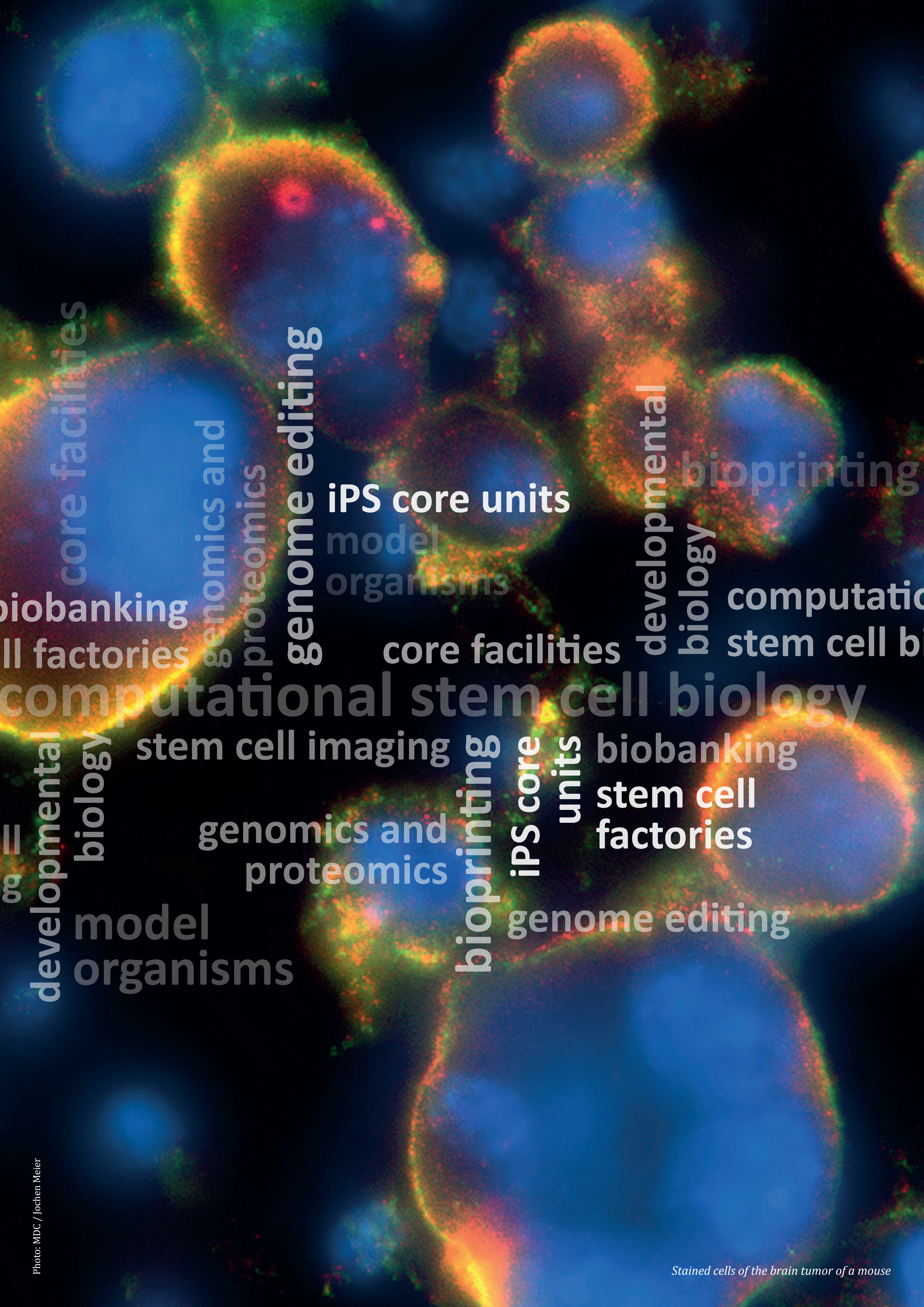


Photo: MDC / Jochen Meier

Stained cells of the brain tumor of a mouse

Stem cells from the factory

Cranking up cell production

The reprogramming technique and sophisticated procedures for generating particular cell types have revolutionized stem cell research. And the field is becoming increasingly institutionalized: many research centers in Germany are now setting up special laboratories or core facilities for cell production. Furthermore, thanks to automated state-of-the-art bioprocess engineering, stem cells are turning into an industrial commodity. With factory-produced cell types, applications in regenerative medicine are becoming a reality.

