TABLE 49.1
Final Supplement Concentrations after Addition to the Medium

Bovine pituitary extract	0.004 mL/mL
Epidermal growth factor (recombinant human)	0.125 ng/mL
Insulin (recombinant human)	5.000 µg/mL
Hydrocortisone	0.330 µg/mL
Epinephrine	0.390 µg/mL
Transferrin (recombinant human)	10.00 µg/mL
$CaCl_2$	0.060 mM

penicillin-streptomycin), then plated in a culture flask. A more detailed description of these isolation procedures can be found in the literature [15–17].

49.3.2 Cultivation

In general, the cultivation of primary keratinocytes is more challenging than the cultivation of fibroblasts. Compared to fibroblasts, keratinocytes are more prone to apoptosis when cell density is too low or to differentiation and senescence when cell density is too high [18].

Keratinocytes can be cultured following two main approaches. The classic method developed by Rheinwald and Green [19] is based on the use of serum-containing media and a feeder layer of lethally irradiated mouse fibroblasts. A more recent approach refrains from using feeder cells, but instead relies on serum-free media that are low in calcium. When using these serum-free media, the chances of contamination with other cells such as fibroblasts or melanocytes is reduced. Thus, this is recommended when the objective is to study the properties of keratinocytes alone. To achieve a satisfying growth rate, the serum-free media have to be mixed with supplements. In case of the medium used in our laboratory, KGM-2, these supplements are provided in an additional supplement pack. The final supplement concentrations after addition to the medium are given in Table 49.1.

Using this medium, keratinocytes are cultured at 37°C in a 5% CO_2 humidified environment. The medium should be changed every 48 hours until about 70% confluency is achieved. Then cells are split in a ratio of 1:7 to 1:10.

For cultivation of fibroblasts, it is possible to use conventional media like DMEM or RPMI-1640, mixed with 10% FBS and 1% penicillin-streptomycin. After isolation, it is possible to add 15% FBS for the first passages to boost cell growth.

The typical phenotype of epidermal keratinocytes and dermal fibroblasts is shown in Figure 49.1.

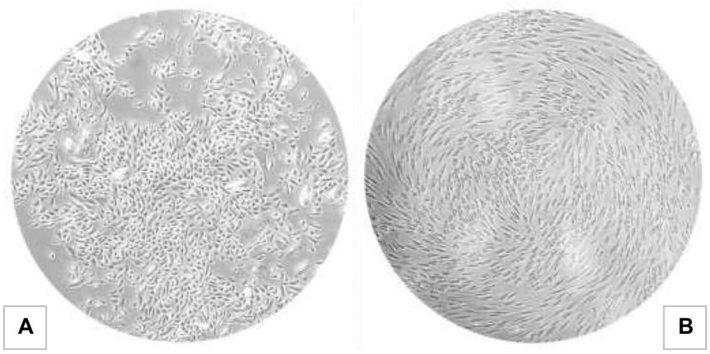

FIGURE 49.1 Microscopic images of primary human keratinocytes (A) and fibroblasts (B); magnification 100×, Nikon Diaphot 300.