Kick-Off Report

2013 REBIRTH

Second Funding Period (2012–2017)
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REBIRTH | Foreword
Dear Reader,

In the summer of 2012 the German Research Foundation (Deutsche Forschungsgemeinschaft; DFG) and the German Council of Science and Humanities (Wissenschaftsrat; WR) decided, after thorough evaluation of the Cluster’s work and conceptual approach by an international review board, to fund the REBIRTH Cluster of Excellence (From Regenerative Biology to Reconstructive Therapy) for a further five years under the Excellence Initiative. Receiving extended funding – with a budget running into the double-digit millions – is a testament to our excellent research and allows us to further expand our expertise, not least for the benefit of patients.

Thanks to outstanding scientific work on new therapeutic concepts for the blood, heart, liver and lungs by our researchers in the first funding period of the Excellence Initiative (2006–2012), REBIRTH has become a major player in the field of regenerative medicine. The interdisciplinary interaction between biologists, chemists, engineers, physicists and medical professionals proved of great value during the Initiative’s first period. In addition to a large number of prestigious publications, there are numerous patent applications and clinical studies – relating, for example, to the therapy of postpartum cardiomyopathy, hepatic cell therapy and gene therapy for genetic immune deficiencies, as well as the implantation of heart valves that grow with the body – that document the work done by the research groups. For this reason, the REBIRTH structure is retained as far as possible in the second funding period (2012–2017), with the focus remaining on the four organ systems. Alongside our established scientists, however, many new faces from our partner institutions as well as national and international universities have joined the Cluster to reinforce its expertise.

Our aims for the next five years are to further translate our scientific achievements into clinical practice and to strengthen interdisciplinary communication both within and beyond the Cluster. In this way, REBIRTH will remain at the cutting edge and become an internationally leading centre – one that is sustainable in the long term – for innovative regenerative medicine.

We are delighted to present you with this report. It will give you an overview of the Cluster’s structural changes, you will get to know both our established and our new scientists, and you will find examples of our achievements and short descriptions of the innovative research projects of each work group.

I hope you find this report an enjoyable and interesting read.

Axel Haverich
Information about the REBIRTH Cluster of Excellence

Key facts

The REBIRTH Cluster of Excellence (From Regenerative Biology to Reconstructive Therapy) has received funding since 2006 under the German Excellence Initiative of the federal and state governments. On the strength of its many years of expertise in the field of regenerative medicine and the successful work of its research groups, it succeeded in winning through against co-bidders from an extremely wide range of research fields both in the initial phase of the programme (in 2006) and in the second (in 2012). REBIRTH’s funding is being continued for the period from 1 November 2012 to 31 October 2017, with resources running into the double-digit millions. The coordinator is Professor Axel Haverich, medical director of the Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG) at Hannover Medical School (MHH).

The aim of the Cluster of Excellence is, through interdisciplinary collaboration between the various scientific disciplines integrated within REBIRTH, to establish ourselves as an internationally renowned institution for regenerative medicine. In 60 different work groups, some 250 researchers are developing therapeutic strategies for the heart, lungs, liver and blood. Drawing on the knowledge gained from fundamental research conducted within REBIRTH and its translation into experimental medicine, new approaches and technologies are to be developed for medical use, which will be applied in everyday clinical routine for the benefit of patients.

By identifying relevant mechanisms involved in regenerative processes, we will be increasingly able to influence undesired processes in the human body and develop regenerative therapies – thus helping the body to regenerate itself. Translational studies will pave the way for clinical application.

Since 2008, REBIRTH has been based in its own building, the Hans-Borst Centre for Heart and Stem Cell Research, the construction of which was funded by the Braukmann-Wittenberg Foundation. The Centre, with a floor area of around 3,300 m², is home to 120 researchers.

The institutions participating in REBIRTH are Hannover Medical School (MHH) and seven other partners (see also page 12):
Germany’s federal- and state-level Excellence Initiative

The idea to strengthen the science landscape in Germany on a lasting basis was developed from 2004 onwards by government and the scientific community, and put in force in June 2005 by Germany’s national and federal-state administrations. The Excellence Initiative is being jointly carried out by the German Research Foundation (DFG) and the German Council of Science and Humanities (WR). There are three funding streams: graduate schools, clusters of excellence and institutional strategies.

Graduate Schools to support young researchers

The idea behind this first funding stream, Graduate Schools, is to ensure that support is provided to promising young researchers. The chief aims are to enhance conditions for doctoral students, help them to be active members of their academic and social communities, and thus contribute to developing internationally competitive locations for science. Graduate schools thus serve as instruments of quality assurance for supporting young scientists. A total of 45 graduate schools are being funded until October 2017.

Clusters of Excellence to promote world-class research

The aim of this second funding stream, Clusters of Excellence, is to enhance the research potential at German university locations through scientific networking and collaboration, and thus to raise their international profile and competitiveness. Funding in conjunction with Cluster of Excellence status is intended to enable the relevant higher-education establishments to set thematic priorities. A total of 43 such clusters are each to receive funding for a five-year period until October 2017.

Institutional strategies to promote top-level university research

The term ‘institutional strategy’ refers to a university’s strategic development in research. These strategies involve focusing on particular thematic areas in cutting-edge research and supporting promising young scientists, and define objectives for the university as a whole. In order to qualify for funding under this third stream, a university must have at least one Graduate School and one Cluster of Excellence. Overall, 11 universities (with their respective institutional strategies) are being funded until October 2017.
Information about the REBIRTH Cluster of Excellence

REBIRTH’s Research Areas A–C and Management Area M: structural diagramme
The Cluster’s structure

The REBIRTH Cluster of Excellence focuses on diseases and disorders of the blood-forming system (including immune conditions), the heart, the respiratory system and the liver. Our animal models encompass a wide spectrum, from rodents to domestic animals and nonhuman primates, with the relevant stem cell technologies incorporated. Carefully planned clinical studies are already being conducted and their scope further expanded. Research priorities range from molecular and cell biology studies of organ regeneration and stem cells to cell and tissue regeneration, and include the following:

- Cell therapies and (embryonic) stem cells, such as induced pluripotent stem cells (iPS),
- Cell reprogramming, differentiation and proliferation,
- Molecular toxicology and genetics,
- Tissue engineering,
- Materials, biomaterials and polymers,
- Nanotechnology, use of lasers, and biophotonics,
- Biothermodynamics and cryotechnology,

- Imaging techniques,
- Biocompatibility,
- Good practice (GXP): good laboratory / manufacturing / clinical practice (GLP | GMP | GCP),
- Clinical studies,
- Ethical and legal dimensions.

The interdisciplinary interaction between biologists, chemists, physicists, engineers and medical professionals proved of great value during the Excellence Initiative's first funding period. In addition to a large number of prestigious publications, there are numerous patent applications and clinical studies.

For this reason, the REBIRTH structure will be retained as far as possible, with the focus remaining on the same four organ systems. All eight institutions will continue to be involved in this research alliance during the second funding period (2012–2017). What is new is that the research groups are di-

![Fig. 1: The illustration shows the coordinated primary functions of the partner institutions involved. On the right, the interdisciplinary and inter-institutional cooperation between the research groups is shown: this cooperation exists within the various Areas and CRUs and extends to the Cluster’s Research Units.](image_url)
Information about the REBIRTH Cluster of Excellence

Table 1: Breakdown of the Areas into the various CRUs.

<table>
<thead>
<tr>
<th>Area</th>
<th>Basic sciences of Regeneration</th>
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<tbody>
<tr>
<td>CRU 1</td>
<td>Stem Cell Biology and Molecular Programming</td>
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<tr>
<td>CRU 2</td>
<td>Organogenesis</td>
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<tr>
<td>Area B1</td>
<td>Regeneration in Disease Models</td>
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<tr>
<td>CRU 3</td>
<td>Liver Regeneration</td>
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<td>CRU 4</td>
<td>Pulmonary and Vascular Regeneration</td>
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<tr>
<td>CRU 5</td>
<td>Myocardial Remodelling and Cardiovascular Regeneration</td>
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<td>CRU 6</td>
<td>Blood and Immune Regeneration</td>
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<td>Area B2</td>
<td>Regenerative Technologies</td>
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<td>CRU 7</td>
<td>Regenerative Materials and Laser Engineering</td>
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<td>CRU 8</td>
<td>Imaging Platform</td>
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<tr>
<td>Area C</td>
<td>Clinical Translation and Regenerative Products</td>
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<tr>
<td>CRU 9</td>
<td>Regenerative Pathology and Pharmacotoxicology</td>
</tr>
<tr>
<td>CRU 10</td>
<td>Regenerative Products, Clinical Trials, Ethical and Legal Dimensions</td>
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Provided into ten scientific platforms (see Table 1). These Collaborative Research Units (CRUs) underline the platforms interactive nature. Within the CRUs, researchers from different disciplines are integrated in order to encourage scientific cooperation between the different research groups and institutions, the idea being that further ‘expertise hubs’ will arise within the scientific Areas A, B1, B2 and C, as well as the new Area M. Each CRU consists of several Research Units which, in turn, bring together scientists from different departments in order to ensure the interdisciplinarity and pooling of the required know-how at all organizational levels (Fig. 1).

Area A, ‘Basic Sciences of Regeneration’, still forms the foundation. The work groups within this Area will investigate the basic principles of the regenerative sciences focusing on basic sciences of regeneration, such as stem cell biology, and organogenesis.

On the basis of scientific developments during the first funding period, however, a slightly altered structure for Area B was recommended. Area B (formerly ‘Reconstructive Ther-
apy in Preclinical Models’) is now divided into Area B1, ‘Regeneration in Disease Models’ and Area B2, ‘Regenerative Technologies’, in order to strengthen these thematic areas. In Area B1, the researchers are pursuing the biomedical approach more intensively, in order that regenerative therapies can be transferred more rapidly into clinical practice. In the technology- and method-oriented Area B2, collaboration between numerous experts within the Cluster will give rise (among other things) to a new imaging platform.

In order to ensure that new therapeutic and study concepts can be reliably implemented and rapidly made use of, an expansion of Area C is planned. Instrumental to this will be the active involvement of scientists in defining safety and efficacy guidelines that take into account ethical, clinical and legal standards.

As a result of developments during the first funding period, Area D and Management have been consolidated. The newly formed management platform is responsible for training programmes, personnel development, finance, communication and public relations. One of the strategic aims of Area M is to strengthen interdisciplinary communication both within and beyond the Cluster. This necessitates proactive interaction both between scientists and with the public. Numerous measures such as specifically encouraging communication between the scientists, as well as press releases, events and schools outreach work are aimed at helping REBIRTH to raise its profile, on a lasting basis, as a research alliance in the scientific world and among the general public. In this way, REBIRTH will remain at the cutting edge and become an internationally leading centre – one sustainable in the long term – for innovative regenerative medicine.

Coordinator: Prof. Dr. Dr. h. c. Axel Haverich

The coordinator for the Cluster of Excellence is Professor Haverich, medical director of the Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG) at MHH. REBIRTH’s success in the face of nationwide competition can be put down to Professor Haverich’s crucial role in initiating the project. Since 1996, Professor Haverich has headed up this Department, where he has expanded the scope of its main speciality, heart and lung surgery.

In 1995, Professor Haverich received the Leibniz Endowment Award for German Scientists from the DFG. He used these prize funds for setting up the Department’s basic research lab facility, the Leibniz Laboratories for Biotechnology and Artificial Organs (LEBAO). Numerous outstanding, internationally significant advances in the field of cardiac, thoracic, transplantation and vascular surgery are directly attributable to his tireless dedication in both clinical and research work.
Information about the REBIRTH Cluster of Excellence

Hannover Medical School (MHH)
Hannover Medical School (MHH) is the only independent medical university in Germany and is the central REBIRTH institution. It was founded in 1965 and is an academic and clinical organization that holds a prestigious position among the country’s elite in the fields of medicine and the natural sciences. In recent years – not least owing to Lower Saxony’s initiative to enhance higher education – special support and funding have been received in three priority areas:

- Infection, immUnity and inflammation research,
- Transplant and stem cell research,
- Biomedical technology and implant research.

This targeted support of selected priority fields has enabled MHH to become one of the front-runners in the top flight of institutions providing medical higher education. This hospital, a multi-speciality tertiary care facility with 18 medical centres, annually treats around 57,000 patients on an inpatient basis and some 415,000 outpatients.

The School is among the world’s leading centres for transplantation medicine. It heads the nationwide statistics on numbers of organ transplants per annum (more than 490) and is among the global leaders in lung transplants. Around 140 bone marrow and blood stem cell transplants are also performed here every year. MHH’s interdisciplinary research and training programmes also receive a large number of individual grants, including funding from the German Research Foundation (DFG), the German Federal Ministry of Education and Research (BMBF) and the European Union. In terms of average funds received per professor, MHH is among the very top medical teaching institutions in Germany.
The Leibniz University of Hannover (LUH)

With a student body numbering around 21,000, the Leibniz University of Hannover (LUH) is the second-largest higher education institution in Lower Saxony. Students have around 75 different degree subjects to choose from. The university’s history goes back to 1831, when it was founded as a higher vocational school. Today, the LUH is widely known on the strength of its many years of experience in both the natural and material sciences. The participating faculties of the LUH have forged interdisciplinary research links with MHH, as shown by their receiving joint funding from the DFG and the BMBF. The various research groups are instrumental in the commercialization of research findings in close collaboration with national and international industrial partners.

