SKIN DEEP

Our skin protects us from cold, heat, radiation, and pathogens. Injuries to the skin require a fast response. At its Tissue Engineering Project House Evonik develops innovative materials and processes to improve growth of tissue in the laboratory, which may allow the establishment of new therapies to heal human skin

TEXT **MICHAEL STANGE**

The hope of millions of people is barely visible to the naked eye. Only 10 to 15 micrometers across—ten times thinner than a human hair—these skin cells are surrounded by a liquid carpet of nutrients, salts, sugars, trace elements, and amino acids. There's a total of over 300 ingredients, but no one knows the cells' favorite recipe in its entirety. Not yet. But if cells are to multiply, that is exactly what's needed. It's a recipe that could improve the lives of people all over the world suffering from severe burns or open wounds. This is why Evonik scientists are studying new materials for culturing cells and growing artificial tissue.

The researchers in question are part of the Tissue Engineering Project House, which Evonik established in Singapore. The name refers to an interdisciplinary field of research encompassing engineering and chemistry fundamentals on the one hand and biological sciences on the other. The goal is to develop the next generation cell nutrition ingredients, carrier materials and culturing processes which will allow the industry to commercialize biological replacement materials that will restore, maintain or improve tissue function.

A GLOBAL TEAM

Through the Tissue Engineering Project House, Evonik aims to better understand how to use cells grown in culture for producing man-made biological tissue and to develop novel solutions. "Both for non-medical purposes and for innovative therapeutic approaches—regenerating human skin following accidents or disease, for example," explains Alexander König. A chemist by education, 38-year-old König has worked at Evonik for four years, and one year ago was given the opportunity to direct the new project house. Two weeks later he was on a plane bound for Asia.

König has since traveled regularly between Singapore, the USA, and Germany, coordinating the international team. In Singapore, up to 20 Evonik research- →

Cells are stored at temperatures around -196°C

ers from different units within Evonik work closely with colleagues in Birmingham/Alabama and Darmstadt, where Evonik has already developed extensive competencies in the fields of medical and cosmetic applications (see infobox). König and his team are to work for at least three years on new functional materials, processing technologies, and even methods for culturing tissue types. Cell therapy is one focus for the team; artificial skin is another.

> **“There would be interesting applications for our customers in the pharmaceuticals and cosmetics industries”**
>
> ALEXANDER KÖNIG,
> DIRECTOR OF TISSUE ENGINEERING PROJECT HOUSE

MANY OPEN QUESTIONS

“Singapore has top universities and lots of experts in applied biomedical sciences,” says König. “We work on several open questions. That’s why we need access to experts from a wide variety of disciplines.” One of these experts is Zee Upton. As the head of the Institute of Medical Biology at the Agency for Science, Technology and Research (A-Star) in Singapore, the 56-year-old biochemist conducts internationally renowned research in the field of wound healing and tissue growth and repair. “Chronic wounds such as diabetic foot ulcers, venous leg ulcers, and bedsores are a huge health problem,” she says. “Two to six percent of the world’s population suffers from them.”

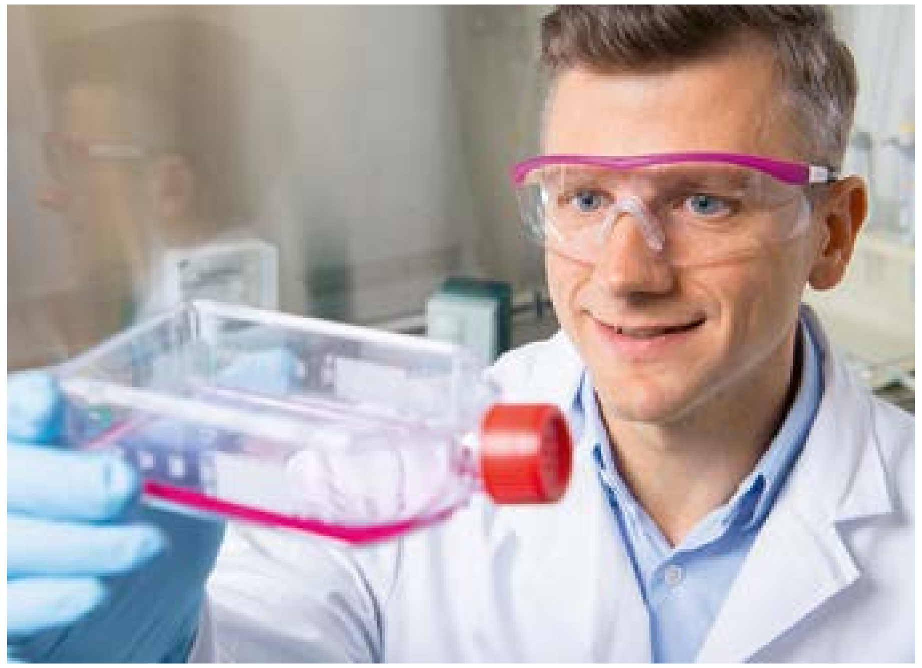
Upton is calling upon researchers to intensify their search for the best possible ways of treating chronic wounds. Although specialized dressings and bandages are available, she remarks, “there’s no satisfactory treatment for healing chronic wounds.” Upton’s comments reflect just one of many challenges that could be tackled with the help of tissue engineering—challenges that the researchers at the project house are now addressing.

