., with a multiwell plate reader at 450 nm in case of the EZ4U assay and at 370 nm in case of the BrdU assay, to quantify the respective reaction products. For more detailed information, see [16, 17].

Low viscosity of the formulation of interest is vital for the success of cell viability assays. The investigated submicron-sized emulsions were of the oil-in-water type, containing 10% w/w oil and 5% w/w of surfactant. Formulations containing phospholipid-type surfactants were evaluated in parallel to formulations stabilized by sodium dodecyl sulfate (SDS). For the oil phase, common cosmetic oils such as jojoba oil, medium-chain triglycerides, and sunflower oil were used.

Different incubation times were tested for comparative purposes. Since prolonged experiment times were found to be less informative due to reinstigated cell proliferation, shorter incubation times of a few hours only are recommended. The results of the cytotoxicity assays using an incubation time of two hours are given in Figure 49.2. Cell viability of both keratinocytes and fibroblasts was generally quite high after treatment with phospholipid-based nanoemulsions, especially when compared with the corresponding SDS-based formulations. The latter generally exhibited high cytotoxicity, basically leading to survival rates around 0%. It can be summarized that cell viability as observed with both the EZ4U and BrdU assay was significantly higher for the three tested lecithin-based nanoemulsions than for their counterparts containing SDS as surfactant ($p < 0.05$ in all cases).

In case of primary keratinocytes (Figure 49.2a), cell viability rates were comparable for all three lecithin-based nanoemulsions. Cell viability was observed to be generally high, with values over 80% in the case of the EZ4U assay and over 60% in the BrdU assay. With the EZ4U assay, the observed cell viability was generally higher than with the BrdU assay, but not to a statistically significant extent. In conclusion, results of the two different cytotoxicity assays were in good agreement for the keratinocyte cultures.

In the case of primary fibroblasts (Figure 49.2b), more differences between individual lecithin-based formulations were observed. The highest cell viability was observed for S75-mct (98% with EZ4U, 96% with BrdU), followed by S75-jojo (90% with EZ4U, 66% with BrdU) and S75-sun