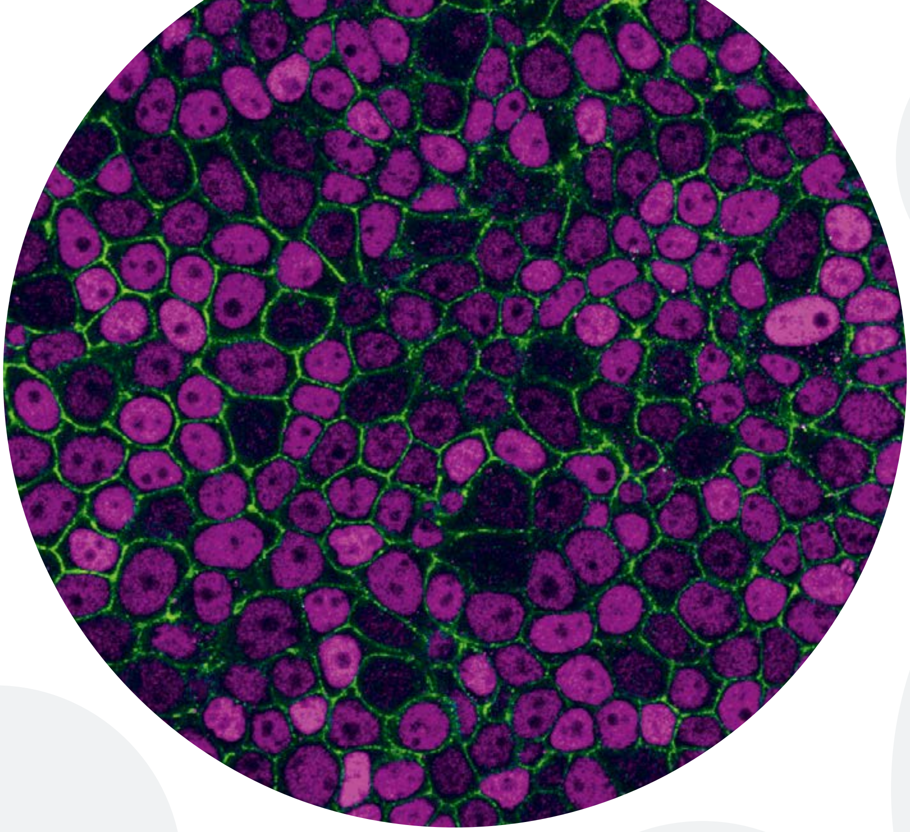
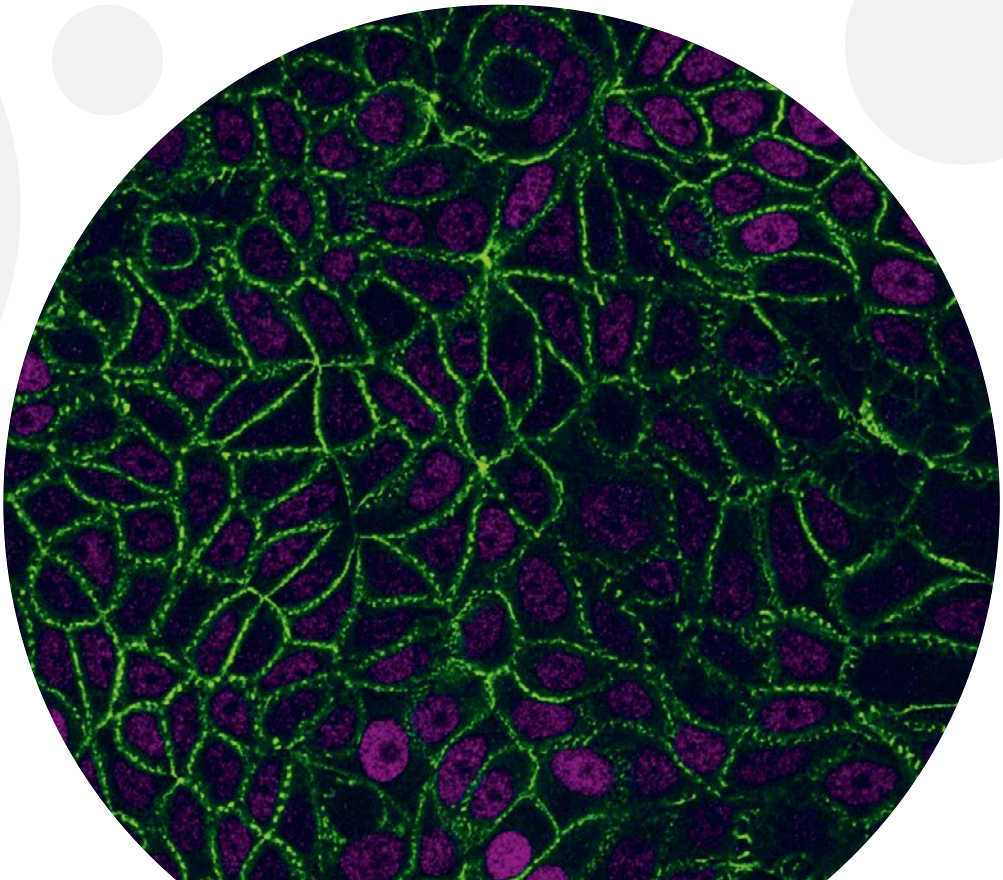


Shining life. Fluorescent cells are analyzed using the “E-Morph” test.

