Construction of three-dimensional human skin model involving dendritic cells and its application to skin sensitization test

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The three–dimensional human skin models already used did not have many dendrite cells so they were difficult to apply to an *in vitro* skin sensitization test. We therefore constructed a three–dimensional human skin model consisting of three different cells, dendritic cells, keratinocytes and fibroblasts (KDF-Skin), exposed immune-sensitizing compounds (DNCB, etc.) and non-sensitizers (Tween80, etc.) to KDF-Skin and investigated the effect of these compounds on cytokine release and the expression of CD86. In addition, in order to cut off the incubation period and the number of cells, a new three–dimensional human skin model using collagen vitrigel membrane was constructed.

Normal human skin fibroblasts (NHSF46) were seeded in collagen gel and cultured for 7 days. After incubation, the normal human dendrite cells (NHDC) seeded in collagen gel were put into a glass ring on collagen gel containing NHSF46. Next, human epidermoid carcinoma (A431) was seeded in the glass ring. After 2-day incubation, the surface of the KDF-Skin was exposed to air and cultured for 13 days, and then immune-sensitizing or non-sensitizing compounds were exposed for 1 h. After 24 h incubation, 10% formalin neutral buffer solution was added to KDF-Skin which was then stained with HE and CD86. NHSF46 were seeded on collagen vitrigel membrane and cultured for 2 h. After incubation, NHDC seeded in the collagen gel were put into a glass ring on the other side of collagen vitrigel. Next, A431 were seeded in the same way. Tthe skin model was cultured for 16 days using the same procedure.

Due to immune-sensitizing compounds, the KDF-Skin significantly released cytokine and significantly expressed CD86. On the other hand, non-sensitizers did not induce IL-1 α , IL-2 and IL- 4 release and the expression of CD86. These results suggest that KDF-Skin is suitable for studying an alternative to animal testing using immune-sensitizing compounds. The incubation period for construction of the new skin model was 7 days shorter than that of KDF-Skin and the amount of NHSF46 used for the new skin model was 4-times less than that of KDF-Skin.