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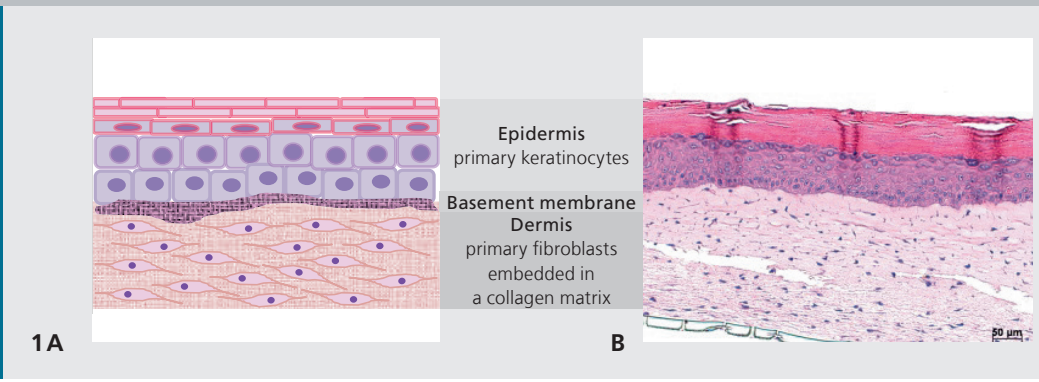

1 *Schematic structure (A) and histological section (B) of a 3D skin model.*

THREE-DIMENSIONAL PRR REPORTER SKIN MODEL

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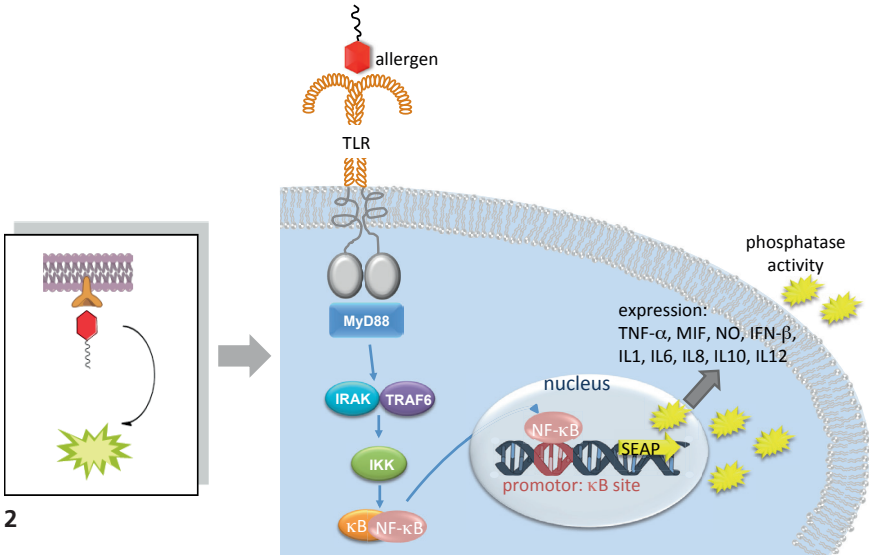
Skin models replace animal testings

The 7th Cosmetics Directive, which demands to replace animal testings for cutaneous resorption with *in vitro* testings by 2009, and the testing of chemicals for harmful cutaneous side effects, as required by the REACH Regulation, have contributed significantly to the advancement of *in vitro* full thickness skin models. For the screening of potential therapeutic substances for local application as human related indicators of efficacy such highly complex, standardized test systems are also in demand.

The human 3D skin model already established at the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB is the basis for a new patented reporter skin model. It consists of primary dermal fibroblasts, embedded in a collagen matrix, as well as a fully differentiated epidermis composed of keratinocytes (Fig. 1).

Changes in cell vitality, morphology and differentiation state of the epidermis in the 3D skin model allow first conclusions, for example, in infection studies, wound healing studies and studies of the mode of action of potential drugs. For more extensive studies, however, time-consuming and costly procedures such as gene expression analyzes or immunostaining of tissue sections are usually required. The aim was therefore to develop a skin model that easily detects the binding of substances to receptors of the innate immune system (pattern recognition receptors, PRRs) via a reporter gene.

2 *Immune-stimulating substances are detected via endogenous receptors expressed by the cells (PRRs) or via receptors introduced by means of stable transfection with subsequent reporter gene activation by NF-kB. In primary immortalized cells used for this purpose, the NF-kB inducible reporter gene was stably transfected.*



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How does the reporter skin model work?

To establish the 3D reporter skin model (DE 102011121556.9), a reporter gene, for example LacZ, a fluorescent protein or a secreted alkaline phosphatase (SEAP), was integrated into primary immortalized fibroblasts and keratinocytes (Fig. 2). The reporter gene is under the control of the transcription factor NF-kB and is induced when e.g. endogenous or ectopically expressed receptors of the human innate immune system are activated by allergen contact.

Applying the substance to be tested to the 3D reporter skin model, its immunomodulating effect can be analyzed directly by expression of the reporter gene. The detection of an activation of the immune system is carried out microscopically or photometrically, for example via the color change of a substrate of the alkaline phosphatase such as pNPP or BCIP in the culture medium of the skin model. The receptor activation can also be detected temporally or spatially resolved depending on the choice of the reporter gene. Time- and cost-intensive immunostainings are omitted.

Allergens as example for application

Pattern Recognition Receptors (PRRs) are known to detect conserved molecular patterns of microorganisms (Pathogen-Associated Molecular Patterns, PAMPs). In addition to PAMPs, some of these PRRs also detect specific allergens such as β-glucan, LPS or chitin from the environment of house dust mites, to which more than 15–20 percent of the population in the industrialized countries react hypersensitive. The allergenic asthmatic reaction is triggered by the β-glucan of the house dust mite. It is recognized by C-type lectin, a receptor of the innate immune system (see table 1). Such allergens as wells as receptor antagonists can be identified using our PRR-specific 3D-reporter skin model.

Advantages

Activation of the immune receptors via substances (antagonists and agonists) can be detected directly through the expression of a reporter gene. Thus, the PRR-specific 3D reporter skin model, which consists of cells with reporter genes, is a unique, fast and flexible tool for identifying lead compounds for drug development in dermatology, detecting allergens in foods and cosmetics, or performing biocompatibility tests.

Applications

- Food industry: Detection of immunostimulating substances
- Pharmaceutical Industry/Medicine: Searching for PRR agonists/antagonists, substances that block the toll-like receptors, are increasingly used in dermatology, especially for topical application. The 3D reporter skin model can be used for drug screening of new TLR agonists/antagonists, which can be used in a variety of medical disciplines.
- Cosmetics industry: biocompatibility studies
- Studies on allergens

Examples of innate immune allergens and receptors (PRRs) that specifically recognize these allergens

Allergen	Ligand	PR receptor
Nickel	Ni^{2+}	TLR4
Peanut extract	Ara h 1	DC-SIGN
House dust mite	β-1,3-Glukan, Der p1	TLR2/TLR6, C-type lectin receptor
Hay fever/flower and grass pollen (allergic rhinitis)		TLR2
Mugwort (allergic rhinitis)	Art v 1	TLR9
Cat hair	Fel d 1	TSLP