Hannover Laser Centre (LZH)

As an independent non-profit research institute for photonics and laser technology, the Laser Zentrum Hannover e.V. (LZH) stands for innovative research, development and consulting. The focus of its applied research in the field of photonic technologies is on optical components and systems, optical production technologies and biomedical photonics.
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University of Veterinary Medicine Hannover, Foundation (TiHo)

The University of Veterinary Medicine Hannover (TiHo) is run under the auspices of a public foundation. Founded in 1778, today it is the oldest independent college of veterinary medicine in Germany. The TiHo has close ties with both the LUH and MHH. The participating departments combine clinical diagnostics and therapeutic procedures with innovative research in biomedicine and biomedical engineering. The TiHo has unique expertise in diagnostic and therapy models, which are of great importance as models for diseases and disorders in humans.
Helmholtz Centre for Infection Research, Braunschweig (HZI)

The Helmholtz Centre for Infection Research (HZI) is a non-university research institution with more than 600 staff. An important player in the Helmholtz Association, the HZI has placed its scientific focus on infection research. The main theme around which the participating researchers’ work revolves is the complex interplay between infection pathogens and the immune system. There are numerous successful research ties with MHH, especially with all of its present collaborative research centres (SFBs). The HZI's involvement is quite crucial for the fields of immunology and cell biology and for associated technology platforms such as genomics, proteomics, mouse genetics, development of good manufacturing practice (GMP) and the production of proteins, cell and gene therapy products, and in vivo imaging. The HZI and MHH receive joint funding from the DFG, the BMBF, the EU, and the Bill and Melinda Gates Foundation.
Friedrich-Loeffler-Institute
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Max Planck Institute for Molecular Biomedicine, Münster (MPI)

More than 150 scientists from over 15 countries are engaged in research at the Max Planck Institute for Molecular Biomedicine (MPI). The institute, founded in 2001, comprises three departments: Vascular Cell Biology, Cell and Developmental Biology, and Tissue Biology and Morphogenesis. One of the MPI's chief research focuses is on the molecular biology of pluripotent stem cells and germline development. This involves strategies for reprogramming somatic cells into a pluripotent state by means of somatic cell nucleus transfer or other techniques such as cell fusion. Many national and international collaborations have been firmly established, including those with the Institute of Farm Animal Genetics in Mariensee, and MHH.

Institute of Farm Animal Genetics, Mariensee, Friedrich Loeffler Institute (FLI)

The Friedrich Loeffler Institute, Germany’s Federal Research Institute of Animal Health (FLI), is an independent higher federal authority run under the aegis of the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV). Work here centres around promoting and monitoring the health of farm animals and protecting people from infections that can be transmitted from animals to humans. The FLI is divided into seven organizational Units housing eleven sub-institutes. The Mariensee site is home to the Institute of Farm Animal Genetics. Prominent research projects at the FLI laboratories relate to the biomedical production and the characterization of transgenic cattle and pigs, to studies on cell reprogramming by somatic cell nucleus transfer on the basis of cloning, and to stem cell culture. The Institute of Farm Animal Genetics has enjoyed long and successful cooperation with MHH (in the form of a collaborative research centre). The Institute has unique expertise relating to large-animal models and tissue regeneration. These aspects are of great importance for the development of innovative approaches to cell therapy and tissue engineering, and their comparative evaluation.

Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover (ITEM)

The Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM) is an institution of the Fraunhofer Society for the Advancement of Applied Research (FhG). The spectrum of research and development activities includes preclinical / clinical pharmacological research and development, allergy and asthma research, and investigations into tissue and environmental toxicology and consumer protection, as well as the testing and registration of chemicals, biocides and pesticides. The ITEM has also collaborated successfully with MHH, the LUH and the HZI for many years.

Focusing on inter-university and cross-disciplinary work in this way has enabled the participation of non-university institutions, which was desired by government and the scientific community alike – as well as the scientific networking and collaboration that this involves within the REBIRTH Cluster of Excellence, with its strong partners – to become reality, and in a quite outstanding way.
Area A | Basic Sciences of Regeneration
Area A
The Area

Area A is covering the basic science research activities of the REBIRTH Cluster of Excellence, and uses a broad experimental repertoire to elucidate molecular mechanisms relevant to the endeavours of regenerative medicine. In particular, we are studying various aspects of murine, non-human primate, porcine and human stem cell biology, as well as mouse and zebrafish models of development, regeneration or immune regulation.

With respect to stem cell biology our main research activities are related to exploring fundamental principles in stem cell self-renewal, to basic mechanisms of epigenetic reprogramming, and to regulatory mechanisms of stem cell maintenance and differentiation in cells of various origins. Animal models are used to unravel molecular mechanisms underlying organogenesis, cell differentiation, proliferation and senescence.
The ultimate aim of our projects is to deepen our knowledge of mechanisms that underlie normal differentiation and regenerative processes as a basis for translating these into novel therapeutic solutions.

More specifically, research in the field of stem cell biology focuses on the generation of safe and therapeutically applicable induced pluripotent stem cells and their use in disease modelling, pharmacotoxicological screenings or cell / tissue replacement therapies of cardiac, pulmonary, hepatic or haematopoietic diseases. In this context innovative gene therapy approaches are developed that allow for the permanent correction of mutated genes. Additionally, the glycan recognition potential and the glycomics of stem cells are investigated in normal development and diseases.

Projects in the field of developmental biology and organogenesis investigate the function and mechanistic aspects of fundamental pathways involved in cell fate decisions, such as Notch signalling. Investigating principles of organogenesis, we will perform further research on ciliogenesis to characterize novel proteins that are important for cilia / basal body function.

Furthermore, with respect to the transcriptional control of organogenesis, we intend to elucidate the role of T-box genes during the regionalization and morphogenesis of the heart as well as during development of other organs. Our work on regenerative immunology aims to understand fundamental processes of lymphoid cell development in order to develop targeted therapies for immune regeneration.
Goals

Patient-specific iPSCs for Hepatic Therapies
Our long term goals is to study human iPS cell-derived hepatic transplants in xeno-tolerant mouse and pig models to address sustained functional capabilities and safety of the transplant. Furthermore, we will establish in vitro disease models based on patient-specific iPSCs, which will allow (high-throughput) screening of putative therapeutic substances or which could serve as valid model to evaluate the feasibility and safety of gene therapeutic approaches in preclinical studies.

Achievements / Planning

Mechanism of iPSC Generation
Inter- and intrasample variations can occur on various levels during iPSC generation (Fig. 1A). Modulation of miRNAs can improve the efficacy of iPS cell generation (Fig. 1B+C). Furthermore, we analyzed the effect of different Nanog variants in the alternative ‘Thomson-factor cocktail’.

Disease Modeling in iPS Cells
Murine and human iPSCs were investigated as models for α1-antitrypsin deficiency, targeted with shRNAs (Fig. 2A+B). Lentiviral gene correction allowed the generation of healthy mice from FAH<sup>+/−</sup> iPSCs after 4n embryo aggregation (Fig. 2C).

Planning
We will continue our collaborative projects on translational hepatology and stem cell biology, which will be substantially extended to large animal models and will be complemented by studies on hepatic transprogramming. Within the next year, we aim to provide a proof of concept for the ‘in vivo transprogramming’ of hepatic cells from liver fibroblasts in mice.
Concepts in stem cell-based translational hepatology.

Cooperations

- T. Moritz, Institute of Farm Animal Genetics, Mariensee, FLI; Institute of Experimental Haematology, MHH, Unit 1.3 iPSC based Haematopoietic Regeneration.
- A. Vogel, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.1 Molecular Mechanisms of Endogenous Liver Regeneration.
- A. D. Sharma, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.2 miRNA in Liver Regeneration.
- M. Ott, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.5 Hepatic Cell Transplantation and Genetic Manipulation.
- U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung Regeneration and Repair.
- T. Thum, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.
- JH. Klusmann, Pediatric Hemat. & Oncology, MHH, Patient-specific iPSC cells to study leukemia predisposing factors.
- A. Zeug, Institute for Neurophysiology, MHH, FRET analyses.
- R. Henschler, Transfusion Medicine LMU, Munich, Adhesion and migration characteristics of iPSC-derived MSCs.
- U. Martin, BMBF-consortium “CARPuD”, R. Bals (Homburg / Saar), M. Mall (Heidelberg), G. Hansen (MHH).
- T. Heinemann, Vallendar, H.-G. Dederer, Passau, BMBF-consortium “induced totipotency”.

Publications


Grants, awards, patents, outreach

- 2009–2014: ≈1,860,000 € extramural funding (BMBF, DFG, others).
- 2011: Pro Scientia Award of Eckhart Buddecke Foundation.
- 2012: Workshop for high school teachers: zellux.net.
- 2012: Invited Speaker at the NIH workshop on “Improving Animal Models for Regenerative Medicine”.
- 2012: Program Committee of the International Congress of the Transplantation Society (Berlin).
Unit 1.2 | IPSCs for Disease Modelling, Drug Screening and Cell Therapy

Goals

In this Unit, we focus on three topics. Firstly, we have generated iPSCs from Cynomolgus monkeys (cyiPSCs), which show high similarities to humans at physiological, cellular, and molecular levels. These cyiPSCs will be applied for investigation of basic biological aspects of primate stem cell biology and in preclinical large animal models. Secondly, we focus on optimizing the genetic correction and engineering of patient-derived iPSCs via zinc finger nuclease (ZFN)- and TALE nuclease (TALEN)-based approaches, including establishment of a TALEN assembly platform. The ability to efficiently introduce transgenes at specific safe genomic loci represents a straightforward approach for generation of clinically applicable transgenic pluripotent stem cell lines and will enable the parallel expression of several tran genes with defined expression levels. Finally, with respect to potential risks and therapeutic potential of iPSCs we are investigating the origin and emergence of genetic abnormalities in pluripotent stem cells, as well as their potential oncogenicity. In particular, we perform comparative examinations of genetic abnormalities / mutations of iPSCs derived from young vs. old somatic cells.

Achievements / Planning

We successfully generated cyiPSCs from skin fibroblasts, resulting in almost identical growth characteristics as compared to cynomolgus embryonic stem cells. We were also able to differentiate these cells into functional cardiomyocytes and to generate transgenic cyiPSC clones with stable reporter expression to enable cell tracking in recipient animals. Cardiac differentiation of the cyiPSCs is currently optimised (Unit 5.7). Additionally, our cooperation partner E. Curnow (Seattle, USA) will use the produced reporter cell lines for generation of chimeric transgenic animals.

Both the ZFN- and the TALEN-based approaches have already been successfully used in our lab to correct for genetic defects in patient-derived iPSCs. Additionally, we are currently establishing a TALEN assembly platform to generate various TALENs for several reporter cell lines for cardiomyocyte and lung-specific differentiation (Mesp, NKx2.1, CFTR). Until now, we generated two TALEN pairs for a knock-in in exon 1 of the CFTR gene, including antibiotic and fluorescence markers. The verification concerning the cutting efficiency of the generated TALENs in iPSCs is ongoing (T7 assay). We will construct TALENs specific for various known safe harbor sites, establish efficient TALEN-based targeting and comparatively assess transgene expression levels at these sites.

We were able to generate iPS cell clones from somatic cells isolated from newborns and adults. Interestingly, reprogramming rates of cells from young cell sources are considerably higher. Notably, first karyotype analyses revealed chromosomal aberrations in iPS clones from elderly patients, whereas the karyotype in clones from young sources was normal. More detailed analyses of the genetic integrity of iPS clones by karyotyping, arrayCGH and exom sequencing is ongoing.

(left): typical cyiPSC colonies (scale bar 100 µM).
(right): cyiPSC-derived cardiomyocyte on day 21 of differentiation (scale bar 100 µM). Staining: antisarcomeric α-actinin (red) and DAPI (blue).
Establishment of a ZFN-based gene targeting protocol...

...resulted in up to 1.2% eGFP<sup>negRedStar<sup>pos</sup> human iPSCs.

Cooperations

- T. Cathomen, Universitätsklinikum Freiburg (ZFN).
- E. Curnow, Washington National Primate Research Center (cyiPSCs).
- Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH (old somatic cells).
- Department of Gynaecology and Obstetrics, MHH (young somatic cells).
- G. Göhring, D. Steinemann, Institute of Cell and Molecular Pathology MHH, Unit 9.5 Cytogenetic Profiling, Unit 6.6 Genomic profiling, (Karyotyping, Array CGH).

Grants, awards, patents, outreach


Publications

Overview

Projects of the group combine work on pluripotent stem cells (PSCs) and haematopoietic stem cells (HSCs). The PSC-part is based on the work of 2012 nobel laureate Shinya Yamanaka, who introduced induced pluripotent stem cells (iPSCs) as a novel and powerful tool to modern biomedical research. In this context our group works on the reprogramming of somatic cells from mice and humans but also pigs and cattle (the latter in cooperation with Prof. H. Niemann’s group at Mariensee). With respect to HSCs and more differentiated haematopoietic cells (also see Unit 6.1) interests of our group primarily involve HSC gene therapy and HSC stem cell biology.

Research Focus

Major topics in the PSC field include the use of miRNAs to optimize reprogramming processes, the suitability of ubiquitous chromatin opening elements (UCOE) to stabilize transgene expression in PSCs and their progeny, and the development of iPSC-based gene therapy strategies for diseases caused by malfunctions of haematopoietic cells such as Pulmonary Alveolar Proteinosis (PAP).

