

core facilities  
genomics and proteomics  
genome editing  
iPS core units  
model organisms  
developmental biology  
biobanking  
stem cell biology  
computational stem cell biology  
core facilities  
bioprinting  
iPS core units  
genomics and proteomics  
model organisms  
stem cell imaging  
biobanking  
stem cell factories  
genome editing

## 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

## Institute of Reconstructive Neurobiology

From disease modeling to stem cell therapies

The Institute of Reconstructive Neurobiology at the University of Bonn Medical Centre focuses on the use of pluripotent stem cells for the study and treatment of neurological disorders. Based on a broad technology portfolio including cell reprogramming, neural differentiation, direct cell fate conversion, stem cell industrialization and neurotransplantation, the Institute develops stem cell-based model systems for disease-related research and drug development as well as novel cell therapy regimens. It closely interacts with LIFE & BRAIN GmbH, a transla-

tional hub of the University of Bonn providing stem cell products and services for pharma, biotech and academia.

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universität **bonn**





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 Göt-

tingen 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.

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.

Robot arm in the StemCellFactory



Photo: Life & Brain



# hexcell Berlin GmbH



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**Our pharmaceutical biotechnology customers use our products in basic scientific research, in the pharmaceutical industry and in stem cell therapy.**



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- Fetal Bovine Serum EU approved, Fetal Bovine US origin, AB Human Male (off the clot),
- HSA, human serum albumin 20 % therapeutic grade Inject.

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.


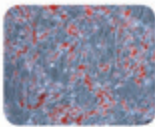
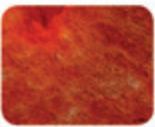

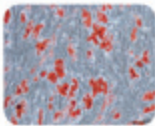
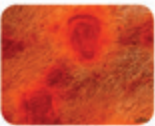

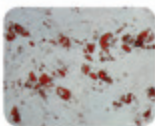
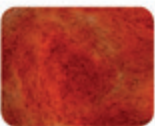

“We can currently produce up to 50 million cells in a volume of 100 milliliters,” says Zweigerdt. The researchers are now aiming to move to a larger scale. “We want to produce around a billion cardiomyocytes in a one-liter bioreactor.” That is roughly the quantity that is destroyed and therefore needs 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.

Biological Industries Releases Human Mesenchymal Stem Cell Differentiation Kits for Health Therapies



Biological Industries (BI), a worldwide leader in the design and manufacture of life science products for the biopharmaceutical industry, has launched innovative hMSC differentiation kits for research and stem cell based therapies.

“This is a unique line of serum-free and xeno-free differentiation kits, providing the ability to efficiently differentiate hMSC from various sources into adipocytes, chondrocytes and osteoblasts,” said David Fiorentini, Director of Research & Development.

 Biological Industries Culture of Excellence	Adipogenesis	Osteogenesis	Chondrogenesis
hMSC-AT			
hMSC-BM			
hMSC-CT			

All three kits are serum free and xeno free:

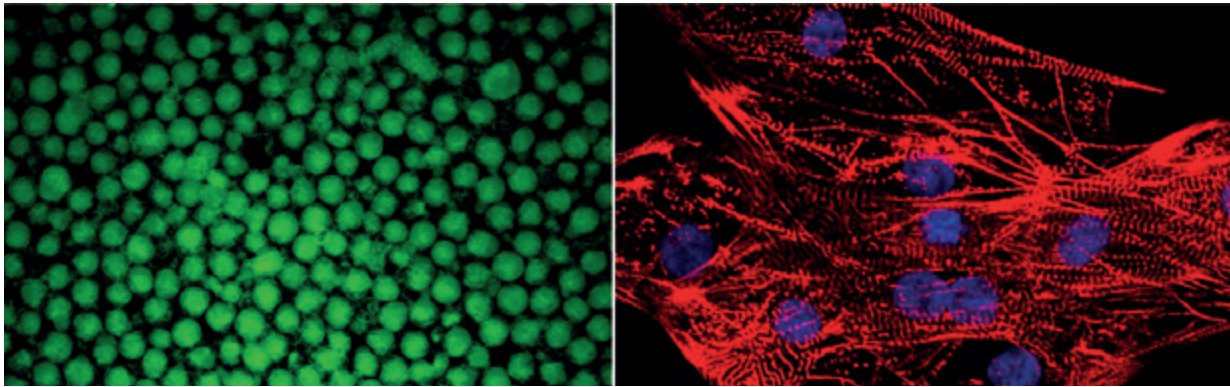
- MSC Go Osteogenic XF™ – Rapid differentiation of osteogenic (Complete, ready-to-use)
- MSC Go Adipogenic XF™ – Basal medium and supplements mix
- MSC GO Chondrogenic XF™ – Basal medium and supplement mix

“The Differentiation media contain all the growth factors and supplements necessary for the direct differentiation of hMSC,” Fiorentini said.

The kits are designed to be user friendly with all necessary ingredients included. They offer a complete system for multipotency evaluation of hMSCs and reliable induction of hMSCs into adipocytes, chondrocytes and osteoblasts/osteocytes. No adaptation is required from cell expansion cultures using MSC NutriStem® XF Medium.

The kits were validated for hMSC from various tissues including BM-hMSC, AT-hMSC, and UCT-hMSC.

BI’s previous release was the MSC NutriStem® XF Medium, a gold standard serum-free, xeno-free medium developed for the growth and expansion of human mesenchymal stem cells (hMSC) isolated from a variety of sources including bone marrow, adipose tissue and umbilical cord tissue; BM-hMSC, AT-hMSC, UCT-hMSC.



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).

### 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*

Photo: MHH / Robert Zweigert

## Max Delbrück Center for Molecular Medicine (MDC)

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 system. 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.

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