AS REALISTIC AS POSSIBLE

One goal of the scientists is to use cells grown in culture as a basis for creating artificial tissue that would mimic its natural counterpart—the skin—extremely closely. Their plan involves different types of human cells interacting just as they do in the body. The most realistic possible model of the skin would need to contain the two layers of the epidermis, or outer layer of skin, and the dermis, or corium, below that—and ideally even 3D-printed blood vessels that would supply the laboratory tissue with adequate oxygen and nutrients. “If we can do that,” says König, “researchers like Zee Upton won’t be the only ones to benefit—there will be interesting applications for our customers in the pharmaceuticals and cosmetics industries too.” Among these applications are new approaches to using cell therapy for wound healing, skin models for in vitro testing, and culturing transplants from the patient’s own cells—the greatest challenge

But first things first: cells—or even skin tissue—grown in culture in the lab could be used for novel cell therapies for healing wounds. This would involve taking a patient’s cells and culturing and/or multiplying them in vitro. The cells would then be reintroduced to accelerate the healing of the damaged organ—the skin in this case.

The next application, i.e. skin models, would be to create replicas of the skin that are as realistic as possible and that would only be used for research and testing purposes. While a few commercial providers are already selling this kind of laboratory-grown tissue, “Those products don’t yet mimic reality well enough,” König points out. “A lot of test results can’t be correlated one-to-one to human beings.” In many models, the protective barrier is around 100 times more permeable than is the case in nature, and the dermis lacks blood vessels. Evonik hopes to help optimize skin models for more reliable results when it comes to testing new medications, cosmetic agents or cleansers. →



Alexander König checks the solution, which consists of a carrier material and a culture medium in which skin cells reproduce

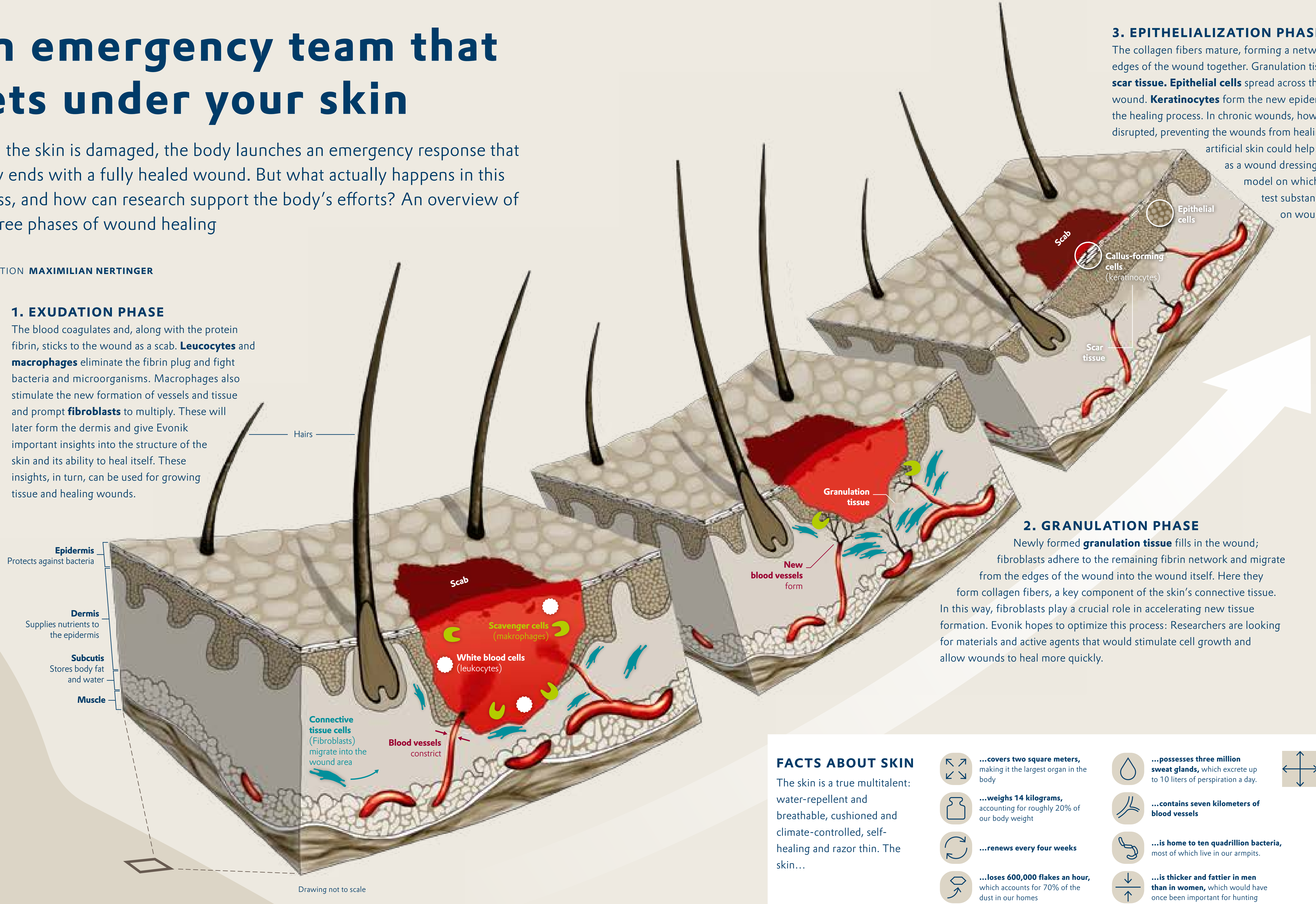
The foundation on which project house researchers can build