In the field of HSC gene therapy our group aims to develop efficient haematopoietic cell based gene therapy approaches for disease states caused by haematopoietic cell malfunction or deficiency such as Pulmonary Alveolar Proteinosis PAP or anti-cancer chemotherapy-induced myelosuppression. Furthermore, we evaluate the use of humanized mouse models to assess the efficacy as well as the safety (genotoxicity) of haematopoietic cell gene therapy approaches. In terms of HSC stem cell biology our projects include the ex vivo generation of primitive and differentiated haematopoietic cells from ESCs / iPSCs, a systematic analysis of the role of miRNAs in primitive reconstituting HSCs, as well as the generation of suitable „humanized“ disease models to analyse and compare normal and leukaemic stem cells.

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Fig. 1: Structure of the A2UCOE and example of UCOE-containing lentiviral vectors for stabilization of transgene expression. Fig. 2: Scheme of patient specific iPSC-based gene therapy. Fig. 3: “Humanized” mouse model to assess the genotoxicity of viral vectors in the context of MGMT-mediated in vivo selection. Fig. 4: Scheme of HSC-based gene therapy with drug resistance (CTX-R) genes. Fig. 5: A screening assay allowing for the identification of novel small interfering RNAs modulating iPSC generation.
Group members: Thomas Moritz, Sebastian Brennig, Iris Winter, Rhui Phaltane, Mania Ackermann, Steffi Liebhaber, Adele Mucci, Nico Lachmann, Reinhard Hämmerle (lower row; from left to right).
Kevin Czarnecki, Alexandra Kuhn, Sarah Kleingeld, Miriam Hetzel, Doreen Lüttge, Nils Pfaff (upper row: from left to right).

Publications


Grants, awards, patents, outreach

- “Eva Luise Köhler Research Award for Rare Diseases 2013” dedicated to Gesine Hansen, Christine Happel, Nico Lachmann and Thomas Moritz, February 2013, for the project “Innovative Gentherapie bei seltenen monogenen Erkrankungen der Lunge”. 

Cooperations

- T. Cantz, A. D. Sharma, Department of Gastroenterology, MHH, Transgene expression during endodermal differentiation, role of miRNA in reprogramming and haematopoietic differentiation, shared platforms for iPSC-generation, joint group meetings, etc.
- H. Niemann, W. Kues, Institute of Farm Animal Genetics, Mariensee, FLI, Generation of porcine and bovine iPSCs, evaluation of porcine iPSC-derived therapeutic cells in syngenic large animals.
Goals

**Cell Surface Glycosylation during Stem Cell Differentiation**

Cell surface proteins and glycans are involved in almost all cellular differentiation and communication processes. It is the aim of this project (I) to investigate how pluripotency of stem cells and their differentiation can be characterized, influenced, or regulated by specific glycosylation patterns and (II) to use glyco-engineering approaches to aid in reprogramming or differentiation of stem cells by glycan modifications.

Achievements / Planning

- DPY-19 Mediates C-Mannosylation of Proteins on WXXW Motifs.
- GXYLT1, GXYLT2 and XXYLTI are Xylosyltransferases Acting on Notch.
- LARGE2 Generates a Xylose and Glucuronic Acid-Containing Glycan on Dystroglycan.
- Wnt Signalling is Affected by 3D Culture of hPSCs.
- Identification of Proteomic Changes Associated with Cardiac Differentiation.
- Disease Modelling of Congenital Disorder of Glycosylation by hiPSCs.
- Unravelling the Biological Role of Protein C-Mannosylation.
- Glycoproteomic Analyses of Selected Mitogens and Receptors During Differentiation of hPSCs into Cardiomyocytes.
- Glycoproteomic Comparison of 2D and 3D Culture of hPSCs.
- Analysis of Wnt-Signalling of hPSC Grown in 2D or in 3D.
Cooperations

- R. Zweigerdt, Institute of Experimental Haematology, MHH, Unit 10.2 Mass production of Pluripotent Stem Cells and their Derivatives.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.
- R. Haltiwanger, New York, USA, Notch glycosylation.
- N. Nifantiev, Moscow, RUS, Synthesis of glycan structures.
- H. Jafar-Nejad, TX, USA, Notch glycosylation.

Publications

Goals

The aim of the Research Group 'Differentiation' is the \textit{in vitro} generation of surrogate somatic cells from pluripotent stem cells for cell replacement therapy of human diseases. Our group focuses on the \textit{in vitro} differentiation of mouse and human ES cells into insulin-producing cells with similar characteristics of pancreatic beta cells as a potential cell source for the treatment of Diabetes mellitus.

Achievements

**Human ES and regenerative medicine**

The differentiation of ES cells requires a guided procedure forcing ES cells through key developmental stages from a pluripotent cell into definitive endoderm, pancreatic endoderm, and finally into a pancreatic insulin-producing cell. We have designed a new cell culture technique to develop almost pure populations of ES cells committed to the endoderm by sequential activation of the Wnt- and Nodal-signalling pathway. ES cells closely follow developmental pathways during \textit{in vitro} differentiation.

Further differentiation yielded pancreatic progenitor cells with exocrine and endocrine potential well suited for potential cell replacement therapies.

Planning

We plan to investigate the specific roles and interactions of the canonical Wnt-, TGFbeta-, and BMP-signalling pathways during later stages of mouse and human ES cell differentiation during the specification of bona fide endocrine progenitor cells.
Cooperations

- T. Thum, J. Fiedler, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration.
- M. Funaki, Clinical Research Center for Diabetes, Tokushima University Hospital, Tokushima, Japan.

Publications

Goals

Regenerative biology demands a profound knowledge of normal developmental processes. We seek to understand at a molecular and cellular level how mouse embryos develop their vascular system and functional epithelia of the lung. More specifically, we investigate structural requirements of the NOTCH ligand DELTA (DLL) during angiogenesis and identify and characterize novel factors essential for the formation of ciliated epithelia.

Achievements / Planning

**DLL1/DLL4–NOTCH signalling in the developing vascular endothelium**

The two paralogous NOTCH-activating ligands DLL1 and DLL4 show overlapping expression in the fetal vascular endothelium and are important for proper angiogenesis. We have generated Dll1Dll4 knock-in mice that express Dll4 in place of Dll1, which is disrupted in these mice. Additionally, we have generated mice in which Dll1 or Dll4 can be conditionally overexpressed.

Analyses of these lines collectively show that despite of their structural similarities, Dll1 and Dll4 function differently during angiogenesis: Dll4 cannot rescue Dll1-deficient blood vessels; and overexpression of either Dll1 or Dll4 causes embryonic lethality but at very different developmental stages (E9.0 vs. E13.5). Using an *in vitro* NOTCH activation system and mutated Dll alleles, we now set out to pinpoint the ligand domain that mediates the functional divergence of both paralogues.

**Novel ciliogenesis-associated genes**

In a set of several genome-wide microarray screens, we identified 378 genes that are putatively activated by FOXJ1, a key regulator of ciliogenesis, during lung development. 58 of these genes also depend on NOTO, the upstream activator of FOXJ1 in the node (a transient structure that forms and functionally depends on motile cilia like the respiratory epithelium). Currently, we are validating our candidates.

We have started a detailed examination of one particularly promising hit, namely FAM183b, a factor of unknown function, conserved throughout mammals. We could show that Fam183b is indeed activated by NOTO and FOXJ1 and is strongly expressed in the lung epithelium. FAM183b specifically localises to a sub-compartment of the distal centriole and initial analysis in cultured cells suggests that both its knock-down and overexpression may affect centrosome biogenesis.

**Validation of novel ciliogenesis-associated genes identified by microarray-screening.** (A) Section-in situ-hybridisation showing fetal expression of a candidate gene and its dependence on FOXJ1. (B) β-gal reporter expression of a second candidate.
FAM183b specifically localises to a distal compartment of centrioles. (A) Co-staining of endogenous FAM183b and centriole markers in IMCD3 cells. (B) ImmunoGold labelling of endogenous FAM183b on sections through centrioles in IMCD3 cells. (C,D) Schemes summarising results like the ones shown in (A,B).

Cooperations

- E. Bengal, Technion, Institute of Technology, Israel, Characterization of ciliary genes.
- O. Dittrich-Breiholz, Physiological Chemistry, MHH, Microarray screens.
- H. Domingos, University Lisbon, Dll1 / Dll4 function.
- D. Hilfiker-Kleiner, Department of Cardiology and Angiology, MHH, Unit 5.2 Endogenous Regeneren Mechanisms of the Heart.
- U. Just, University Kiel, NOTCH target genes.
- A. Kispert, Institute of Molecular Biology, MHH, Unit 2.2 Transcriptional Control of Organogenesis.
- E. Kremmer, GSF, Munich, Generation of monoclonal antibodies.
- A. Krueger, Institute of Immunology, MHH, Unit 2.3 Regenerative Immunology.
- H. Lickert, IDR, Munich, Fluorescence-labelling of cilia and basal bodies in vivo.
- B. Macek, Proteome Center, Tübingen, Mass Spec analyses.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.

Publications


Grants, awards, patents, outreach

- NMWK / Technion: Identification and characterization of novel components required for cilium formation and function.
- DFG Normalverfahren: Functional characterization of a novel basal body / centrosomal protein.
Goals

Transcriptional control of organogenesis by T-box proteins

Our previous work has shown that Tbx2/Tbx3, Tbx18 and Tbx20 control important processes in the development of the heart, the lung and the liver. We wish to analyze their regulation and function in organ development by

- Phenotypic analysis of the role and potential of Tbx18 in the myocardium.
- Identification of protein interaction partners and target genes of Tbx18.
- Identification of protein interaction partners and target genes of Tbx20.
- Phenotypic analysis of the function of Tbx2/Tbx3 in lung development.
- Identification of upstream regulators and downstream targets of Tbx2/Tbx3 in the lung.

We have shown that

- an activating form of Tbx18 induces premature smooth muscle cell differentiation of epicardial cells (Greulich et al., 2012).
- Tbx18 and Wt1 are required for formation of a complete pleuropericardial membrane (Norden et al. 2012).
- Tbx2 and Tbx3 define the myocardium and induce cushion formation in the atrioventricular canal (Singh et al., 2012).
- a myocardial fate of epicardial cells cannot be deduced from Wt1-cre based fate mapping approaches (Rudat and Kispert, 2012).
- Canonical Wnt-, Hh-Fgfr1 / 2-signalling is dispensable for epicardial development (Rudat et al., manuscript in revision).
- atrial and ventricular misexpression of Tbx18 is deleterious for heart function.
- Tbx2 maintains proliferation in the mesenchyme of the developing lung (Lüdtke et al., 2013).

We plan to

- finish the analysis of the role and potential of signalling pathways (Wnt-, Pdgf-, Fgf- and Shh) in epicardial and pericardial development.
- continue and finish the analysis of mice with conditional misexpression of Tbx18 and Tbx18VP16 in the developing atria and ventricles.
- validate the relevance of Tbx18 and Tbx20 Y-2H candidates by pull down assays and start the functional analysis of some of these interactions.
- complete the phenotypic analysis of the role of Tbx2 in the mesenchyme of the developing lung in loss-and gain-of-function situations in vivo.
- get first insight into the combined function of Tbx2 and Tbx3 in lung development using a conditional double knockout approach.
- test the role of Shh-signalling as upstream regulator of Tbx2/Tbx3 in lung mesenchyme.
- describe a set of genes controlled by Tbx2 in the lung mesenchyme by microarray analysis of Tbx2-deficient lungs.
Publications


Cooperations

- J. Heineke, K. Wollert, K. Schuster-Gossler, Department of Cardiology and Angiology, MHH.
- V. Christoffels, Academic Medical Center, Amsterdam.
- M. Mark Taketo, Kyoto University, Global COE Program.
- M. Mommersteeg, London.
- D. Epstein, University of Pennsylvania, Perelman School of Medicine, Philadelphia.
- L. Pasano, IBDM Development Biology Institute of Marseille.
- Wang, Beijing.

Grants, awards, patents, outreach

- Niedersachsen-Israel grant submitted.
- Thyssen grant submitted.
- DFG grants in preparation.
Goals

**Regeneration of the adaptive immune system**

More than 50 years after its inception bone-marrow transplantation to treat lethal diseases such as leukemias/lymphomas remains associated with a prolonged phase of immunodeficiency due to failure of efficient regeneration of the adaptive immune system. It is our long-term goal to better understand the fundamental processes of lymphoid cell development in order to ultimately harness this knowledge to develop targeted therapies for immune regeneration.

Achievements

- We discovered that miR-181a/b-1 is a critical regulator of iNKT cell development by directly interfering with T cell receptor signalling in the thymus.
- We identified a novel regulatory circuit linking immunoglobulins as products of the adaptive immune system to differentiation of dendritic cells. This novel regulatory circuit might be harnessed to optimize immune reconstitution in bone-marrow transplantation settings.

