Faster to Active Ingredients with

By now, most consumers have heard the terms “bioactive substances” or “active ingredients.” The terms were originally used in the pharmaceutical industry only, but are now applied to many other health-promoting materials that are gaining importance in fields such as nutrition and wellness. A comparable trend has been observed for a while now in the cosmetics industry, where Degussa is a leading globally operating supplier of raw materials, additives and, increasingly, active ingredients for cosmetics. Identifying substances that have positive effects on age-related changes in the skin are a particular focus of R&D departments. Recently, Degussa researchers have also been using DNA chip technology to track down effective active ingredients. One good example of their success is salicyloyl phytosphingosine. This active ingredient, tested by using a DNA chip, is proven to regenerate the skin and soften wrinkles.

Degussa is the first manufacturer of cosmetic active ingredients to use DNA chip technology in the search for bioactive substances. The active ingredient salicyloyl phytosphingosine, which was tested using this technology, is proven to accelerate the regeneration of skin and minimize the effects of aging.
DNA CHIPS

Worldwide demand for beauty products is on the rise. Even if we exclude perfumes and colognes, the total "personal care" market exceeded US $150 billion in 2005. Cosmetic active ingredients – defined as ingredients that have a positive effect on skin cells – including vitamins, UV filters and plant extracts, already account for over US $2 billion of that figure. One quarter comprises specific active ingredients with defined and, for the most part, quite complex structures ("specialty actives"), which are currently growing at considerable rates of eight to ten percent per year.

In recent years, the consumer has also come to associate prevention of skin aging with high-quality skin care products. The cosmetics industry has responded with the development of increasingly refined and complex products targeted not only for the premium segment, but also for the mass market. This is a dynamic business segment, which always needs new incentives for further growth. Therefore the industry increasingly draws on concepts and scientific discoveries from areas like life sciences, dermatology, materials sciences and biotechnology.

Scientific innovations, then, supply the basis for advances within the cosmetics industry. And because of the enormous variety of naturally occurring or synthetically manufactured structures that can serve as starting points, the search for bioactive compounds is one of the most exciting chapters in cosmetics research. Although some of these substances often show highly efficient and also specific effectiveness even in low concentrations, the targeted search for active ingredients remains a gamble. Frequently, the outcome relies on chance, since the molecular structure and the concrete area of application have to match.

To significantly increase the number of positive hits in a large quantity of test substances, Degussa’s Personal Care Business Line is the first supplier of cosmetic active ingredients to introduce DNA chip technology for selecting potential compounds. This technology enables us to see a total picture of the action of a bioactive substance, and thereby identify specific active ingredients that preferably address a definite group of targets. In the area of DNA chips, Degussa is working closely with Prof. Gerd Schmitz at the Institute for Clinical Chemistry and Laboratory Diagnostics of the University of Regensburg.

The groundwork for this innovative technology was laid by the Human Genome Project, which created scientific history in February 2001. Two research companies simultaneously announced the sequencing of the human genome, which the media hailed as the unlocking of our genetic material. The sequence analysis of the genetic information forms the basis for a whole host of advanced work aimed at understanding how genetic information is transformed into cellular characteristics. DNA chips, also called biochips or DNA microarrays, can be used not only for the expression analyses of individual genes and complex gene groups, but also for diagnostic purposes. Additionally, we now have a methodology available for identifying the influence of substances down to the genetic level, and highlighting the key genes that switch certain metabolic processes on and off, or regulate them up or down.

Cosmetics are primarily for use on the skin. With a surface area of about two square meters, the skin is by far the largest organ of the body, contributing about 15 percent of its total weight. Also in skin cells, genetic information is converted into cellular characteristics when it is translated into proteins. Because the skin has a very complex structure, it does not react to the topical administration of skin care formulations based on a simple black-and-white model, but by exciting a number of physical, chemical, and/or biochemical processes. These processes take place in certain cell types, and in turn influence the regeneration of the skin barrier of the uppermost layer of skin, the epidermis. However, they also influence the synthesis of key proteins, such as collagen and elastin structures in the next highest layer, the dermis.

