

Preclinical stem cell research

Good models needed

Before stem cell-based therapies can be tested on patients, they must first be extensively investigated in a preclinical development phase. Not all processes in the body can be simulated in the test tube or on the computer, so experiments on suitable animal models are essential. But stem cell technologies can also help

to reduce the need for animal experiments in drug development. Cell cultures produced using pluripotent stem cells enable toxicology tests to be performed at an early stage, and multi-organ chips may soon make it possible to investigate the human body in miniature format.



*Stained
facial muscles
of a mouse*

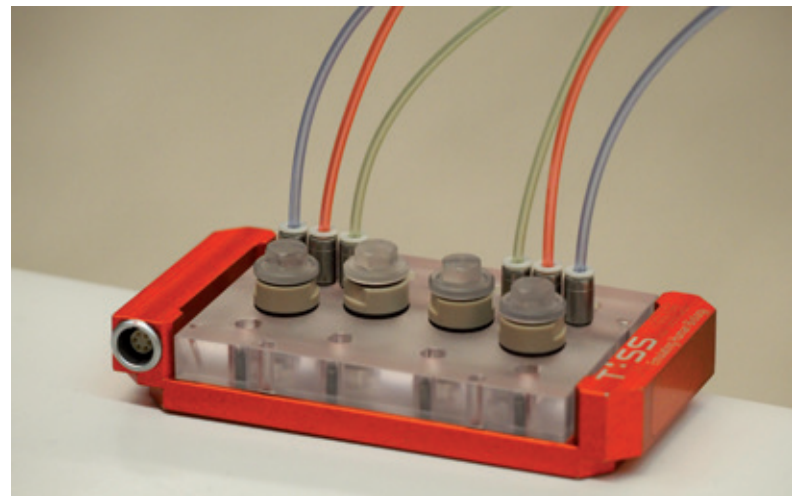
Any attempt to use cells as medical products involves dealing with highly complex, living biological systems – which makes working with them a special challenge. Before any clinical application can be trialed on humans, the safety profile and mode of action of the cell therapies must be thoroughly tested in living organisms. Animal experiments are controversial, but for the foreseeable future they will remain indispensable for biomedical research and application, because not all processes in the body can be simulated in the test tube or on the computer.

Innovative cell therapies differ fundamentally from conventional pharmaceutical substances in terms of their pharmacology and toxicology. This means that developers of cell-based medical products must draw up individual concepts for their products in order to provide the authorities with convincing data.

Small-animal models are of limited usefulness

But which animal model should be used to obtain the most informative results? The regulatory authorities favor what are known as homologous animal models. This means that the therapeutic effect of cells of a certain species is researched in a corresponding disease model – such as rat cells in rats with myocardial infarction. In other cases, heterologous models can be used – for example if human cell types are transplanted into rodents with a suppressed immune system or into “humanized” animals in order to prevent rejection.

Small-animal models – especially rodents such as rats and mice – are used in particular for early feasibility studies. “But in the later stages of development the use of appropriate large-animal models such as sheep or miniature pigs is unavoidable,” says Thomas Braun, Director of the Max Planck Institute for Heart and Lung Research in Bad Nauheim. Braun, a developmental biologist, works exclusively in basic biomedical research; he studies muscle stem cells and the regeneration of heart muscle. Mice are some of his most important subjects



This Four-Organ-Chip contains an intestine, a liver, a kidney and a skin module.

New requirements bring extra bureaucracy

According to figures from the Federal Ministry of Agriculture, 2.8 million animals were used in scientific studies in 2014 – 870,000 of them in basic research and 333,000 in translational research. While the overall number of laboratory animals has fallen slightly, the number of transgenic rodents continues to rise – a trend that is ascribed to the steadily growing use of genetic engineering.

As a result of the new EU directive on the protection of animals used for scientific purposes, Germany’s animal protection law has been amended. The new legislation came into force in 2013, with notable consequences. “The work involved in obtaining approval for animal experiments has increased dramatically,” says Braun. “Regardless of whether we are using mice or zebra fish, we must now assess each individual transgenic stem for possible stress and in some cases obtain approval for an animal experiment from the authorities.” Since breeding animals are also covered by this assessment, the overall documentation requirements

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German Cancer Research Center (DKFZ)

Research for a life without cancer

The German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ), located in Heidelberg, is a member of the Helmholtz-Association and is the largest biomedical research institute in Germany.