Planning

- To develop and employ a quantitative model of lymphoid development using cellular bar coding coupled with high-throughput sequencing.
- To define miRNAs that act as principal modulators or parts of regulatory networks controlling lymphoid development.
- To develop a spatio-temporal model of early lymphoid development.
- To optimize humanized mouse models to identify and characterize human T cell progenitors.
Cooperations

- I. Prinz, Institute of Immunology, MHH.
- M. Eder, M. Scherr, Department of Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.7 Molecular Control of Granulocytic Differentiation.
- A. Gossler, Institute of Molecular Biology, MHH, Unit 2.1 Notch Signalling and Ciliogenesis.
- J. Bohne, Institute of Virology, MHH.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.
- S. Weiss, Molecular Immunology, Helmholtz Centre for Infection Research, Braunschweig.
- U. Kalinke, Twincore, MHH.
- R. Naumann, MPI-MCBG, Dresden.
- C. Klein, LMU, Munich.
- S. Herzog, Freiburg University.
- J. Tsang, NIH, Bethesda, USA.
- A. Liston, KU Leuven, Belgium.
- CYTHERIS S.A., France.

Publications


Grants, awards, patents, outreach

[Image of GRANTS, AWARDS, PATENTS, OUTREACH]

[Image of SFB 738]

Head of Department
Prof. Dr. Reinhold Förster
Department
Institute of Immunology, MHH
Unit 2.4 | Zebrafish Cardiovascular Developmental Genetics

Goals

We are studying the embryonic zebrafish heart, a relatively simple organ compared with its mammalian counterpart, to understand the assembly of the early heart tube. We would like to understand: How do myocardium and endocardium communicate during cardiac morphogenesis? What determines the differentiation of endocardium into its different morphological derivatives? In collaboration with clinical researchers, we develop animal models for human cardiovascular diseases. Our long-term interest is to understand how the cellular mechanisms controlling zebrafish cardiogenesis shape the human heart and its associated blood vessels.

Achievements / Planning

TGF-β signalling in cardiogenesis

An important part of our research is to elucidate the signals that regulate the morphogenesis of myocardium and endocardium and to understand to what extent these two tissues communicate during heart formation. One focus is on the mechanisms by which TGF-β signalling (mainly Nodals and BMPs) control cardiac morphogenesis. Recently, we found that Nodal, via modulating the ECM within the left cardiac field, dampens the efficiency of BMP signalling. Another research aim is to decipher the molecular signalling events that determine the differentiation of endocardium into its different morphological derivatives such as cushion cells.

Cerebral cavernous malformations

In another study, we have used zebrafish ccm mutants and human patient cavernoma tissue to identify the underlying molecular mechanism in this group of diseases. Our study revealed that the CCM pathology involves increased TGF-β and angiogenesis signalling and that Krüppel-like factor 2 (Klf2) activity is a central component in these pathways. We are now isolating bioactive compounds that suppress the ccm mutant phenotype or other disease phenotypes in zebrafish with potential therapeutic and translational benefits for the human patient.

(A) Schematic diagram illustrating that the Nodal target Hyaluronan synthase 2 dampens Bmp activity within the left cardiac field which causes lower expression of non-muscle myosin II and higher cardiac progenitor cell motility. (B) Cross section through the cardiac cone (myocardial cells marked green; F-actin, red).
Cooperations

- M. Affolter, The Centre of Molecular Life Sciences, University of Basel, Claudin-5.
- C. Albiges-Rizo, Department of Cell Differentiation and Transformation, Institute Albert Bonniot, Grenoble, Vascular pathologies.
- J. Bakkers, Hubrecht Institute, Utrecht, BMPs in cardiac development.
- J. Fielitz, Experimental and Clinical Research Center (ECRC) at the Max-Delbrueck Center for Molecular Medicine (MDC) Berlin-Buch, MURFs in cardiac development.
- V. Haucke, Department Molecular Pharmacology and Cell Biology, FMP, Berlin, Wnt signalling.
- A. Haverich, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Vascular pathologies.
- J. V. Kries, FMP, Berlin, Pharmacological screens in zebrafish.
- F. Rosenbauer, Institute of Molecular Tumor Biology (IMTB), Medical Faculty of the WWU Münster, PU1, miRNAs and macrophage development.
- U. Sure, Clinic for Neurosurgery, Universitaetsklinikum Essen, Vascular pathologies.
- P. Vajkoczy, Department of Neurosurgery with Pediatric Neurosurgery, Charité, Berlin, Vascular pathologies.

Publications


Grants, awards, patents, outreach

- **DFG Normalverfahren**: Genetics of endocardial-myocardial interactions during zebrafish heart development.
- **DFG Normalverfahren**: Investigations on the roles of Claudins during zebrafish cardiovascular development.
Area B1 | Regeneration in Disease Models
Area B1
The Area

Regeneration in Disease Models – this is the common goal of 25 REBIRTH Units in Area B1. They are organized into four collaborative research Units corresponding to the organ systems targeted in REBIRTH, namely the liver, the lung (including vessels), the heart, and the blood system (including research focusing on immunity).

All Units follow a translational and interdisciplinary approach to developing new regenerative therapies. Based on cell culture models, we are investigating potential therapeutic approaches while moving forward from rodent and large-animal models to first-in-man trials. Our novel treatment strategies are based, *inter alia*, on small molecules, RNAi, recombinant proteins or vectors enabling reversible, inducible and dose-controlled interventions. Novel findings in stem cell biology and strong interaction with researchers from Area A facilitate new strategies for cellular therapies including iPSCs or ESCs. Advanced tissue engineering approaches will be used to reconstitute organ integrity in conjunction with novel strategies to induce immune tolerance against transplanted cells and tissue-engineered constructs. It is the aim of Area B1 to advance therapeutic concepts based on endogenous regeneration, gene and cell therapy, tissue engineering, or biohybrid devices, and to carefully choose and adjust these strategies according to the requirements for regeneration in the relevant organ system.
In **CRU 3** we aim to explore novel therapeutic strategies for liver regeneration and inherited metabolic liver diseases. Our research involves different animal models; our sights are, however, very much on clinical translation. For example, liver regeneration is investigated in humanized mice. These transgenic animals accept human hepatocyte transplants and can be used to study the molecular mechanism of cell cycle proliferation of human cells. Our research also focuses on the role of the immune system and miRNAs for liver regeneration, and includes hepatobiliary regeneration. Different cell sources are analysed regarding their potential for gene and cell therapies as well as for drug-testing purposes, including pluripotent stem cells, human adult liver stem cells and cells generated by transprogramming of hepatic phenotypes.

For lung regeneration and repair in **CRU 4**, we focus both on device development and on cell therapy strategies. The concept of biofunctionalization is applied in the development of an endothelialized extracorporeal membrane oxygenator (ECMO). This strategy should prolong the (hitherto limited) time the device is functional for gas exchange before its hollow-fibre surface is blocked by adhesion of blood components. In the long run, this could lead to the development of an implantable bio-artificial lung. Further research aims at *ex vivo* lung regeneration using the Organ Care System (OCS) and at *ex vivo* gene therapy for cystic fibrosis and other genetic diseases, with functional repair using cell therapy. CRU 4 also develops new strategies for vascular regeneration; here we investigate, for example, the relationship between senescence, endothelial dysfunction and cardiovascular disease. Cell engineering could allow the generation of tunable ‘synthetic’ cells such as conditionally immortalized human endothelial cells, which form perfused vessels after transplantation into mice.

**CRU 5** combines Units with different strategies for regeneration of the heart. Novel secreted factors can be identified using bio-informatic secretome analyses and will be implemented for non-cell-based strategies toward cardiac regeneration. Our research on the regenerative milieux of the heart will give new insight into the mechanisms of endogenous regeneration, the role of paracrine factors and micro-RNAs in different cardiac cell types, and the importance of cellular cross-talk. In addition to gene therapy approaches to promoting cardiac function for the treatment of heart failure and pathological hypertrophy, vascular remodelling and regeneration will also be targeted using antagonirs, LNAs, or small molecules, as well as specialized myeloid cell populations for arterial regeneration. Our tissue engineering strategies for the heart aim to provide heart valve prostheses and bio-artificial cardiac tissue for reconstructive therapy, which are tested in small- and large-animal models of cardiovascular disease. For clinical application, we plan to establish non-immunogenic or fully autologous grafts.
For regenerative therapies of the blood and immune system, CRU 6 has a strong focus on cell and gene therapy. Novel strategies include enhanced and synthetic cells with improved engraftment and reconstitution potential. We investigate several ‘key players’ in the haematopoietic system, such as haematopoietic stem cells, but also dendritic cells and myeloid cell lineages including granulocytes and monocytes / macrophages. The identification of mechanisms of myelopoiesis and crucial factors for lymphatic regeneration should lead to novel therapeutic concepts based on gene therapy, microRNAs or cellular vaccines. At the same time, we are developing safer systems for gene therapy and new strategies for improved graft survival based on HLA-silenced cells and tissue for transplantation. Our research in CRU 6 focuses both on preclinical validation in animal models, including humanized mice, and on clinical translation.

Projects in Area B1 strongly rely on the regenerative technologies that are developed in Area B2. Our tissue engineering efforts are supported by the development of new regenerative materials; laser engineering facilitates cell and tissue modification; and many regenerative processes could not be analysed in detail without the help of our imaging platform. In the future, we expect even closer interaction with Area C as well, to further advance the ongoing validation and clinical translation of the presented strategies.

By now, research in Area B1 has demonstrated the therapeutic benefit of a number of new strategies in proof-of-concept-studies targeting, for example, various forms of leukaemia. New treatment options have been developed in close collaboration with strong national and international partners. These have been the basis for ongoing clinical trials such as ‘ESPOIR’, a European Clinical Study for the Application of Regenerative Heart Valves, and ‘CATCH-AMI’, which is investigating CXCR4 AnTagonism for Cell Mobilization and Healing in Acute Myocardial Infarction. In addition, researchers have filed a number of patents, among them patents for several miRNA-based therapies to improve cardiac remodelling in mouse models of cardiac disease.
CRUs and Units

**CRU 3**

**Liver Regeneration**

Unit 3.1  Molecular Mechanisms of Endogenous Liver Regeneration
Unit 3.2  Hepatobiliary Regeneration
Unit 3.3  miRNA in Liver Regeneration
Unit 3.4  Hepatic Cell Therapy - Patient Liver Stem Cells
Unit 3.5  Hepatic Cell Transplantation and Genetic Manipulation

**CRU 4**

**Lung and Vessel Regeneration**

Unit 4.1  Biohybrid Lung
Unit 4.2  Lung Regeneration and Repair
Unit 4.3  Senescence in Vascular Regeneration
Unit 4.4  Rational Cell Engineering

**CRU 5**

**Myocardial Remodelling and Regeneration**

Unit 5.1  Secreted Factors and Non-Cell-Based Strategies for Cardiac Regeneration
Unit 5.2  Endogenous Regeneration Mechanisms of the Heart
Unit 5.3  Myocardial Cellular Crosstalk and Gene Therapy
Unit 5.4  Vascular Remodelling and Regeneration
Unit 5.5  miRNA in Myocardial Regeneration
Unit 5.6  Tissue Engineered Valves
Unit 5.7  Myocardial Tissue Engineering
Unit 5.8  Large Animal Models for Myocardial Repair
Unit 5.9  Regenerative Agents

**CRU 6**

**Blood and Immune Regeneration**

Unit 6.1  Enhanced and Synthetic Cells for Regeneration
Unit 6.2  Regenerative Gene Therapy
Unit 6.3  Tolerogenic Cell Therapy
Unit 6.4  Regenerative Immune Therapies Applied
Unit 6.5  Engineered Antigen-presenting Cells and Artificial Lymph Nodes
Unit 6.6  New Regulatory Mechanisms of Myelopoiesis
Unit 6.7  Molecular Control of Granulocytic Differentiation
Goals

- Liver regeneration in humanized mice.
- Dissecting the role of the immune system for liver regeneration.

Achievements / Planning

Liver regeneration in humanized mice

FAH\(-/-\)/Rag2/Il2rg\(-/-\) mice have been generated, which accept human hepatocytes. Liver repopulation can be continuously monitored in these mice by measuring human albumin levels in the serum and by FAH\(-/-\) immunohistochemistry once the mouse is scarified.

We will optimize the transplantation protocol to achieve a robust and reproducible repopulation with human hepatocytes.

Once the protocol is established, we will use the humanized mice to study molecular mechanism of cell cycle proliferation in human hepatocytes.

Dissecting the role of the immune system for liver regeneration

We generated several immunosuppressed mouse models in the FAH\(-/-\) background. We identified three mechanism of liver regeneration in Fah\(-/-\) mice: stem cell activation, hepatocyte proliferation (hyperplasia) and hepatocyte growth (hypertrophy). The immune system is required for efficient liver regeneration.

Elucidating the molecular mechanism by which the immune system contributes to liver injury and regeneration.

Tyrosine catabolism.

Humanized mice 22 weeks after transplantation.
Cooperations

- R. Geffers, Helmholtz Center for Infection Research, Braunschweig, Genome Analytics Group.
- T. Longerich, Institute of Pathology, University Hospital Heidelberg, Heidelberg.
- S. Herzig, University Heidelberg, Heidelberg.
- C. Dorrell, Department of Genetics, Oregon Stem Cell Center, Health and Science University, Portland, USA.
- A. Sharma, T. Cantz, M. Ott, Department of Gastroenterology, MHH, Hannover.
- A. Gross, Weizmann Institute, Rehovot, Israel.

Grants, awards, patents, outreach

- Deutsche Forschungsgemeinschaft SFB / Transregio 77.
- Wilhelm-Sander Stiftung.
- DFG.
- GIF.

