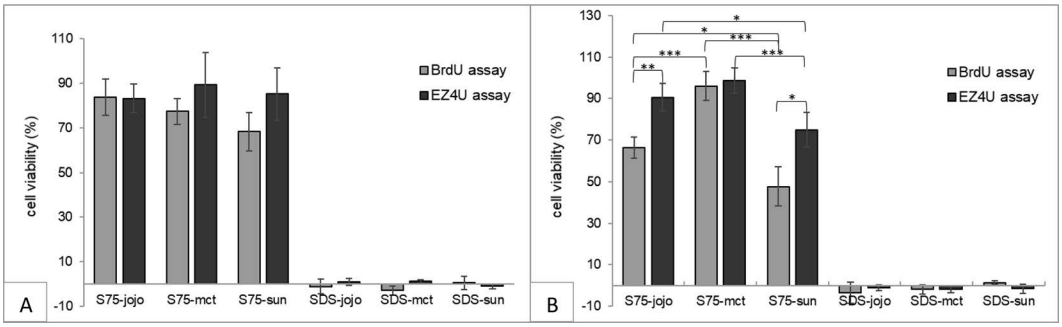

FIGURE 49.2 Cell viability of human epidermal keratinocytes (A) and dermal fibroblasts (B) after 2 hours of incubation with nanoemulsions. Data are expressed as % viability ± SD, $n = 4$ with 12 parallel experiments per formulation. Gray bars represent the results obtained by BrdU assays, black bars those obtained by EZ4U assays. S75: Lipoid S75 (soybean phospholipids with phosphatidylcholine content of 70% w/w); SDS: sodium dodecyl sulfate; jojo: jojoba oil; mct: medium-chain triglycerides; sun: sunflower oil. Nanoemulsions consisted of 5% w/w of surfactant (S75 or SDS), 10% w/w of oil component, and water. (Image reprinted from [17] with permission of Elsevier.)

(75% with EZ4U, 48% with BrdU). The results of the EZ4U assay indicate significantly lower fibroblast viability rates after treatment with S75-sun when compared to the corresponding formulations S75-jojo and especially S75-mct ($p \leq 0.05$ and $p \leq 0.001$). The results of the BrdU assay confirm this observation; significantly lower fibroblast viability was observed for S75-sun, with even larger differences to S75-jojo and especially S75-mct ($p \leq 0.001$). Thus, good agreement of the results obtained with the two different assays was confirmed for the fibroblast cultures despite differences in absolute values (tendency to higher absolute values in cell viability for EZ4U, which reach statistical significance in the case of S75-jojo and S75-sun).

49.3.4 Investigation of Wound Healing Using Scratch Assays

Taking these results one step further, Norway spruce resin and betulin were incorporated into phospholipid-based nanoemulsions to investigate their potential for wound healing applications. Since the described phospholipid-based formulations exhibited good compatibility with both epidermal and dermal human cells, they are promising candidates for stabilization of formulations to be used on sensitive or damaged skin. Formulations containing the previously mentioned wound healing agents are currently under investigation. Resin from Norway spruce (*Picea abies*) is known to have wound healing properties; ointments prepared from Norway spruce resin have been used in Scandinavia for centuries to treat acutely and chronically infected wounds [22]. Extract from birch bark (*Betula pendula*) has a long-lasting history as a traditional remedy to accelerate wound healing. Betulin is the main compound of these extracts and has been shown to accelerate wound healing *ex vivo* and *in vivo* [23]. At the moment, these formulations are being tested in cell viability assays and additional scratch assays *in vitro*. The latter is a simple, cost-efficient, and well-developed method to measure cell migration. After creating a standardized scratch in a cell monolayer, images are recorded at defined time intervals to observe and quantify cell migration during this simulated wound healing process [24]. Formulations can be applied after the scratch to analyze their effects on wound healing. Preliminary studies with nanoemulsions containing Norway spruce resin delivered promising results (Figure 49.3). After induction of an artificial injury to a confluent fibroblast monolayer and treatment with the test formulations, cell migration into the wound region was observed in regular intervals over 20 hours. First results indicated enhanced cell migration in the case of formulations with the test compound.