Revealing bubbles

Hormones are essential, actually. But an excess can be harmful. BfR scientist Dr. Sebastian Dunst and his team have developed an animal-free test method that can detect the undesired hormonal effects of chemicals.



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Microscopic images: Dr. Sebastian Dunst and his team at the German Centre for the Protection of Laboratory Animals are investigating changes in the cell membrane.

If you look closely, you can see them. Tiny honeycombs sandwiched between fine green shimmering lines. “The honeycombs remind me of bubbles in bubble wrap,” says Dr. Sebastian Dunst, describing the microscopic image. We are in the blacked-out microscopy room at the BfR’s German Centre for the Protection of Laboratory Animals in Berlin-Marienfelde. It is crammed with computers and modern, electronically controlled microscopes with which even the smallest details of a cell can be visualised.

A microscopic image is projected onto the screen. The fine green lines are the cell membranes, the outer envelope of human cells. Tightly packed, the cells pave the screen. They combine to form a tissue that is only interrupted by the “air cushions” between the cells. For biologist Dunst and his team, these bubble-like changes in the cell membrane are at the centre of a new test method they have developed, and which they have named “E-Morph”. E-Morph effectively checks whether the cell tissue is holding together tightly or is too loose.

Molecular ropes

To understand the principle of E-Morph, you have to dig a little deeper. The contacts between the cells are mainly mediated by the thread-like protein E-cadherin, which is evenly anchored in each cell’s membranes. Like molecular ropes, the E-cadherin proteins now bind cell envelopes of neighbouring cells tightly together. However, if the cells are brought into contact with certain chemical substances, the E-cadherin ropes change their organisation. The former even distribution turns into clusters, thereby creating those gaps in the cell membranes that appear as “air cushions” or bubbles in the microscopic image.

Surprisingly, this does not weaken the connections between the cells. “As soon as the bubbles form, the cells become more stable and stick to each other much more firmly,” says Dunst, explaining the process. “In the case of cancer cells, this can mean that in this state, the formation of metastases is hampered because the cells can no longer migrate from the tumour tissue.” The tissue, therefore, remains firmly linked.

The E-Morph test looks at how different chemicals or active substances influence cell cohesion and, in doing so, the risk of metastasis (formation of secondary tumours). Primary focus is on the female sex hormone oestrogen and substances that mimic or weaken its effects. Oestrogen makes the tissue “looser”; it can trigger the formation of metastases. Therefore, oestrogen-like substances can increase the risk to develop malignant cancer. Conversely, chemical compounds that suppress oestrogen activity reduce the risk and, for this reason, are also used to treat cancer.

This sounds theoretical, but it has practical significance. There are a number of man-made or natural substances found in the environment that have a hormone-like activity, sometimes with oestrogen-like effects. For example, in unfavourable cases, these substances can impair fertility or create favourable conditions for diseases such as cancer.

Tested for hormonal effects

In the EU, chemicals and pesticides must, therefore, be tested to see whether they have potentially harmful hormone-like effects. Such substances are known as “endocrine disruptors” in specialist terminology. The E-Morph test can help to detect them.

Dunst’s procedure for detecting the hormonal effects of chemicals is mostly automated. Human breast cancer cells are used. There are two reasons for this: firstly, these cells have typical characteristics of intact mammary gland cells so they are susceptible to the effects of oestrogen. Secondly, unlike healthy, “normal” tissue, cancer cells can be easily grown in the laboratory.

Oestrogen makes cells loose

The cells are initially brought into a state in which they form the characteristic bubbles between the cell membranes with the help of an oestrogen blockade using the cancer drug Fulvestrant. The substance to be tested is then added. Now it is checked whether the cell contacts loosen and the bubbles disappear as they would under the influence of oestrogen. This may suggest that the test substance has a hormone-like effect and increases the risk of tumours.

The robot-assisted test evaluation takes place quickly and facilitates the testing of many substances in a short time. Sebastian Dunst hopes that the procedure will help to use chemicals safely and to identify new substances for cancer treatment. It is no coincidence that the test has been developed at the German Centre for the Protection of Laboratory Animals at the BfR because it is a possible alternative method to the so far mandatory animal experiments for chemical and drug testing. "When we can completely replace these animal experiments is another question," says Dunst. "We are not yet there."

Recently, Dunst and his team filed a worldwide patent application for the E-Morph test. "The procedure provides information on how cells and tissues actually change under the influence of hormones or hormone-like substances," says Dunst. "It therefore provides more realistic conditions than tests that only cover a few individual aspects of hormone activity under more simplified conditions." E-Morph may be interesting for two applications – firstly, for testing new active substances in medicinal products and secondly, for testing chemicals that are expected to enter the market.