Publications

Goals

Molecular and Cellular Mechanism of Biliary Regeneration and Differentiation

Biliary Regeneration is an underinvestigated issue in liver regeneration upon acute and chronic liver damage. Noteworthy, misregulation of bile duct homeostasis is one of the major causes of chronic liver graft failure, known as ischemic-type biliary lesions (ITBL), after liver transplantation. Multiple other critical conditions can lead to secondary sclerosing cholangitis (SSC) exhibiting severe bile duct destruction (Fig. 1).

In our collaborative project we will elucidate the endogenous regeneration of bile duct cells form common hepatic progenitor cells, i.e. the differentiation of stem cells into functional bile duct cells in vitro and in vivo. Furthermore, we will analyze the molecular regulation of bile duct regeneration after liver damage (metabolic liver damage, partial hepatectomy, toxic injury) in our experimental models and in clinical specimens. Both approaches aim for novel therapeutic approaches for the prevention and treatment of bile duct-associated pathologies (Fig. 2).

Achievements / Planning

Corner Stones of Hepatobiliary Regeneration

Within the REBIRTH cluster our three groups actively collaborate in various projects with a strong focus on hepatic stem cell biology, hepatic cell transplantation, and liver regeneration. In particular, we have comprehensive expertise in endoderm differentiation of pluripotent stem cells, in molecular characterization of liver regeneration and in manipulating hepatic regeneration by means of microRNAs and shRNAs transfection in vivo and in vitro.

Biliary Differentiation from Hepatic Precursor Cells

With respect to biliary differentiation we will establish protocols to isolate und propagate biliary cells from murine livers and evaluate protocols for the differentiation of stem cell-derived bipotent hepatic progenitor cells into biliary cells. Furthermore, we will analyse microRNA profiles of stem cell-derived biliary cells in comparison to undifferentiated pluripotent stem cells, fetal progenitor cells and mature bile duct cells to identify microRNAs that promote and maintain biliary differentiation capabilities.

Molecular Characterization of Biliary Regeneration

In our established experimental models of biliary disorders (Mdr2⁻/⁻-mice, toxic injury: DDC/CDE diet) gene expression profile will be performed to identify relevant biliary genes and microRNAs during hepatobiliary regeneration. This data will be correlated to data from clinical specimens, obtain from patients suffering from secondary sclerosing cholangitis (SSC), ischemia-type biliary lesions (ITBL) and other biliary disorders.

Tissue-specific expression of transgenes and small RNAs in Bile Duct Cells

In previous experiments a transductions systems for bile duct cells was established, which takes advantage of a biliary promoter driving the transgene expression. This construct can be applied in vitro and in vivo using an Adeno-Associated Virus 8 (AAV8) vector system.
understanding of the pathologic changes in hepatobiliary regeneration as fibrotic tissue remodelling and the rarefication of small bile ducts a deeper. For future therapies addressing the resulting sclerosing cholangitis ischemia, chronic inflammation, or other mechanisms lead to (secondary)

In various disease entities the physiologic regeneration is impaired and / or hepatocytes to maintain proper hepatobiliary functions.

A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy. Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration.

Cooperations

T. Cantz, MPI-Cell and Developmental Biology; Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 1.1 Translational Hepatology & Stem Cell Biology.

T. Moritz, Institute of Farm Animal Genetics, Mariensee, FLI; Institute of Experimental Haematology, MHH, Unit 1.3 iPSC based haematopoietic regeneration.

A. Vogel, Department of Gastroenterology, Hepatology and Endocrinology, MHH Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit3.1 Molecular Mechanisms of Endogenous Liver Regeneration.

A. D. Sharma, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.2 miRNA in Liver Regeneration.

M. Ott, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.5 Hepatic Cell Transplantation and Genetic Manipulation.

U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung regeneration and repair.

T. Thum, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration.

A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.

Fig. 2: The mature hepatocytes and cholangiocytes as well as tissue-specific progenitor cells, namely “oval cells” are the most important cell types contributing to Hepatobiliary Regeneration. During fetal liver development, bipotent liver progenitor cells occur and give rise to bile duct cells and later mature cholangiocytes as well as to hepatoblasts and later mature hepatocytes. Stem cell-based hepatobiliary regeneration aims to mimic the differentiation of pluripotent stem cells into such hepatic progenitor cells and their subsequent specification into functional cell types, such as cholangiocytes.

Normal bile ducts display an even lumen that ensures an unimpaired bile flow, which is necessary for proper detoxification metabolism of the liver. Diseases affecting the hepatocytes, which contribute to the bile canaliculi with their apical (canalicular) membrane surface, as well as conditions affecting the cholangiocytes directly lead to proliferation of cholangiocytes and / or hepatocytes to maintain proper hepatobiliary functions.

In various disease entities the physiologic regeneration is impaired and ischemia, chronic inflammation, or other mechanisms lead to (secondary) sclerosing cholangitis. For future therapies addressing the resulting fibrotic tissue remodelling and the rarefication of small bile ducts a deeper understanding of the pathologic changes in hepatobiliary regeneration as well as in concepts for cell-based therapies is of imminent importance.

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Goals

Every year more than 70,000 patients in Europe die from various forms of liver failure. Therefore, our long term goal is to identify novel stimulatory cues that enable generation, expansion and proliferation of hepatocytes for the treatment of end stage liver diseases. To achieve this, we aim to identify specifically, microRNAs (miRNAs), small non-coding posttranscriptional regulators, in order to enhance hepatocyte proliferation and liver regeneration to overcome obstacles of cell-based therapies for liver disease.

Achievements / Planning

- We were the first to demonstrate that miRNAs are required for normal hepatocyte proliferation during liver regeneration. (Song*, Sharma* et al. Hepatology 2010).
- We were the first to provide evidence that ectopic expression of miR-221 promotes survival of hepatocytes during acute liver failure. (Sharma et al. Hepatology 2011).
- We were the first to establish the role of miR-221 as a promoter of hepatocyte proliferation during liver regeneration. (Yuan et al. Hepatology 2012).

<table>
<thead>
<tr>
<th>Year</th>
<th>Milestones to be achieved</th>
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<tbody>
<tr>
<td>2013</td>
<td>Identification of novel differentially regulated miRNAs during liver regeneration.</td>
</tr>
<tr>
<td>2014</td>
<td>Elucidation of roles of identified miRNAs in primary mouse and human hepatocytes.</td>
</tr>
<tr>
<td>2015</td>
<td>Elucidation of the functions of identified miRNAs in mouse and human hepatoma cells.</td>
</tr>
<tr>
<td>2016</td>
<td>In vivo assessment of miRNA modulation on liver regeneration in mouse models.</td>
</tr>
<tr>
<td>2017</td>
<td>Evaluation of miRNA-modulated human hepatocyte proliferation in humanized mouse models of liver regeneration.</td>
</tr>
</tbody>
</table>

Three ongoing research areas in laboratory.
Cooperations

- T. Cantz, MPI-Cell and Developmental Biology; Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 1.1 Translational Hepatology & Stem Cell Biology.
- T. Moritz, Institute of Farm Animal Genetics, Mariensee, FLI; Institute of Experimental Haematology, MHH, Unit 1.3 iPSC based haematopoietic regeneration.
- M. Ott, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 1.3 iPSC based haematopoietic regeneration.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.
- A. Vogel, Department of Gastroenterology, Hepatology and Endocrinology, MHH Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.1 Molecular Mechanisms of Endogenous Liver Regeneration.
- T. Cathomen, University Medical Center Freiburg: Development of targeted genetic engineering for treatment of liver diseases.

Publications


Grants, awards, patents, outreach
Goals

Patient Specific Adult Liver Stem Cells
Our work focuses on human hepatic progenitor cell populations isolated from liver cell suspensions obtained from partial hepatectomies by our clinical cooperation partner. Following the optimization of the isolation procedures of these cells their thorough genetic and functional characterization will be performed together with the examination of their maturation and differentiation potentials.

Achievements / Planning

In vitro culturing
Enrichment, purification, culture and N2-storage of human liver stem cells are now possible. Cells can be passaged at low ratios and show no visible signs of senescence. The cells exhibit stunning morphologic plasticity (Fig. 1) and sensitivity to the slightest changes of culture conditions, strongly indicating an undifferentiated stem cell phenotype.

Genetic Characterization
A panel of numerous calibrated RT-qPCR primers is used that provides a reliable overall phenotypic picture by showing the expression of genes specific for hepatocytes and their metabolism, transcription, secretion, or maturation state. We compared our progenitor cell population to primary hepatocytes kept in culture for up to 168 hours. EpCAM, Wnt2, Wnt10b and CK19 are up-regulated in the progenitor cell population, while the expression of Albumin and most liver metabolic markers (and also AFP) is greatly decreased but measurable in this cell type. (Fig. 2)

Future Goals
In vivo functional testing of the adult liver stem cells in immunodeficient FAH mouse models will be carried out with our cooperation partners. For a live and long term staining of progenitors, EpCAM specific gold nanoparticles made by our collaboration partner will be applied. Also, upon in vitro hepatocytic maturation, cells will be used for drug and drug-toxicity testings.
Cooperations

- F. Vondran, Clinic for General, Abdominal and Transplant Surgery, MHH, Primary human hepatocytes.
- A. Vogel, Department of Gastroenterology, Hepatology and Endocrinology, MHH Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.1 Molecular Mechanisms of Endogenous Liver Regeneration.
- L. Sajti, Laser Zentrum Hannover, Unit 7.3 Nanoparticles for durable live-staining of stem cells in tissue culture.
- K. Niehaus, CeBiTec, Bielefeld, Metabolomic analysis of in vitro matured human hepatocytes.

Publications

Goals

**Goal 1**
- Ex vivo and in vivo somatic cell transprogramming of hepatocyte and pancreatic β-cell phenotypes.

**Goal 2**
- Targeted in vivo AAV8 mediated genome editing with zinc finger nucleases for the gene therapy of inherited metabolic liver diseases.

Achievements

**Goal 1**
- RIP activation by PDX-1, MAF-A, NGN-3 in human liver cells.
- Conversion of human fibroblasts into hepatocyte like cells by overexpression of HNF-1, HNF-3 and Gata-4 and HNF-4.
- In vivo expression of HNF-1, HNF-3,Gata-4 and HNF-4.

**Goal 2**
- Targeted integration of the FAH gene into the Rosa26 locus has been shown by qPCR and serial hepatocyte transplantation in FAH(−/−) mice.

Planning

**Goal 1**
- Transplantation of β-cell surrogate cells in diabetic mice.
- Adenovirus mediated transprogramming of myofibroblasts in fibrotic mice.

**Goal 2**
- Treatment of mouse cohorts with AAV-FAH or AAV-FAH + AAV-Znf and survival analysis.
- Genome editing in humanized mouse liver.

Transprogrammed hepatocyte-like cells (iHeps).
Clonal expansion of gene corrected cells.

Genomic insertion of FAH into ROSA26 gene locus.

Group members: Michael Ott, Asha Balakrishnan, Martin Pacher, Sabine Brandes, Norman Junge, Viola Stückemann, Qinggong Yuan (from left).

Cooperations

- T. Cantz, MPI-Cell and Developmental Biology; Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 1.1 Translational Hepatology & Stem Cell Biology.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.
- A. D. Sharma, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.2 miRNA in Liver Regeneration.
- U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung regeneration and repair.
- H. Niemann, Institute of Farm Animal Genetics, Mariensee, FLI, Unit 9.1 Large Animal Models.
- B. Pützer, Universität Rostock.
- H. Büning, ZMMK, Cologne.
- S. Ferber, Sheba Medical Center, Tel Aviv, Israel.
- S. Strom, Karolinska Institute, Department of Laboratory Medicine, Stockholm, Sweden.
- Cytonet GmbH, Weinheim, Germany.
- Corline, Uppsala, Sweden.

Grants

- EU, BMBF, DFG, Industry.

Publications

Goals

At present, the only curative therapy option to treat end stage lung diseases remains lung transplantation. Due to the shortage of donor organs there is a high mortality rate of patients on the organ waiting list. For bridging the time between lung failure and organ transplantation extracorporeal membrane oxygenators (ECMOs) can be applied to support the function of the diseased lung. However, the contact of the patients blood with the artificial gas-exchange hollow-fibre membrane of state-of-the-art ECMOs, results in thrombus formation and subsequent failure of these devices within days to weeks. To overcome this limitation the aim of Unit 4.1 is to develop an implantable biohybrid lung with improved biocompatibility that can be used as a long-term lung substitute. Therefore endothelialisation of the different blood-contacting surfaces of the device is considered to be the most effective strategy. Furthermore, shape and size of the prospective bioartificial lung, as well as cannulation techniques have to be developed to fit for testing in animal models. The results of these experiments will be implemented in the concept of the fully implantable biohybrid lung prototype for preclinical studies.

Achievements

The materials used for the different blood contacting components of the biohybrid lung necessitate the use of various surface coatings or treatment strategies, in order to enable the adhesion of an endothelial cell (EC) monolayer onto blood contacting surfaces. In addition to other coating techniques, dip coated Albumin / Heparin and covalently bound cRGD peptides showed to effectively mediate the formation of an EC monolayer on PMP gas exchange membrane films. In the case of TPU, which is a potential material for the housing and cannulae, improved cell adhesion could be achieved by embedding 0.1 wt% Au-nanoparticles. Albumin / Heparin coating was also effective in the formation of a confluent EC monolayer on 3D hollow fibre membranes. Assessment of further coating strategies is currently in progress (Fig. 1). Furthermore, the clinical application of an endothelialised biohybrid lung requires high quantity and quality of immunotolerable cells.