The Tissue Engineering Project House represents another step in Evonik’s efforts to strategically develop its activities in the field of medical applications. The former Medical Devices Project House is now an established competence center unlike any other in the world. Its focus is on developing new biocompatible materials and the technologies for processing those materials to make implants for orthopedic and cardiovascular applications. A range of products has already been successfully positioned on the market, and the team at the Tissue Engineering Project House can now build on that work through its focus on growing entire tissues artificially. The research work is complemented by technologies and products from Evonik that have been established for years. These include amino acids and derivatives produced without the use of animals as well as the additives (known as boosters) that Evonik makes for animal-free, cell-based production of biological actives and the company’s expertise in cosmetics testing and skin models.

An emergency team that gets under your skin

When the skin is damaged, the body launches an emergency response that ideally ends with a fully healed wound. But what actually happens in this process, and how can research support the body's efforts? An overview of the three phases of wound healing

ILLUSTRATION **MAXIMILIAN NERTINGER**



1. EXUDATION PHASE

The blood coagulates and, along with the protein fibrin, sticks to the wound as a scab. **Leucocytes** and **macrophages** eliminate the fibrin plug and fight bacteria and microorganisms. Macrophages also stimulate the new formation of vessels and tissue and prompt **fibroblasts** to multiply. These will later form the dermis and give Evonik important insights into the structure of the skin and its ability to heal itself. These insights, in turn, can be used for growing tissue and healing wounds.

2. GRANULATION PHASE

Newly formed **granulation tissue** fills in the wound; fibroblasts adhere to the remaining fibrin network and migrate from the edges of the wound into the wound itself. Here they form collagen fibers, a key component of the skin's connective tissue. In this way, fibroblasts play a crucial role in accelerating new tissue formation. Evonik hopes to optimize this process: Researchers are looking for materials and active agents that would stimulate cell growth and allow wounds to heal more quickly.

3. EPITHELIALIZATION PHASE

The collagen fibers mature, forming a network that pulls the edges of the wound together. Granulation tissue converts to **scar tissue. Epithelial cells** spread across the surface of the wound. **Keratinocytes** form the new epidermis and complete the healing process. In chronic wounds, however, this process is disrupted, preventing the wounds from healing. This is where artificial skin could help: as a transplant, as a wound dressing or as a laboratory model on which Evonik could test substances for their impact on wound healing.

Drawing not to scale

FACTS ABOUT SKIN

The skin is a true multitalent: water-repellent and breathable, cushioned and climate-controlled, self-healing and razor thin. The skin…

…covers two square meters, making it the largest organ in the body

…weighs 14 kilograms, accounting for roughly 20% of our body weight

…renews every four weeks

…loses 600,000 flakes an hour, which accounts for 70% of the dust in our homes

…possesses three million sweat glands, which excrete up to 10 liters of perspiration a day.