Our knowledge of the various mechanisms of the metabolic processes related to aging processes, both on a clinical (for example, how stress and UV light lead to the development of wrinkles) and a molecular level (for
example, modulation of cellular communication processes) is also growing. It was a goal of the Degussa researchers to be able to define and comprehensively investigate the influence of bioactive ingredients on the skin early on. This is where DNA chip technology flexes its muscles. It can be used to parallel test the action of substances on a total of 35,000 known genes. The enormous wealth of data generated by the tests is evaluated using processes from system biology that have been developed by Degussa’s Care & Surface Specialties Business Unit in close cooperation with the Feed Additives Business Unit and external partners. Bioinformatics and, especially, mathematical models are supplying the first information about which gene groups are influenced by certain active ingredients.

More than 35,000 gene probes on a checkerboard of a few square centimeters

The DNA chips have a substrate made of glass or silica and measure about two square centimeters. Manufacturing processes from microelectronics, solid-phase and combinatorial chemistry, molecular biology, and robotics are combined in their production. In the final analysis, ordered arrays are generated in a photo-lithographic process on which single-stranded oligonucleotides with a defined sequence are fabricated using lithographic masks and light-sensitive protective groups. This in situ synthesis attaches these DNA probes in fixed places on the surface of the chip. At the end of this procedure, the chip, which is manufactured by the Affymetrix company in the United States, has up to 250,000 array elements per square centimeter, with which the activity of all of the approximately 35,000 human genes can be determined at the same time. But not all the genes in a cell are actively synthesizing proteins at the same time. The regulation of gene activity depends much more on certain environmental conditions, and hence significantly influences the properties of a cell.

The information needed to manufacture proteins is stored in the DNA. When the organism needs a particular protein, the genetic information of the DNA is converted into proteins by transcription and translation. Effective active ingredients for cosmetics can either stimulate (e.g., more collagen for an anti-wrinkle effect) or reduce (e.g., fewer cytokines with irritable skin) gene expression, and therefore the quantity of protein available in the organism. DNA chip technology can be used to study the influence of an active ingredient on gene expression.
During a chip experiment, the first step is the extraction of the synthesized transcripts of the gene sequences (single-stranded messenger ribonucleic acid or mRNA) from a biological sample. These kinds of “working copies” or transcripts are used by the cells to manufacture the proteins that regulate metabolism, produce connective tissue, or communicate with other cells. In the laboratory experiment, this mRNA is multiplied in its totality, marked with a fluorescent dye, and sorted with the help of the DNA chip. The principle is based on mRNA’s property of forming double strands of DNA by joining the single-stranded DNA of complementary sequence present on the chip (hybridization).

The quantity of each individual available messenger RNA that allows researchers to draw conclusions about the activity of a gene can then be determined through fluorescence analysis, in which the intensity of the light emitted is proportionate to the quantity of available transcripts. A comparison of samples that, for instance, were treated with or without a bioactive substance, can then allow researchers to draw conclusions about the biological activity of the sample. A single run-through therefore replaces the thousands of individual experiments that once had to be carried out painstakingly one after the other. In the processing of the extensive data this generates, Degussa is also cooperating in the personal care area with Genedata AG of Basel, Switzerland, which specializes in the evaluation and modeling of bioinformatics findings.

In addition to the biological effectiveness, and as “condition sine qua non,” researchers also study a sample’s potential toxicity: If the sample turns out to be poisonous, all additional work is pointless. This is why they sift through the mountains of data specifically for anything that indicates these effects.

Checking results on skin models

The effects observed on the genetic level in the cell culture are then selectively tested on artificial three-dimensional skin models. The skin used in these models is cultured in vitro on a layer of polypropylene in a special medium. Researchers are able not only to apply potential active ingredients in pure form on the “model,” but also finished creams with and without new active ingredients, and examine the biological effectiveness no matter the dose administered.