Along these lines, two cell sources are currently under investigation, including human cord-blood-derived ECs and β2-microglobin-silenced ECs as a potential allogeneic but immunotolerable cell source. Moreover, REBIRTH Unit 4.2 is currently investigating the generation of ECs from human induced pluripotent stem cells.

Planning

Following the successful endothelialisation of the gas exchange hollow fibre membranes, their performance regarding efficiency in gas transfer and long term haemocompatibility will be assessed in vitro. Also, ECMO prototypes for both rat and sheep animal models will be established. Owning to this, cell isolation and seeding strategies, as well as device geometry and cannulation techniques will be adapted to accommodate animal models.

Fig. 1: Vital stain of surface treated hollow fibre membranes 3 days after seeding with ECs indicates that strategy B seems to be superior. A) Albumin / Heparin, B) strategy B; scale bar: 5mm.
Cooperations

- G. Dräger, Institute of Organic Chemistry, Leibniz University Hannover, Unit 7.1 Functionalized Polymers and Regenerative Agents.
- B. Chichkov, Laser Zentrum Hannover, Unit 7.4 Nanosurfaces.
- R. Blasczyk, Institute for Transfusion Medicine, MHH.
- J. Seume, Institute of Turbomachinery and Fluid Dynamics, Leibniz University of Hannover.

Publications


Grants, awards, patents, outreach

- DFG – Preventing the rejection of allogeneic ECs by HLA-class I silencing.
- 3rd price in the challenge “Oxygen in Action”, PreSens Precision Sensing GmbH.
Goals

We aim at the development of induced pluripotent stem cell (iPSC)-based therapies for the treatment of various diseases of the respiratory system. To achieve this, we will generate patient-derived iPSCs (1-3), correct genetic defects (4), develop improved protocols for targeted differentiation of iPSCs into endothelial cells (ECs) and respiratory cell lineages (5), and scale up the production of ECs. Finally, these iPSC-derivatives will be applied for biofunctionalisation of a biohybrid lung (Unit 4.1), ex vivo cell replacement using the Organ Care System (OCS), and application in preclinical animal models.

Achievements

We efficiently generated iPSCs (from different species and patient-derived). Both embryonic and induced pluripotent stem cells were successfully differentiated into endothelial cells, as well as into airway and epithelial (progenitor) cell lines. We were the first to clinically apply the OCS system for preservation of donor lungs. For experimental purposes, we also initiated a small animal model for the OCS (rat). Additionally, a pig lung tumor model was established, allowing explantation, treatment, and subsequent autotransplantation.

Planning

Additionally to the establishment of human reporter iPS cell lines and the genetic correction of cystic fibrosis (ΔF 508) patient-derived iPSCs via ZFN- and TALEN-based approaches, we will continue our efforts to develop protocols for efficient respiratory differentiation of iPSCs. Moreover, we plan to develop a protocol for scalable targeted differentiation of hiPSCs into functional endothelial cells and further purification through MACS. Furthermore, we will investigate different approaches for the ex vivo decellularisation and reseeding of the vascular compartment of pig lungs (maintained in the OCS) including functional assessment. Finally, ex vivo tumor resections and autotransplantation will be established in a preclinical pig model.

The OCS is presented to Angela Merkel.

Publications


Patents, public relations

- Presentation of OCS lung system to the German chancellor, Angela Merkel on November 27, 2012.

- Patent application WO 2012/104400: Development of efficient protocols for the differentiation of murine ESCs and iPSCs in Clara- and ATII cells by applying specific (growth) factors.

Cooperations

- TransMedics Inc, Development of Organ Care System, Andover, UK.

- Genea, Sydney, Australia (respiratory differentiation).
Goals

Accumulating evidence points towards an important role of senescence in aging, degeneration and for the response to certain injuries in vivo. The hypothesis is that senescence exhausts the reserves of somatic cells that are involved in cell-division and thereby cell-renewal – cellular skills indispensable for repair, integrity and regeneration. There are several observations suggesting senescence as mediator of or even the cause of cardiovascular complications.

Our goals are:

- To prove the causal relationship between senescence and endothelial dysfunction and cardiovascular disease.
- To show that strategies that interfere with senescence lead to endothelial and vascular regeneration.

Achievements

**Human autopsy specimen (aortas and coronary arteries)**

p16^{INK4a} and p21^{CIP1} expression is significantly elevated in vessels from adult patients with chronic kidney disease as compared to control subjects (Fig. 1).

**Mouse aortic rings**

p16^{INK4a} and p21^{CIP1} expression is significantly elevated in aortas from old wildtype mice (24–28 months) as compared to young mice (4–6 months, Fig. 2).

Aortas from old wildtype mice show only marginal histological differences (e.g. only slight increase in collagen deposition, see Fig. 3A), but they show a significant decrease in Acetylcholine-dependent vasodilatation (Fig. 3B).

Planning

Breeding of endothelium-specific p16^{INK4a} KO (p16^{INK4a} EC KO) mice (in cooperation with Prof. Heineke, Unit 5.1).

Functional impact of loosing a major senescence pathway in endothelial cells investigated by:

- **Ex vivo organ bath experiments using aortic rings**
- **Three-dimensional aortic ring angiogenesis assay**
- **Cross-breeding with ApoE KO mice**

**Hypothesis:** Improvement of endothelium-dependent vasodilatation

**Hypothesis:** Improvement of cellular proliferation, microvessel branching, perivascular recruitment and remodelling

**Hypothesis:** Improvement of athero-sclerotic phenotype
Cooperations

- T. Thum, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 mRNA in Myocardial Regeneration.
- D. Hilfiker-Kleiner, Department of Cardiology and Angiology, MHH, Unit 5.2 Endogenous Regeneration Mechanisms of the Heart.
- J. Heineke, Department of Cardiology and Angiology, MHH, Unit 5.3 Myocardial Cellular Crosstalk and Gene-Therapy.
- A. Haverich, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH.
- M. Stiesch, Clinic for Dental Prosthetics, MHH.
- U. Tegtbur, Sports Medicine, MHH.
- R. Schmitt, Clinic for Nephrology, MHH.
- C. S. Falk, Transplantation Immunology, IFB-Tx, MHH.

Publications


Grants, awards, patents, outreach

Goals

This project aims at the development of intelligent, tunable cell based therapeutic tools for regenerative approaches. Synthetic regulatory circuits designed to sense external or physiological input signals are interfaced with cellular regulation pathways to control cell fate or function. Transplantation of such cells in animals is being followed to substitute or modulate endogenous functions to overcome a diseased phenotype in a self-controlled or externally controlled manner, respectively.

Achievements

Control of cell expansion

Based on synthetic regulatory modules, strict control of cell proliferation has been achieved (May et al., 2010). Controlled cell expansion allowed to establish novel cell lines that preserve the inherent functionality. This has been proven upon transplantation of conditionally immortalized human endothelial cells into immunocompromised mice: formation of perfused vessels that are constituted from human cells indicate functionality. Currently, this concept is validated for hepatic cells.

Predictable transgene expression

Based on a recently developed, highly efficient strategy for targeting expression cassettes into defined chromosomal sites in mice (‘safe harbors’) (Sandhu et al., 2011) we synthetically activated antigen expression to explore the immune response in sterile, non-infectious conditions (Cebula et al., 2013). Moreover, targeted integration of synthetic expression modules was successfully employed for controlled induction of induced pluripotent stem cells (Haenebalcke et al., 2013a, b).

Planning

To establish novel synthetic systems, synthetic expression circuits are developed as building blocks and integrated into precharacterized sites in the genome to give predictable expression. Synthetic circuits are designed and interfaced with cellular regulatory networks to modulate relevant processes such as differentiation and / or proliferation. Implementation of these cell systems into animals is followed to validate such cell systems as cellular prostheses in vivo.
Cooperations

- M. Ott, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.5 Hepatic Cell Transplantation and Genetic Manipulation.
- M. Bock, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.4 Hepatic Cell Therapy - Patient Liver Stem Cells.
- T. Cantz, MPI-Cell and Developmental Biology; Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 1.1 Translational Hepatology & Stem Cell Biology.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.

Grants, awards, patents, outreach


Publications

Goals

Acute myocardial infarction (AMI) is the leading cause of heart failure worldwide. Members of the Division of Molecular and Translational Cardiology are investigating the mechanisms that promote heart failure development after AMI. Our goal is to develop more effective diagnostic and therapeutic strategies to prevent heart failure after AMI. Within REBIRTH, we aim to develop non-invasive strategies to improve wound healing, regeneration and clinical outcome in patients with AMI.

Achievements / Planning

The development of heart failure after AMI is determined by the extent of the infarct, the wound healing response, and chronic remodelling. The wound healing response provides a largely unexploited therapeutic window of opportunity to improve outcome in AMI. Wound healing after AMI involves multiple cell types that are interacting with each other in a highly orchestrated manner. This inter-cellular communication occurs primarily through secreted proteins. The importance of paracrine signalling in the infarcted heart suggests that individual secreted proteins might be developed as therapeutic agents after AMI. Protein based therapeutics have several potential advantages, including ease of standardisation and large-scale production; the potential for off-the-shelf, systemic, and repetitive administration, and the potential to design protein cocktails tailored to specific disease settings. We are conducting bioinformatic secretome analyses in various (progenitor) cell types to identify novel secreted proteins controlling wound healing after AMI. In a complementary approach, we are exploring the therapeutic potential of a new progenitor-cell mobilizing agent in mouse and pig AMI models, a concept that will be soon tested in a placebo-controlled, multicenter trial in AMI patients.

Further Research Projects

- DFG-project: Function and therapeutic potential of newly identified secreted proteins after AMI.
- DFG-project: Role of multifunctional mediators of inflammatory wound healing after AMI.
- DFG-project: BOOST 2 clinical trial.
- DFG-project: Protective and immune-modulatory function of cytokines in viral myocarditis (T. Kempf).
- German-Israeli Foundation (GIF): Metal homeostasis in ischemic heart disease (K. Wollert, T. Kempf in cooperation with M. Chevion, University of Jerusalem).
- EU FP7-program: Biomarker for Cardiovascular Risk Assessment in Europe (BiomarCaRE).
- EU FP7-program: The effect of intracoronary reinfusion of bone marrow-derived mononuclear cells (BM-MNC) on all cause mortality in acute myocardial infarction (BAMI).
Cooperations

- A. Schambach, REBIRTH Unit 6.2 Regenerative Gene Therapy, Experimental Haematology, MHH.
- F. Bengel, REBIRTH Unit 8.3 Radionuclide Molecular Imaging, Dept. of Nuclear Medicine, MHH.
- I. Gruh, G. Kensah, REBIRTH Unit 5.7 Myocardial Tissue Engineering, LEBAO, MHH.
- J. Heineke, REBIRTH Unit 5.3 Myocardial Cellular Crosstalk and Gene Therapy, Dept. of Cardiology and Angiology, MHH.
- M. Bobadilla, Roche Pharma Ltd., Basel, CH.
- K. Dembowski, Polyphor Ltd., Basel, CH.

Publications

Goals

- Advance the knowledge on regenerative processes especially with regard to regenerative milieus of the heart.
- Characterizes the cardiac micromilieu in health and disease.
- Identify therapeutic tools to influence the cardiac micromilieu.
- Develop gender specific regenerative strategies.

Achievements

- Discovered novel paracrine factors and microRNAs in exosomes altering the cardiac microenvironment with regard to disease development (*Nature* 2012; *JCI* 2013).

Planning

- Establishing human keratinocyte culture for iPSCs to study alterations in secretom of diseased cardiomyocytes.
- Analyze role of EMT for cardiac progenitor cells.
- REBIRTH-active for women.

(top) Irina Gorst is analysing heart sections of LNA-miR-146a treated mice.

(bottom) Lea Greune is isolating Keratinocytes from human hair follicles.
Cooperations

- M. Scherr, Department of Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.7 Molecular Control of Granulocytic Differentiation.
- A. Hilfiker, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Unit 5.6 Tissue Engineered Valves.
- A. Gossler, Institute of Molecular Biology, MHH, Unit 2.1 Notch Signalling and Ciliogenesis.
- M. Gaestel, Institut for Physiological Chemistry MHH.
- D. Manstein, Institute for Biophysical Chemistry, MHH.
- T. Thum, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration.
- J. Heineke, Department of Cardiology and Angiology, MHH, Unit 5.3 Myocardial Cellular Crosstalk and Gene-Therapy.
- J. Bauersachs, Department of Cardiology and Angiology, MHH.
- U. Tegtbur, Sports Medicine, MHH.
- C. Bara, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH.
- R. Gerardy-Schahn, Institute for Cellular Chemistry, MHH.
- G. Heusch, Essen.
- R. Schulz, Giessen.
- T. Braun, Bad Nauheim.
- M. Böhm, Homburg.
- S. Rohrbach, Giessen.
- GP Meyer, Hamburg.
- A. Lichtenberg, Düsseldorf.
- K. Sliwa, Giessen.
- I. Strumann, Liége, Belgium.
- Z. Arany, Boston, USA.
- G. Christensen, Oslo, Norway.
- P. Aukrust, Oslo, Norway.
- M. Mayr, London, Great Britain.
- F. Favret, France.
- W. Muller, Manchester, Great Britain.
- M. Ernst, Melbourne, Australia.
- G. Conderelli, Milano, Italy.