The engineering of human induced pluripotent stem cells (iPS cells) and cell types derived from them is now at the heart of the activities of many stem cell researchers. The iPS technique has revolutionized stem cell research in just a few years. Using the process first described by Shinya Yamanaka in 2006, molecular biologists can revert cells to a quasi-embryonic state simply by introducing four specific reprogramming factors. The resulting pluripotent stem cells can be propagated almost indefinitely and can theoretically give rise to every other cell type in the body.

The potential of these artificially produced stem cells is vast: cells from patients can now be propagated in the Petri dish and used to study the molecular mechanisms of disease. Such stem cell-based disease models are promising tools for drug research. iPS cells are also viewed as an important source of material for cell-based therapies. The first clinical study to be based on iPS cells was launched in Japan in 2014.

iPS technology as a service

The techniques and processes used in the generation of iPS cells are becoming increasingly robust and productive. However, there is still great demand for expertise in dealing with stem cells and for the right laboratory equipment. More and more major biomedical research institutions in Germany have started to set up in-house core facilities for stem cell production.

For example, with the opening of the Berlin Institute of Health (BIH), Berlin will be home to an international center of excellence for translational and system medicine that pools the strengths of Charité – Universitätsmedizin Berlin and the Max Delbrück Center for Molecular Medicine (MDC). The work of this new "super institute" is supported by seven technology platforms. One of them is the Stem Cell Core Facility. At the MDC's site in Berlin-Buch, biologist Sebastian Diecke and his team of four are in the process of setting up this special laboratory. Biotechnologist Harald Stachelscheid is in charge of the counterpart facility at the Charité campus Virchow-Klinikum. "Our task is to support basic and clinical research by providing all the technology for the use of human iPS cells," explains Diecke. He arrived in 2014 from Stanford University in California, where alongside his post-doc activities he worked for a similar service facility funded by the California Institute of Regenerative Medicine (CIRM).

The mission of the core facility at the BIH, which is initially funded until 2018, is broad and includes derivation, differentiation, characterization and distribution of the iPS cell lines. "A technique that is increasingly in demand is that of genome editing," says Diecke (see the chapter on Genome Editing, page 32). In this field, too, the researchers possess expertise and experience. The BIH experts also plan to store important cell lines in a biobank. In essence, the staff at the Stem Cell Core Facility do not simply see themselves as service providers; they want to be on hand to support the work of researchers with advice and practical assistance. "We provide standardized protocols and techniques, advise on projects, and offer regular practical courses in the various methods," says Diecke.

Reprogramming in a joint effort

Micha Drukker is in charge of the core facility – the hiPS Cell Unit – in the Institute of Stem Cell Research at Helmholtz Zentrum München. This, too, is an experimental platform where patients' iPS cells can be cultured, scrutinized and differentiated into particular cell types. The main function of the core facility is to serve researchers from the Helmholtz Association, but research partners in the Munich area can also make use of it. Among other things, the researchers of the Drukker group have specialized in a reprogramming technique based on artificial mRNA molecules.

Another unit, the Stem Cell Unit – Göttingen is a non-commercial facility of the University Medical Center Göttingen (UMG) that is funded by it and the German Center for Cardiovascular Disease (DZHK). The director of the unit is stem cell researcher Kaomei Guan-Schmidt, who also leads her own research group. At the Stem Cell Unit, patient-specific iPS cells are derived, characterized and provided to the Göttingen research facilities and DZHK partners. The Göttingen researchers specialize in disease models of heart failure and the differentiation of iPS cells into heart muscle cells.