In contrast to liquid cultures, skin models have cells from all the stages that skin goes through over the course of a development cycle – from the dividing basal cells all the way to the keratinized barrier cells. The genetic and biochemical reactions that occur here are already very similar to those of the skin, which makes the results easily comparable to the in vivo situation. The properties that receive special attention are regeneration, the influence on sensitive skin, the anti-wrinkling effect, roughness reduction, as well as tautness and elasticity. In cooperation with Degussa’s Skin Care Business Line, researchers developed different test systems that can, for example, yield more precise information on the metabolism of the skin, and also on the effects of substances on damaged skin.
Degussa not only views itself as a manufacturer and supplier of active ingredients, but is positioning itself in the attractive cosmetics market as a development partner for outstanding system solutions. In line with its company-wide “Solutions to Customers” business model, early cooperation with customers, which can begin as early as the development phase of a product, is often essential. At this point, work focuses on the selection of the “right” raw materials, and on a formulation aspect that takes a variety of factors into account. One of these factors is the stability of the active ingredients in a cream, which can sometimes call for encapsulation to protect the active ingredients against the influence of temperature, moisture, oxygen content, pH value, and UV light.

Another important question in the development of cosmetic active ingredients is how fast or deep they penetrate into the skin. This penetration behavior is tested on samples of pig skin, which are fixed in special mounting devices (Franz cells), and supplied with the potential active ingredients formulated into different creams. Researchers then determine which cream system ensures an optimal penetration of the active ingredient to the site of action.

After DNA chips and skin models – the effectiveness of an active ingredient must also be proven in vivo

Before a product can be launched on the market, researchers must also evaluate the effectiveness of the active ingredient in vivo. For these purposes, Degussa maintains a pool of 60 to 70 employees who have volunteered as test persons. Depending on the activity profile, a number of different in vivo methods can be used, including determining transepidermal water loss (TEWL), and the moisture, smoothness, and irritation of the skin. When researchers are certain they have found positive effects, they bring in independent testing institutes that work for such organizations as the Stiftung Warentest (Germany’s leading consumer safety group) to participate in the final evaluation. They also rely on the expertise of leading dermatologists.

A perfect example of the successful use of DNA chip technology at Degussa is the proven effectiveness of salicyloyl phytosphingosine, a derivative of the naturally occurring phytosphingosine, and structurally related to ceramides. Salicyloyl phytosphingosine displays three optically active carbon atoms, which theoretically generate eight different structures. However, only one of these forms is highly active – a form Degussa manufactures in the bioreactor with the help of yeast. This fermentative process is superior to chemical synthesis, which yields a mixture of all eight variants. The substance obtained from this process is then linked to salicylic acid.

After targeted screening in various in vitro and in vivo studies, this active ingredient has shown itself to be highly effective for activating a variety of skin processes, and has also been proven to minimize signs of age. Salicyloyl phytosphingosine (PS SLC) has demonstrably accelerated skin regeneration. Skin treated with this active ingredient regenerates about twice as fast as untreated skin. A formula containing as little as 0.2 percent PS SLC promotes the production of key ceramides that perform important protective functions as lipid layers in the outermost layer of skin (stratum corneum). It also improves the barrier properties of the skin, and effectively reduces transepidermal water loss. Other application tests show that PS SLC has an astounding ability to smooth the skin and minimize wrinkles. Within as few as four weeks, the deepest, most obvious wrinkles were reduced by about 10 percent. These were the results of an external study using 30 test subjects, each of whom had been treated on one half of the face with PS SLC, with the other half left untreated.

The successes achieved with salicyloyl phytosphingosine are proof that there are effective active ingredients out there that can give new impetus to today’s cosmetics industry. In this case, too, the clear activity signals shown in the DNA chip study provided the starting point. This method has proven its usefulness not only for discovering new active ingredients, but also for predicting the function of compounds with as of yet undefined properties, and will be used even more in the future. The existence of this new tool and the active ingredient itself are in line with the trend that customers are demanding increasingly and demonstrably more effective cosmetics. As a system supplier, Degussa is well positioned to meet this demand, and has once again proven its innovative ability as a trendsetter, even for the highly promising growth opportunities in the cosmetics market.