

Immortalised cell line derived from the normal human skin tissue



Summary

An human keratinocyte cell line immortalized by means of at least one functional tumor gene of retroviral origin characterised in that it is (1) non-tumorigenic, (2) conserves the capacity for differentiation and for the expression of proteins and of enzymes expressed by normal differentiated keratinocytes even after an elevated number of passages in culture, and (3) forms a stratified and polarised epithelium having a stratum corneum ortho-keratinocyte, if cultivated in an organo-typical culture in a medium without serum and without a layer of nourishing cells. An improved process to immortalize human skin cells to obtain immortalized keratinocytes is characterised in that it comprises the following steps: (i) isolating a sample of human skin; (ii) preparing the sample of skin for in vitro culture; (iii) obtaining keratinocytes from this prepared sample of human skin and seeding in a growth medium without serum with said keratinocytes, on culture plates provided with a coating comprising fibronectin, collagen type I and SAB, which facilitate the fixation and the growth of cells; (iv) replacing the medium in a way sufficient to obtain an optimal confluent growth of cells in culture, continuously maintaining the coating of the plate; (v) transferring keratinocytes into a selection medium without serum on culture plates coated previously in a similar manner; (vi) infecting keratinocytes using functional tumor genes of at least two different retrovirus; (vii) transferring the resulting immortalized keratinocytes to a proliferation medium suitable for the proliferation of immortalised keratinocytes on culture plates coated in a similar way; (viii) transferring the resulting proliferated keratinocytes into a differentiation medium having a high content of calcium on culture boxes coated in a similar way. Use of keratinocytes for immunological, pharmacological, photo- and chemical-toxigological analyses of skin reaction and for expression of heterologous genes and for the construction of artificial skin.

Fig. 1