Robot arm in the StemCellFactory

Photo: Life & Brain

There are other core facility initiatives for iPS production in Aachen, Bonn, Hamburg and Münster, and similar facilities are planned in Dresden and Hannover. Another iPS initiative covers university sites within the Bavarian research network ForIPS. "Each site has of course developed special knowledge in particular fields," says Sebastian Diecke. He and his colleagues Harald Stachelscheid and Micha Drukker have launched a scheme aimed at strengthening the links between core facilities in Germany and improving the exchange of knowledge. The idea is that combining expertise will make it easier to compare the different facilities' standards in producing the cells and datasets. The purpose is also to discuss and share protocols and new techniques and prevent the creation of redundant structures.

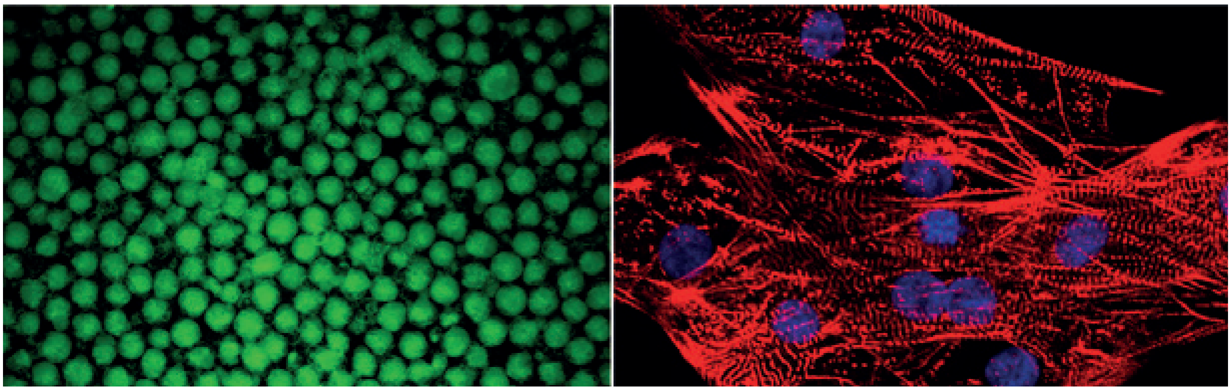
Bioreactors for mass production

Pluripotent stem cells are an important basis for the production of defined cell types. However, enormous quantities of such cells are needed for tests in industry, regenerative cell therapies, and the generation of replacement tissue *in vitro.* The team headed by Robert Zweigerdt of the Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO) at Hannover Medical School (MHH) is working on the mass propagation of pluripotent stem cells. In recent years, the team has used and refined bioreactor systems that work on the same principle as the stirred-tank reactors used in biopharmaceutical production. "Unlike in a conventional 2D culture, the cells float in a 3D suspension culture and are stirred," says Zweigerdt. "As a result, the culture conditions are highly homogeneous and we can observe the cell growth continuously online." The researchers have tweaked several aspects of the process, adapted the media, defined the culture conditions, and adjusted the stirrers. In matters of bioreactor technology, the researchers from Hannover have worked closely with Eppendorf subsidiary DASGIP.

Stem cells cling to one another

The stem cells floating in the bioreactor definitively cling to one another. "They form pure cell aggregates; that makes upscaling easier," says Zweigerdt. In their bioprocess, the researchers can track the formation of the cell clumps and control their density and size. In the bioreactors from Hannover this enables undifferentiated cell aggregates to be produced from human embryonic stem cells (ES cells) or iPS cells. Through the exchange of culture media these differentiate in the bioreactor into cell types such as heart muscle cells and endothelial cells.