At the DKFZ, more than 1,000 scientists work together in order to develop novel strategies aimed at improving the prevention, diagnosis and treatment of cancer. Several research laboratories investigate normal and cancer stem cells (CSCs) as well as their respective niches. The goal is to develop strategies to monitor and

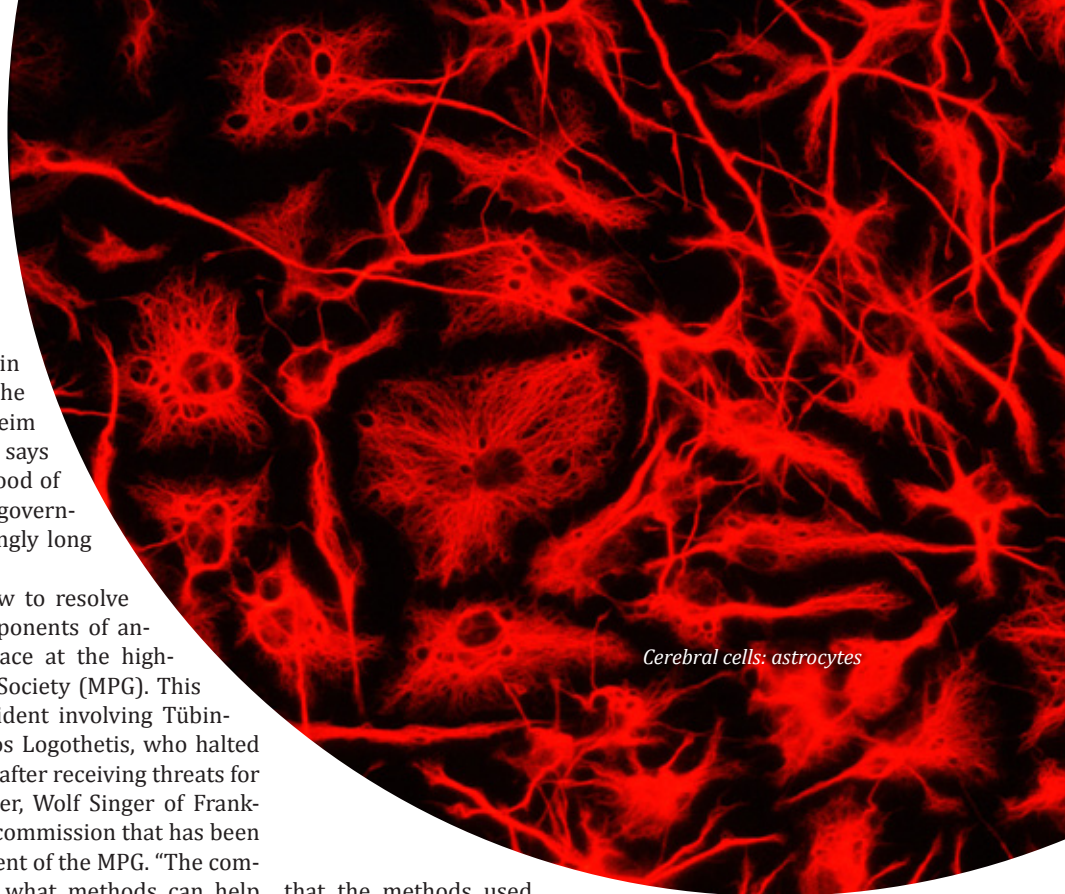
target CSCs in primary cancers and metastasis. Together with the Dietmar Hopp Foundation, the DKFZ is a shareholder of HI-STEM, the nonprofit Heidelberg Stem Cell Institute and organizes the biannual Heinrich-Behr-Conference on „Stem Cells and Cancer“, which attracts international experts in the field.

With the Heidelberg University Clinic, the DKFZ has established the National Center for Tumor Diseases (NCT), to clinically translate innovative basic cancer and stem cell research discoveries into clinical therapies.

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Cerebral cells: astrocytes

represent a significant increase in bureaucracy. To deal with this, the Max Planck Institute in Bad Nauheim has taken on extra staff. Braun says that the situation is creating a flood of paperwork, which the regional government offices take a correspondingly long time to process.

Meanwhile, deliberations on how to resolve the entrenched conflict with opponents of animal experiments are taking place at the highest level within the Max Planck Society (MPG). This was partly triggered by the incident involving Tübingen-based brain researcher Nikos Logothetis, who halted his experiments on apes in 2015 after receiving threats for months. Another brain researcher, Wolf Singer of Frankfurt, is heading an international commission that has been specially convened by the President of the MPG. "The commission is currently discussing what methods can help overcome the polarization," says Braun.