Publications


Grants, awards, patents, outreach

- Obtained DFG Einzelantrag Hi 842/8-1.
- Applied for several FP7 initiative with focus on paracrine factors and small molecule treatment.
- Report on PPCM in “Spiegel online” in April 2013.
Achievements / Planning

Intercellular / Interorgan Crosstalk

We found that

- endothelial cell GATA2 inhibits cardiomyocyte hypertrophy and dysfunction (plan to publish 2014/2015).
- endothelial CTRP9 inhibits pathological cardiac hypertrophy (plan to publish 2013/2014).
- We established a bioassay to measure myostatin (derived from muscle and heart) in serum of patients (plan to publish 2013).
- We identified cardiomyocyte GATA4 to be important for infarct healing (plan to publish 2016). (Gene) therapy in heart failure.
- We identified CIB1 as an important regulator of pathological hypertrophy. We are testing / establishing an AAV-shCIB1 for gene therapy (plan to publish 2014/2015).
- We identified TIP30 as inhibitor of mRNA translation. TIP30 gene therapy inhibits pathological hypertrophy (plan to publish in 2013/2014).
- Oral therapy with Finasteride inhibits pathological hypertrophy and heart failure in mice (plan to publish 2013/2014).

Angiogenesis

- We identified the transcription factors GATA6 as master regulators of angiogenesis (JBC, 2011; and plan to publish 2014/2015).
Cooperations

- K. Wollert, Department of Cardiology and Angiology, MHH, Unit 5.1 Secreted Factors and non-Cell-Based Strategies for Cardiac Regeneration.
- D. Hilfiker-Kleiner, Department of Cardiology and Angiology, MHH, Unit 5.2 Endogenous Regeneration Mechanisms of the Heart.
- A. Kispert, Institute of Molecular Biology, MHH, Unit 2.2 Transcriptional control of Organogenesis.
- U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung regeneration and repair.
- M. Eder, M. Scherr, Department of Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.7 Molecular Control of Granulocytic Differentiation.
- T. Thum, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration.
- I. Gruh, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 5.7 Myocardial Tissue Engineering.
- G. Theilmeier, Department of Anaesthesiology, MHH.
- J. Bauersachs, Department of Cardiology and Angiology, MHH.
- J. D. Molkentin, Cincinnati Children’s Hospital, USA.
- S. Potter, Cincinnati Children’s Hospital, USA.
- H. Xiao, College of Human Medicine, Michigan State University, East Lansing, USA.
- M. Mayr, King’s College, London, UK.
- S. A. Duncan, Medical College of Wisconsin, Milwaukee, USA.
- S. A. Camper, University of Michigan Medical School, USA.
- A. Abor, Michigan, USA.
- S. Conway, Indiana University, USA.
- L. Field, Indiana University, USA.
- I. Kehat, Technion, Haifa, Israel.
- O. Müller, Heidelberg.

Grants, awards, patents, outreach


Publications
Goals

Vascular remodelling processes result in vascular pathologies like atherosclerosis or post-angioplasty restenosis. The resulting vascular occlusive and thrombo-embolic events like myocardial infarction, stroke and peripheral artery disease represent the most common cause of death in industrialized countries. The aim of our research efforts is to generate a more precise understanding of the mechanisms that trigger vascular remodelling processes and consequently to develop novel strategies for their prevention and therapy.

Specifically we aim on the:

- Identification of cell-specific and disease-specific regulated molecules during vascular remodelling processes in different vascular cells during the development of atherosclerosis and restenosis (signalling molecules; miRNAs).
- Development of novel, specific and selective therapeutic strategies for the treatment of vascular proliferative diseases (antagomirs, LNAs, small molecules).

Achievements / Planning

Identification of novel target molecules

In previous studies we determined the differential expression of miRs during the development of atherosclerosis and restenosis in mice as well as in human atherosclerotic plaques or neointimal tissues. In addition, we determined the cell specific differential miR-expression in endothelial cells (EC), smooth muscle cells (SMC) or monocytes of diseased vessels. Following the temporospatial expression pattern, we identified cell- and disease-state specific miRs.

Epigenetic regulation of gene expression during vascular remodelling

Since the epigenetic regulation of differential gene expression seems to have major impact on developmental and regenerative processes, we aim to identify key players of these processes during vascular remodelling processes: In one project we assess the role of BET-Bromodomains on endothelial and smooth muscle cell functions by using novel highly selective small molecule inhibitors. These molecules might represent promising agents for future therapeutic clinical strategies. In a further project we focus on the family of sirtuin proteins which have been reported to prolong the lifespan in several disease models and even primates. Modulating sirtuin function thus might for the first time offer a modality to rejuvenate the aged vasculature.

Cell- and disease-specific targeting

In the upcoming projects, the effects of inhibitors of distinct microRNAs as well as BET-bromodomains or sirtuins will be assessed. In vitro, the proliferation, migration, adhesion and resistance to apoptotic stimuli of EC and SMC will be determined. In vivo, the effect of small molecule inhibitors or antimir LNAs and antagomirs as well as the effect of an EC-specific knock down of respective miRs (Tie2-Cre; miR(fl/fl)-mice) on re-endothelialization and neointimal lesion formation as well on atherosclerosis development will be assessed in respective mouse models.
Cooperations

- T. Thum, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration, miRNA screening and appl.
- F. Limbourg, Clinic for Nephrology, MHH, Unit 5.9 Regenerative Agents, Angiogenesis models.
- S. Dimmeler, University of Frankfurt, Institute of Cardiovascular Regeneration, miRNA function in vascular cells and knock out mice.
- W. Seeger, S. Pullamsetti, University of Gießen, FoxO1 in pulmonary vascular remodelling.
- H. Langer, University of Tübingen, Modulation of platelet function to prevent vascular remodelling.
- E. Olson, Texas, miRNA knock out mice.
- P. Libby, K. Croce, Small molecule BET-Bromodomain inhibitors.
- E. van Roij, Boulder, specific miR inhibitors (LNAs).
- M. Mayr, King’s College, University of London, Proteomics.

Publications


Grants, awards, patents, outreach

- Excellence Cluster Cardio-Pulmonary System (ECCPS) Gießen.
- SFB547 Kardio-Pulmonales System, Gießen.
- IRTG PROMISE Gießen / Barcelona.
- Albert Fraenkel Award (German Cardiac Society).
- Rudi Busse Award (German Cardiac Society).
Goals

Devlopment of microRNA (miR)-based therapeutics

MicroRNAs (miRNAs) are powerful post-transcriptional regulators targeting multiple targets. Endogenously transcribed, miRNAs repress expression of target genes thus governing control of cellular signalling pathways. Altered miRNA expression is causally related to cardiovascular disease. Identification of miRNA-dependent pathways is therefore an important aim to develop new therapeutic approaches.

Achievements / Planning

Endogenous miR-24 knockdown sustains cardiac vascularity after myocardial infarction (MI)

Hypoxia and cardiac ischemia in mice increased miR-24 expression in endothelial cells (ECs). In vitro overexpression revealed a pro-apoptotic function for miR-24 in ECs. Anti-angiogenic features were also observed in vitro and in the developing vasculature of zebrafish. Therapeutic antagonism of miR-24 in a mouse model of MI improved ischemic remodelling by direct effects on cardiac EC survival.

MiR-24 is an appropriate candidate miR to test in clinically relevant cardiac ischemia-reperfusion (IR) injury models.

Translation of miR-based therapies to large animal models

Based on the findings in the permanent infarction model of mouse MI, we plan to interfere with miR expression in a large animal pig model of cardiac IR. Pharmacological and phenotypic analysis will help to reveal the efficacy of therapeutic miR interference.
Cooperations

- U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung regeneration and repair.
- J. Bauersachs, Department of Cardiology and Angiology, MHH.
- D. Hilfiker-Kleiner, Department of Cardiology and Angiology, MHH, Unit 5.2 Endogenous Regeneration Mechanisms of the Heart.
- T. Cantz, MPI-Cell and Developmental Biology; Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 1.1 Translational Hepatology & Stem Cell Biology.
- T. Moritz, Institute of Farm Animal Genetics, Mariensee, FLI; Institute of Experimental Haematology, MHH, Unit 1.3 iPSC based haematopoietic regeneration.
- M. Pavone-Gyöngyösi, University of Vienna, large animal models.

Grants, awards, patents, outreach

- IFB-Tx, BMBF.
- DFG (TH903/7-2, TH903/10-1, TH903/11-1, RE3523/1-1, LO1736/1-1, SFB-587); Leduqc Fondation (Coordinator).
- Support by various foundations and industry.
- Several Patents for miR-based therapeutics for cardiac disease.
- “MicroRNA-24 regulates vascularity after myocardial infarction”, Franz-Maximilian-Groedel Forschungspreis 2012, Prof. T. Thum and Dr. J. Fiedler, DGK.

Publications

Tissue Engineered Valves

Goals

- Off the shelf availability of non-immunogenic heart valve prosthesis based on modified xenogenic (porcine) decellularized heart valve matrices.

Milestones

- Generation of xenogen reduced porcine natural heart valve matrices by decellularization and enzymatic treatment of the glycocalix.
- Testing of low reactive heart valve matrices in humanized mouse models.
- Functionality tests of low / non-immunogenic natural heart valve matrices in large animal experiments (sheep / non human primate).

Planning

- Effect of modified decellularization protocols in respect to residual α-Gal epitopes and epitopes recognized by human natural xenoantibodies.
- Modification of glycocalix structures on matrices by enzymatic treatment.
- We will develop humanized mouse models to test small matrices samples implanted subcutaneously for xenore-activity.
- Biomechanical tests on successfully modified matrices.

Binding of preformed Xenoantibodies to decellularized matrices (top) HV in decellularisator (bottom).
Cooperations

- A. Krueger, Institute of Immunology, MHH, Unit 2.3 Regenerative Immunology.
- D. Hilfiker-Kleiner, Department of Cardiology and Angiology, MHH, Unit 5.2 Endogenous Regeneration Mechanisms of the Heart.
- S. Cebotari, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Unit 5.8 Large Animal Models for Myocardial Repair.
- D. Hartung, Institute for Radiology, MHH, Unit 8.5 Functional and Molecular MRI.
- H. Niemann, Institute of Farm Animal Genetics, Mariensee, FLI, Unit 9.1 Large Animal Models.
- W. Wolkers, Institute for Multiphase Processes, LUH, Unit 10.3 Biostabilization of tissues and macromolecular assemblies.

Publications


Grants, awards, patents, outreach

- Fördervereinigung der Deutschen Kinderherzzentren e.V.
- DFG TRR 127 “Xenotransplantation” Project C7.
Unit 5.7 | Myocardial Tissue Engineering

Goals

New treatment strategies for failing hearts

Our aim is the generation of bio-artificial cardiac tissue (BCT):
-
- for reconstructive therapy of cardiovascular diseases,
- as 3D in vitro model of cardiac physiology and disease.

Myocardial tissue engineering involves the generation and cultivation of appropriate cell sources, the design of adequate extracellular matrices, the development of bioreactor and transplantation strategies.

The current focus of our work is to enlarge our system of generating BCTs from induced pluripotent stem cell-derived cardiomyocytes to suitable dimensions for the use in the clinical situation.

Achievements / Planning

Myocardial tissue from pluripotent stem cells

The functional assembly of strong bioartificial cardiac tissue in vitro requires chemical and mechanical stimulation. We identified four different parameters critically affecting the formation of miniaturized BCTs from murine and human PSC-derived CM. i) use of purified cardiomyocytes in cardiac body aggregates, ii) the addition of fibroblasts, iii) supplementation with ascorbic acid and iv) the application of growing stretch. We were able to generate BCTs from human ESCs displaying so far unparalleled physiological performance (4.4 mN/mm2). Currently, we are scaling up to generate larger constructs with reasonable sizes for large animal transplantation studies.

Generation of sufficient cardiomyocytes from PSCs by standardized differentiation and selection

Reconstructive cardiac therapies require large amounts of cardiomyocytes. We developed an agarose microwell approach to reproducibly aggregate and differentiate human and murine ESCs and iPSCs. A defined small-molecule based protocol was used for human ESC and iPSC differentiation, resulting in high cardiomyogenic efficiency (up to 65% cardiomyocytes (CM)) and yield (6 CM per input hESC). Genetic selection enabled us to efficiently purify cardiomyocytes (99%), which were used as non-dissociated “cardiac bodies” for tissue engineering. Currently, selectable human and non-human primate iPSC clones are being generated by targeting a genomic safe harbor site with zinc finger nuclease technology.

Strategies for tissue preservation

For the technical implementation of clinical BCT transplantation, it will be necessary to preserve tissues for transport and storage. We are currently developing strategies for short term (up to 4 weeks) preservation of BCTs at 4°C.

Extracellular matrix design

To provide defined ECM-materials for clinical tissue transplantation, we (together with Unit 7.1) established a system of hydrogel blends based on chemically modified alginate, hyaluronic acid and collagen. We identified hyaluronic acid as a factor positively affecting passive and active force development of BCTs based on rat primary and human ESC-derived cardiomyocytes, respectively. Currently we are investigating RGD-modified polymers for myocardial tissue engineering.