"We can currently produce up to 50 million cells in a volume of 100 milliliters," says Zweigerdt. The researchers are now aiming to move to a larger scale. "We want to produce around a billion cardiomyocytes in a one-liter bioreactor." That is roughly the quantity that is destroyed and therefore needs to be replaced in a heart-attack patient. The researchers also aim to produce other cell types needed for regeneration of the heart. The team working with Zweigerdt and LEBAO research director Ulrich Martin are already planning the next step for the heart muscle cells from the bioreactor; preclinical cell therapy studies in large animal models are due to start soon. The Hannover team is also involved in one of the large European consortiums for iPS production, StemBANCC (see the chapter on Archiving Stem Cells, page 42). A key aim here is to provide heart muscle cells in large quantities for cell-based test systems in drug research and safety pharmacology.



Left: Generation of heart muscle cells monitored by the activation of a fluorescent reporter gene. The cell aggregates in the bioreactor emit green light. Right: The generated heart cells show the typical cross striations (red) around the nucleus (blue).

Photo: MHH / Robert Zweigerdt

Automated stem cell factory

The proliferation of cell products on an industrial scale for drug-testing purposes is a vision that is becoming reality at the LIFE & BRAIN research center in Bonn, where Oliver Brüstle is scientific director. In collaboration with the Fraunhofer Institute for Production Technology (IPT), RWTH Aachen, the MPI for Molecular Biomedicine in Münster, HiTec Zang, and Bayer Technology Services (BTS), the facility that has recently been set up focuses on the automated culturing of iPS cells and differentiation of those cells into nerve and heart muscle cells. “The Stem Cell Factory is a fully automated production line that reproduces the entire process from skin cell to finished iPS cell in one facility,” explains Simone Haupt, who heads the Bioengineering Segment at LIFE & BRAIN.

The roughly five-meter-long production line is a closed system: once the skin cells have been introduced, reprogramming, picking of clones, and cultivation proceed automatically. The finished product is ready in ten weeks. “The big advantage is that iPS cells from a large number of patient samples can be cultivated here simultaneously,” states Haupt. The follow-on project Stem Cell Factory II was launched in 2014, once again with the involvement of RWTH Aachen, Fraunhofer IPT in Aachen, HiTec Zang GmbH, and the MPI in Münster. The state of North Rhine-Westphalia is providing funding of €1.2 million for the project, which will see additional modules added to the facility. For example, the bioengineers are developing an automated genome editing process that will enable them to make specific modifications to the stem cell genome (see the chapter on Genome Editing, page 32). For another, external module, researchers are working on creating three-dimensional cell clusters and organoids – such as small particles of human brain or heart tissue – from differentiated cells. In addition, there are plans to develop the stem cell factory into a commercial facility, with the spin-off of a company dealing with the automation of cell culture. “We have already had a very positive response from the biobank scene,” says Haupt.

The artificial creation of stem cells has found a firm place in biomedical research facilities in Germany. And biotechnologists and engineering scientists are continuing to explore how knowledge from stem cell laboratories can be translated into industrial practice.

Text: Philipp Graf

Max Delbrück Center for Molecular Medicine (MDC)

MDC MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN BERLIN-BUCH IN DER HELMHOLTZ-GEMEINSCHAFT e.V.

Located in Berlin-Buch, the MDC carries out high-quality, interdisciplinary research on basic mechanisms and applications in major human health threats including cancer, cardiovascular and metabolic diseases, and disorders of the nervous sytem. These thematic research areas are supplemented by the Berlin Institute for Medical Systems Biology (BIMSB) at the MDC and the MDC-Charité partnership in the Berlin Institute of Health (BIH).

Professor Mathias Treier, senior group leader at the MDC, states: “Opportunities for partnerships with clinical groups, a range of cutting-edge technology platforms, and superb animal facilities for diverse model organisms make the MDC an excellent site for stem cell research.” Recently, MDC and BIH have created a stem cell core facility to offer expertise to derive and manipulate iPSC lines for MDC groups or BIH projects. Alongside assisting groups, the facility is putting an emphasis on training, says Sebastian Diecke, head of the facility.

Max Delbrück Center for Molecular Medicine (MDC), Berlin-Buch
Robert-Rössle-Str. 10, 13125 Berlin
www.mdc-berlin.de