Alternatives to animal experiments

Although it might be desirable from an ethical point of view to replace all animal experiments with alternative methods of drug development, experts regard this as highly unfeasible in the foreseeable future. Nevertheless, in vitro models and simulations in silico are becoming increasingly informative and realistic; in some tests for the cosmetics industry they have already completely replaced animal experiments. Cell cultures enable toxicology studies to be carried out on potential drug candidates, especially when this involves testing their effect on heart and liver tissue in the Petri dish. Cardiotoxicity and hepatotoxicity are among the most common reasons why potentially promising substances are ruled out for further drug development.

Researchers investigating alternative methods use the principle of the 3Rs, developed in the 1950s. The 3Rs stipulate

that the methods used should either avoid or replace the use of animals (replacement), minimize the number of animals used in experiments (reduction), or minimize animal suffering (refinement). The German Centre for the Protection of Laboratory Animals (Bf3R) was established in Berlin in 2015. It forms part of the Federal Institute for Risk Assessment (BfR) and is funded by the Federal Ministry of Agriculture. Bf3R focuses on methods that completely replace animal experiments – including stem cell-based cell culture tests such as the embryonic stem cell test. This uses mouse ES cells that are differentiated into heart muscle cells, nerve cells or bone cells. These cells can then be tested to discover whether substances might be toxic to embryonic development.

Elsewhere, too, scientists are refining stem cell-based methods for use in pharmacology and toxicology research. Pharmacologists led by Thomas Eschenhagen at the University Medical Center Hamburg-Eppendorf (UKE) have succeeded in growing iPS heart muscle cells in ordered,

Photo: ZEBET / Manfred Liebsch

Max Planck Institute for Heart and Lung Research

MPI-HLR

The Max Planck Institute for Heart and Lung Research, located in Bad Nauheim, investigates developmental processes of organs in the cardiovascular system and the lung.

A second focus is molecular and cellular processes during the formation of diseases in heart, blood vessels and lung, including remodeling processes in these organs. Scientists at the institute search for new approaches to support repair and regeneration of the affected organs.

The MPI closely cooperates with universities in Frankfurt, Gießen and Marburg. It has become a major part of various federal and state excellence initiatives and contributes to two "Gesundheitsforschungszentren".

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highly organized 3D networks. The team from Hamburg generates heart muscle strips that can be used to test the effect of pharmaceuticals on heart strength.

A team led by Jürgen Hescheler of the Institute of Neurophysiology at the University of Cologne has bathed cardiomyocytes produced by iPS techniques in a toxic substance and then identified molecular biomarkers that are characteristic of cardiotoxicity. The findings come from a project of the EU consortium DETECTIVE, which is supported in part by the European Cosmetics Association (Colipa).

Working towards the ten-organ chip

In vitro models still have their limits when it comes to assessing the efficacy and safety of substances. For example, they are unable to depict the interaction of organs. In an important new development, scientists are now try-

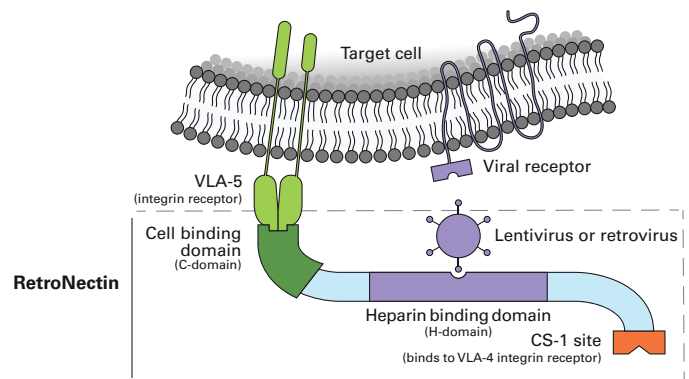
ing to model various connected human organ systems on biochips. Biotechnologists led by Uwe Marx and Roland Lauster of the Technische Universität Berlin and the company TissUse are using iPS technology to engineer human organoids that mimic even the smallest functions of a particular organ. On the chip the human organs are shrunk by a factor of 100,000. The organoids thrive in small chambers on a plate the size of an object slide. The construct is supplied with a fluid-filled system of micro-channels that resembles the bloodstream. The first of these "multi-organ chips" were dual combinations of the skin and liver or the liver and nerve tissue. The most advanced product to come out of the Berlin project is currently a four-organ chip consisting of intestine, liver, kidney and skin modules. The tissue engineers hope to present a multi-organ chip with more than 10 organs in 2018. The research is supported by the BMBF within the GO-Bio-Program. The pharmaceutical industry already regards this miniaturized human test dummy as having great potential for the preclinical research of the future.

Text: Philipp Graf

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² Bone marrow cells.
³ Peripheral blood mononuclear cells.