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Reporter Skin: In-vitro Skin Makes Cell Reaction to Test Substance Measurable in Real Time

The EU has banned animal testing for cosmetics and non-animal alternative methods are preferable for the risk assessment of new chemical substances. At the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, a three-dimensional skin model has now been set up for the first time that directly displays the skin's reaction to substances: The reporter skin.

Thanks to the built-in reporter, the cellular response can be measured precisely and quickly – using a living model. This means that not only cosmetics can be tested effectively, but also allergens and the inflammation-causing or toxic effects of biocides, pesticides and chemicals.

Testing chemicals and cosmetics safely without animal testing

Shampoos, creams and make-up contain a whole range of different substances. Before a new cosmetic product is launched on the market, its ingredients must be tested for safety and efficacy. This is required by European legislation. Animal testing has not been permitted for a long time and, since 2013, cosmetics that have been tested on animals may no longer be marketed in the EU. Pesticides, biocides and all other chemicals must also be tested in accordance with the European Chemicals Regulation (REACH) with regard to their hazard potential, for example an allergenic or inflammation-causing effect – if possible, without animal testing.

Animal-free alternative methods are therefore in demand. Manufacturers use laboratory-grown skin cell cultures, for example, when developing and testing new ingredients or formulations. Here, the skin cells grow in a cell culture dish with a culture medium that supplies them with nutrients and completely surrounds them. This makes it difficult to test solid or oily substances. In-vitro skin models, tissue grown from skin cells in the laboratory, are also already available on the market as test systems. Their disadvantage is that in order to analyze the effect of the test substance, the in-vitro tissue must be prepared and examined microscopically. An immense number of identical skin models are therefore required to investigate a skin-irritating effect, which can only occur after repeated administration of the substance.

More informative value through three-dimensional skin model with skin barrier

Researchers at the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB have established a three-dimensional skin model made of human cells that very accurately depicts the complex physiology of the skin, including an intact skin barrier, and therefore provides much more meaningful results than 2D cell cultures. The patented skin model consists of dermal fibroblasts, the cells of the subcutis, which are embedded in a collagen matrix, and keratinocytes, which form a fully differentiated multi-layered epidermis. "Just like natural skin, this forms a horny layer that acts as an effective barrier against external influences," explains Dr. Anke Burger-Kentischer, Head of the Cell and Tissue Technologies Department at Fraunhofer IGB.

The cells for the skin model, the fibroblasts and keratinocytes, are isolated by the scientists from skin samples that accumulate as waste products during surgical procedures in doctors' surgeries and hospitals. Isolated directly from the original tissue, these cells – in contrast to commercial cell lines from tumor tissue – resemble the cells in vivo and exhibit a normal physiology. The isolated primary cells are then first immortalized so that they can be cultured permanently and are available indefinitely. This allows the skin models to be constructed in a reproducible manner and provide donor-independent results.

Precise test results on a living model thanks to the reporter function

The highlight of the skin model, however, is its built-in reporter function. Using so-called reporters, the expression of a

relevant gene can be monitored easily and reliably. “We link the reporter gene to the signaling pathway for a cellular signaling cascade that plays a role in skin stress reactions and anchor the construct stably in the genome of our immortalized keratinocytes,” says Burger-Kentischer, explaining her pioneering approach. In inflammatory reactions, for example, the receptor associated with the signaling cascade in the membrane of the skin cell acts as an interface to the outside world: if the substance under investigation binds to the receptor, this activates the signaling cascade and, via the coupling of the reporter gene to the transcription factor – as the final domino of the cellular response – the reporter gene is also read and the reporter protein is produced.

As a reporter, Burger-Kentischer uses secreted alkaline phosphatase, an enzyme that converts its substrate into a yellow dye. “For our reporter skin, this means that after applying the test substance, we take a sample from the culture supernatant of the skin model, add the alkaline phosphatase substrate and can measure the color change after just a few minutes to hours, provided the signaling cascade has been set in motion,” says the scientist. As the cells of the reporter skin do not have to be destroyed for the analysis, the time course of the cell reaction can also be tracked.

“With our reporter skin, we can realistically simulate the in-vivo situation and analyze cell reactions in real time. This makes it a very specific and cost-effective alternative to previous models, because the tissue does not have to be fixed, immunohistochemically stained, sectioned and microscoped in many consecutive and time-consuming steps in order to recognize and evaluate the changes in the cells,” says the scientist.

Test system also suitable for water-repellent and solid substances

“With our reporter skin, we obtain much more meaningful results than with models without a skin barrier and, unlike with submerged cell cultures, we can test many more chemicals and substance mixtures, including water-repellent ones that do not form a stable dispersion in the aqueous cell culture medium,” adds Burger-Kentischer. For manufacturers of cosmetics and chemicals, this means that the three-dimensional reporter skin model can be used to test oils or oil mixtures for the first time, as well as solid substances such as textiles and food.

Detection of cytotoxicity, sensitization, inflammation and skin penetration in the same model

Burger-Kentischer and her team have even succeeded in integrating various cellular signaling pathways, each with different reporters, in the same skin model. This means that an entire set of 3D reporter skin models is now available for a wide range of applications.

“This allows us to make statements about the toxicological potential of a substance, investigate the skin penetration of a substance and specifically and quickly read out the activation of different cellular stress signaling pathways by the substance in the same model,” says Burger-Kentischer. In terms of their applicability and informative value, these models significantly outperform existing test systems on the market.

By mapping all three known skin stress signaling pathways, manufacturers of cosmetics and chemicals can quickly find out whether and what type of cell stress a substance causes: for example, whether it has an inflammatory effect or sensitizes the skin, which can lead to allergies in the long term. Or whether it reacts with ER stress, which leads to proteins being folded incorrectly and thus no longer being able to fulfill their biological function.

Integration of further proof of efficacy possible

Burger-Kentischer's team can test in advance in the institute's in-house laboratory whether the reporter skin model is actually suitable for a production company before the in-vitro models are produced in large quantities for interested parties. At the customer's request, the reporter test system can also be supplemented with other cellular signaling pathways or transferred to other organs.

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Further information

Dr. Anke Burger-Kentischer
Head of Department Cell and Tissue Technologies
Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB
Nobelstr. 12
70569 Stuttgart

Tel.: +49 (0) 711 970 4023

Dr. Claudia Vorbeck
Communications
Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB
Nobelstr. 12
70569 Stuttgart
Tel.: +49 (0) 711 970 4031

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