A test for pharmaceuticals and chemicals

So that the patent really becomes effective, it must still be approved separately in each individual country. This is an elaborate process because a separate patent lawyer who has knowledge of the local language and who enforces the patent claims must always be charged with the task. For this reason, it is necessary to choose where the test will be offered. "Countries with a strong chemical or pharmaceutical industry are worth considering first," explains the scientist. This means countries like the USA, Germany, France or Japan.

Dunst has worked at the BfR for around four years; before that he studied biology in Dresden, where he also did his doctorate. He carried out research on fruit flies and, in doing so, discovered his love for microscopy. What he likes about the BfR is that his work is important for both humans and animals. "It's not an abstract science, you can really get things moving and make a change", he says. "It is particularly important for me to work in a team – together we can achieve a lot". The E-Morph test is just one example of this great collaborative work. ◘

More information:
BfR Communication No. 023/2020 of 20 May 2020

BETTER UNDERSTANDING OF CERVICAL CANCER

Cervical cancer is the fourth most common malignant tumour in women worldwide. To better understand the progression of the disease and to test new treatments, junior professor Peter Loskill from Eberhard Karls University of Tübingen is developing "mini organs" using human cells from the cervix. They imitate the disease and its early stages in humans on a "tissue chip" and make it possible to study immune cells that are in contact with the cancer.

BARRIER-FREE? BETTER NOT IN THE BRAIN

The brain is a sensitive organ. It is protected from harmful substances and pathogens by a biological barrier called the blood-brain barrier. Dr. Petra Hundehege and her research group from the University of Münster are investigating the disadvantages of a permeable blood-brain barrier. They develop a computer simulation model to investigate the damage to the blood-brain barrier, as it can occur after a stroke or cranial injury.

FEWER ANIMAL EXPERIMENTS, BETTER RESULTS

Inadequate statistics/biometrics bear the risk of a loss of quality of research results. Furthermore, dubious results might entail unnecessary studies also with regard to animal experiments. Professor Daniel Hofmann's team at the University of Duisburg-Essen wants to remedy this. The group develops the freely accessible software "BASTA", which stands for "Bayes statistics for animal research". BASTA ensures a better calculation of animal numbers and an increase in data evaluation, thereby increasing the impact for publication.

ZEBRAFISH PROTECT BRAIN DEVELOPMENT

Harmful substances can impair brain development in the womb. For this reason, chemicals and drugs must be tested to see if they are toxic to the nervous system (neurotoxic). This testing is required by law and is currently carried out on mice and rats. Zebrafish (Danio rerio) embryos are a possible alternative to rodents. Dr. Stefan Scholz and his research group from the Helmholtz Centre for Environmental Research in Leipzig are testing whether Zebrafish represents a suitable model organisms to analyse neurotoxicity.

FRESH AIR FOR ASTHMA RESEARCH

Asthma is a chronic inflammation of the airways often caused by allergies. To better understand the onset and progression of this widespread illness, Professor Holger Garn and his team at Philipps-Universität Marburg are developing a "miniature version" of the lung in a Petri dish, called an organoid. Tissue from the respiratory tracts of mice and humans serve as the basis for the organoids.

AGEING UNDER THE MICROSCOPE

Idiopathic pulmonary fibrosis is a chronic disease that usually leads to death after a few years. In the process, the lung is constricted due to a proliferating connective tissue (fibrosis). Premature ageing is probably involved in the occurrence of the disease. At the Helmholtz Zentrum München, Dr. Mareike Lehmann and her research group now develop methods based on microscopic tissue sections from the human lungs. On them the team wants to study premature ageing and the formation of fibrosis to develop a test and to examine substances. This may help to replace stressful animal experiments.

Micro-organ instead of animal experiment

Whether fruit flies, cell culture or software, there are many approaches to reduce conventional experiments on animals. An overview of projects that received funding from the German Centre for the Protection of Laboratory Animals at the BfR in 2019.

COMBATTING KIDNEY DISEASE WITH THE FRUIT FLY

The kidneys clean the blood. However, too much circulating insulin, the hormone that lowers blood sugar (as in type II diabetes or adult-onset diabetes), can damage the kidneys' filter systems (glomeruli) and facilitate kidney failure. To better understand these processes and prevent kidney failure, Dr. Lucas Kühne and his team at Cologne University Hospital now study the kidney cells of the common fruit fly (Drosophila melanogaster). This not only has scientific benefits since the animals are easy to breed and reproduce quickly, it can also help to reduce the number of laboratory animals used thus far, such as mice or rats.

EMBRYO 2.0

The embryo grows up hidden from the outside world. This makes it challenging to understand its development in detail. However, Dr. Jesse Veenvliet and his team at the Max Planck Institute for Molecular Genetics in Berlin are convinced that embryonic stem cells from mice can shed more light on the matter. Embryonic stem cells are able to form all kinds of embryonic tissue. Under certain conditions, they can grow into entities that resemble early embryos. Modern research methods will be used to trace the plethora of underlying developmental processes.

More information: www.bfr.bund.de/en > German Centre for the Protection of Laboratory Animals > Bf3R research funding
(The next call for proposals for research funding will take place in spring 2021.)