…contains seven kilometers of blood vessels

…is home to ten quadrillion bacteria, most of which live in our armpits.

…is thicker and fattier in men than in women, which would have once been important for hunting

…contains the following in a single square centimeter:
- Three million cells
- Four meters of nerve fiber
- 100 sweat glands
- 15 sebaceous glands
- 5 hairs

“The cells have to feel like they’re in their natural environment”

ALEXANDER KÖNIG

Yet another development—one that could be used in applications such as transplants—might conceivably come from this research as well. This is another area where some initial successes have been achieved: as early as 1993, Japanese researchers at Kitasato University were the first to grow replacement skin and to transplant it successfully onto a man’s burn wound. “But we still haven’t established methods or materials that would be easily reproducible,” König observes.

The reasons why none of the three applications have been successfully standardized to date are as varied as they are complex. Developing and culturing man-made biological tissue are expensive, complicated processes due in no small part to the complexity of the skin. Skin is the largest organ of the human body, and contains roughly 120 billion cells. The different cell and tissue types have to work together with the top two skin layers to protect the body from pathogens, heat, cold, and UV radiation. Even a copy from the lab would have to serve those functions.

FROM THE CELLS TO THE PIECE OF SKIN

Cells need to do more than just proliferate in the laboratory—they also have to organize exactly the way they would in the human body, and to do that they need the right mix of nutrients, growth factors, and a scaffold. The nutrient solution with all of its ingredients—the cell culture medium, in other words—is first heated to 37 degrees Celsius, because cells feel most comfortable at body temperature. The scientists then dispense the liquid into plastic dishes coated with a gel-like scaffold material, onto which they pipette the cells, one type of cell per dish. The keratinocytes become the epidermis, or top protective layer of the skin, while the other cell type—the fibroblasts—forms the dermis below that. Once they are in their respective dishes, the cells are finally placed in the incubator to mature and multiply. As soon as enough cells have formed, the researchers combine the cell types into a two-layer model while adding growth factors. After that, it’s back to the incubator for two to three weeks until the cells have joined to form a firm, transparent skin the size of a one-cent coin.

Researchers can use a 3D printer to print blood vessels, among other structures, from a variety of materials

Watching the development of the cells, which are just 10 to 15 micrometers across—requires a microscope

“Our first aim is to simplify and speed up this drawn-out process,” König says. “A lot of the steps have to be carried out manually. That can cause the product to vary—and that jeopardizes reproducibility.” This risk rears its head in another area as well: the material.

“The current standard is for both the carrier and the nutrient solution to be based on animal products. That causes the composition to vary from batch to batch, which makes it difficult to obtain government approval for clinical applications.” The effects on research are so far-reaching that they sometimes slow down the culturing process considerably or even ruin the specimen entirely. Moreover, products derived from cows, such as the bovine serum often used in the nutrient solution, harbor the risk of contamination with pathogens such as the one that triggers BSE (bovine spongiform encephalopathy).

“That’s why we’re working to optimize the materials we use in addition to developing innovative methods,” König notes. Chemically produced synthetic materials can replace animal products if they are suitably biocompatible and have the right mechanical and physical properties. “When the cells are on the carrier material, for example, they have to feel like they’re in their natural environment. Thus what we need is a functional material that stimulates cell growth and that we can modulate depending on whether we’re growing bone or skin cells, for example.”

GLOBALLY UNRIVALED COMPETENCE CENTER

This is where König and his team can draw upon know-how that has already been successfully developed by their predecessor—the Medical Devices Project House in Birmingham, which is now an established, globally unrivaled competence center in this field. Examples here include the production and processing of biocompatible plastics. “At the Tissue Engineering Project House, we can use the knowledge they generated on medical devices such as implants, and then develop it still further,” says König.

Evonik’s portfolio also includes another foundation for successful cell culturing: For many years now, the company has been supplying manufacturers of cell culture media with amino acids and derivatives that are produced without the use of animals. Over the past few years, Evonik has also expanded its business in performance-enhancing additives for the cell-based manufacturing of biological active agents. “Here we have already successfully switched to media with no animal-based components. Now we want to explore applying that step to the culturing of skin cells,” König explains.

“Our vision is to take existing competencies, further research activities, and external expertise and to funnel these into efficient, reproducible solutions.” Once the project house is that far along, Evonik intends to offer new carrier materials and nutrient solutions for commercial tissue culturing. “Ideally we would be able to play a part in fulfilling the hopes of millions of people for more effective therapeutic approaches,”König says.