Tissue transplantation in animal models

Next to survival and engraftment of constructs, functional integration and improvement are the desired outcomes of tissue therapy. We are currently investigating the therapeutic effects of transplantation of miniaturized murine ESC and iPSC-BCTs in a xenogenic rat model of myocardial infarction (MI).

Vascularization

For clinical transplantation, we plan to establish vascularized BCTs.
Agarose microwells (AMs) for reproducible aggregation (A) and differentiation (B) of human ESCs.

**Publications**


*# contributed equally

**Cooperations**

- U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 1.2 iPSCs for Disease Modelling, Drug Screening and Cell Therapy.
- T. Moritz, Institute of Farm Animal Genetics, Mariensee, FLI; Institute of Experimental Haematology, MHH, Unit 1.3 iPSC based haematopoietic regeneration.
- U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung regeneration and repair.
- J. Heineke, Department of Cardiology and Angiology, MHH, Unit 5.3 Myocardial Cellular Crosstalk and Gene-Therapy.
- S. Cebotari, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Unit 5.8 Large Animal Models for Myocardial Repair.
- G. Dräger, Institute of Organic Chemistry, Leibniz University Hannover, Unit 7.1 Functionalized Polymers and Regenerative Agents.
- M. Ochs, Institute of Functional and Applied Anatomy, MHH, Unit 8.2 Quantitative Microscopy in Regeneration.
- R. Zweigerdt, Institute of Experimental Haematology, MHH, Unit 10.2 Mass production of Pluripotent Stem Cells and their Derivatives.
- P. Sasse, B. Fleischmann, University Bonn.
- M.Y. Emmert, University Hospital Zurich, Switzerland.
- P. Christalla, D. Eckhardt, Miltenyi Biotec GmbH.
- A. Giacomello, E. Messina, University of Rome, Italy.
- D.A. Elliott, Monash University, Victoria, Australia.
Goals

- Implementation of appropriate animal models for myocardial replacement and regeneration using tissue engineering and cell therapy approaches.

Achievements

- Development of surgical approach and circulatory support protocols to address defects of right and left ventricle.
- First pilot operations using epicardial implantation of Tissue Engineering constructs.
- First pilot operations using transmural introduction of myocardial Tissue Engineering constructs.

Planning

- Development of surgical technique for myocardial replacement of left and right ventricle in large animal.
- Implementation of different techniques for epicardial, intramyocardial or transmural replacement.
- Implementation of different protocols for immunologic suppression for xenotransplantation model.
- Implementation of myocardial infarction model in large animal.
- Implementation of functional investigation tests (Echo-cardiography, Angiography, MRI).

Schematic presentation of the surgical replacement of the right atrium (top). Complex surgical intervention including 2 cavities in pig model (bottom).
Cooperations

- I. Gruh, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 5.7 Myocardial Tissue Engineering.
- A. Hilfiker, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Unit 5.6 Tissue Engineered Valves.
- D. Hilfiker-Kleiner, Department of Cardiology and Angiology, MHH, Unit 5.2 Endogenous Regeneration Mechanisms of the Heart.
- R. Zweigerdt, Institute of Experimental Haematology, MHH, Unit 10.2 Mass production of Pluripotent Stem Cells and their Derivatives.
- FP-7 EU Project “BioCent”.
- DFG Project “Construction and testing of TE myocardial patches”.
- BMBF Project “Transplantation of bioartificial tissue in large animal model”.
- Cortiss Project “Myocardial replacement using autologous vascularized patch”.

Publications

Goals

Linking development and regeneration

To understand and enhance arterial regeneration by studying and applying genes regulating arterial development. We aim to employ genetic programmes for vascular repair and regeneration (e.g. Notch) and specialized myeloid cell populations with stable repair properties.

Background

We study growth and regeneration of blood vessels. Blood vessel growth is essential for the regeneration of damaged organs by restoring perfusion and providing routes for cells. This process is critically regulated and maintained by intricate cell-to-cell communications within vessels, but also between vessels and tissues. However, the regenerative capacity of arteries is limited and vascular disease remains the leading cause of death worldwide.

Our goal is to understand and enhance mechanisms of vascular regeneration. We focus on genetic programmes for arterial morphogenesis and regeneration, but also on specialized myeloid cell populations as regenerative agents.

Planning

Further define branching programme of arteries. Establish new vascular imaging protocols in mice by MRI using dedicated small animal coil. Identify mechanism and effector functions regulating regenerative capacity of macrophages.
Cooperations

- F. Bengel, Department of Nuclear Medicine, MHH; M. Meier, Institute of Laboratory Animal Science, MHH, Hüper, MHH, State of the art vascular and perfusion imaging in mice.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.
- D. Hilfiker-Kleiner, K. Wollert, Department of Cardiology and Angiology, MHH, Angiocrine signalling in cardiac homeostasis and function.
- Keshet, Jerusalem, Macrophage education in vessel regeneration.

Publications


Grants, awards, patents, outreach

- 2011 Rudi Busse Award for Experimental Cardiology (Awarded to group member).
- Grants: Deutsche Forschungsgemeinschaft (DFG), Bundesministerium für Bildung und Forschung (BMBF), German-Israeli Foundation (GIF).
Goals

Our major aim of the project is the generation of enhanced and synthetic cells for regeneration and more specifically haematopoietic cell with improved capabilities. This will include the modification of haematopoietic stem cells (HSCs) as well as differentiated myeloid cells such as monocytes / macrophages and granulocytes. Long term aims of this work are the generation of (i) HSCs with improved engraftment and reconstitution potential, and (ii) genetically modified granulocytes and monocytes / macrophages for the treatment of selected diseases.

Achievements / Planning

In the first part which will focus on the expression of genes that will enhance the engraftment and repopulation ability of HSCs, we will in particular analyze and utilize the functions of genes that we discovered in our extensive studies applying insertional mutagenesis approaches. Genes successful in these studies also will be applied to haematopoietic ex vivo differentiation of pluripotent stem cells. These studies will be guided by our previous experience with HoxB4 expression in ESCs to promote HSC differentiation. Here, in addition to genes encoding for transcription factors, signalling molecules and factors required for survival and genomic integrity also specific miRNAs will be screened for their function in early haematopoietic and myeloid differentiation.

As a second part, we will modify myeloid cells and in particular monocytes / macrophages and granulocytes to (i) enhance site specific homing to specific tissues (e.g. liver vs. lung vs skin etc), or (ii) direct these cells to specific disease sides (inflammation, tumor, vascular occlusions), and / or (iii) to deliver therapeutic molecules (cytokines, toxins etc). In this context inducible expression of the therapeutic molecules will be investigated utilizing vector constructs already optimized for this purpose.

Schematic overview of experiments.
Cooperations

- U. Modlich, LOEWE Center for Cell and Gene Therapy, Frankfurt, analysis of stem cell function in mpl-deficiency models; insertional mutagenesis studies in murine and human HSCs.
- T. Thum, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration.
- T. Cantz, A. D. Sharma, Department of Gastroenterology, MHH & R. Zweigerdt, Institute of Experimental Haematology, MHH, Use of UCOE-elements to improve transgene expression in PSC-derived progeny.
- R. Stripecke, Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.4 Regenerative Immune Therapies; Safety assessment of lentiviral vectors designed for human gene therapy.
- H. Geiger, Ulm & M. Milsom, Heidelberg, retroviral insertional mutagenesis to discover genes conferring radioprotection and / or compensating the Fanconi anemia phenotype.

Grants, awards, patents, outreach

- German-Chinese Junior Research Group, Axel Schambach, Duanqing Pei: “Modern Applications in Biotechnology”, funded by DAAD & BMBF.
- Initiative “REBIRTH goes back to school”: scientific talks for students. Persued in close cooperation with schools of lower saxony such as Gymnasium Burgdorf, Gymnasium Clausthal-Zellerfeld and IGS Albert-Einstein Schule Laatzen. Initiated by Nico Lachmann. (contributors: Mania Ackermann, Nico Lachmann).

Publications

Goals

**Regenerative gene therapy as a modern concept of molecular therapy**

Within our group we aim to reach the following goals:

- Novel gene therapy approaches with improved efficacy and biosafety for use in otherwise difficult to treat diseases.
- Clinical translation of gene therapy for at least one candidate disease.
- Molecular therapeutics to fight immunodeficiencies, infections and cancer / leukemia.
- Understand transcriptional networks governing (stem) cell identity to develop new tools for regenerative medicine, incl. reprogramming, transdifferentiation and disease modelling.
- Support other groups with vector expertise for various genetic interventions.

Achievements / Planning

**What we have achieved:**

- Participation in clinical translation of gene therapy trials, e.g. international multicenter trial for X-SCID.
- Bridge function between basic science and clinical translation, embedded in various (inter)national networks (e.g. SFB738, PidNet, Carpud, ASGCT, TAGTC, CellPID).
- Formal REBIRTH link to Harvard / Boston: AS is Associate Faculty and Visiting Scientist in Pediatrics at Boston Children’s Hospital / Harvard Medical School (coop. with David Williams).

**What we plan to do:**

- Development of efficient and more importantly safer systems for gene therapy.
- Progress in preclinical validation of gene therapy strategies in appropriate mouse models.
- Strengthen REBIRTH’s contribution to (inter)national networks in advanced cell manipulation.
- Improve non-integrating retroviral vectors, incl. retroviral mRNA, episome and protein delivery.
- Analyze retroviral vector-host interactions.

Regenerative gene therapy: A graphical abstract. The maturation cascade from induced pluripotent stem cells (iPSC) via hematopoietic stem cells (HSC) to mature hematopoietic cell types is shown. Inherited disorders with association to specific cell lineage are depicted below.
Severe combined immunodeficiency (SCID). The lack of IL2 receptor leads to incorrect signalling in several interleukin receptors and to a lack of NK- and T-cells. B cells are a functional. Symptoms can be successfully by gene therapy of HSC.

Publications


Grants, awards, patents, outreach

- Grants: BMBF (PidNet, DAAD, iGene, ReGene, IFB-Tx), DFG (SFB738).
- Patent application for alpharetroviral SIN vectors.
- Several travel awards.
- Young investigator award ESGCT 2011.
- German- Chinese junior research group funded by BMBF and DAAD.
Goals

**Generation of universal cell-based products:**
The main goal of this group is the production of cell-based therapeutic products for universal use. We aim at the generation of cell products with equal therapeutic efficiency independent of the genetic background of their recipients. In addition, we aim at the identification of novel mechanisms which might support the immune response during rejection.

Achievements

So far, we have generated HLA-class-I-silenced corneas, endothelial cells, megakaryocytes, and platelets. Furthermore, we have demonstrated that HLA-universal cells efficiently escape the allogeneic immune response *in vitro* and *in vivo*. In addition, we have characterized the role of the protein Semaphorin 5A (Sema5A). Soluble Sema5A showed to significantly enhance the immune response and may be considered as a biomarker for rheumatoid arthritis. Also, another Semaphorin member (Sema7A) showed a critical role in the regulation of CD34+ cell differentiation.

Planning

Further evaluation of the functionality of HLA-silenced platelets in small and large animal models. In addition, the production of HLA-silenced platelets will be established under GMP conditions. Also, the capacity of HLA-universal endothelial cells to survive in an allogeneic environment will be evaluated. Finally, the role of Semaphorins in the regulation of CD34+ cells differentiation will be further investigated.
Cooperations

- R. Stripecke, Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.4 Regenerative Immune Therapies, consulting on the use of lentiviral vectors.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy, Design of GMP-compliant vectors.
- A. Haverich, B. Wiegmann, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Unit 4.1, HLA class I silencing in endothelial cells for the biohybrid lung.
- H. v.d. Leyen, Hannover Clinical Trial Center, MHH, Unit 10.6, consulting on translation related strategies.
- Engelmann, M. Valtink, Institute for Anatomy, TU Dresden: HLA silencing in the cornea.
- R. Jacobs, Clinic for Clinical Immunolgy, MHH, Natural-Killer cells.
- T Witte, Clinic for Immunology and Rheumatology, MHH, Semaphorins in rheumatoid arthritis.
- S. Apte, USA, Sema phorin 7A interactions.
- A. Bajor, MHH, cornea immunology.
- C. Domingos-Hadamitzky, Department of Plastic, Hand and Reconstructive Surgery, MHH, miRNAs in skin scarring.
- C. Guzman, K. Schulze, Helmholtz Centre for Infection Research, Braunschweig, HZI, mouse models.

Grants, awards, patents, outreach

- Julia Bodmer young scientist award.
- Next Generation award.
- Several Best abstracts and poster awards.
- Severa Travel awards.
- DFG, HILF, Habilitationsförderung, Stiftung für Immuntherapie.

Publications

Goals

- **Rational genetic reprogramming for development of induced Dendritic Cells (iDC):** DC are professional antigen presenting cells (APCs) that play fundamental role in the development, regeneration and memory of immunity. We use genetic vectors (lentivirus) to program DC.

- **Preclinical validation of human vaccines in vitro and in humanized mouse models:** One relevant pre-requisite for approval of human iDC vaccines for use in humans is to demonstrate their potency and safety in cell culture systems or in animal models.

- **Clinical development:** Under good-manufacturing practices of iDC vaccines is ongoing for autologous immune therapy against cancer and after stem cell transplantation.

Achievements

- We showed feasibility of iDC production tailored with different antigens and cytokines for therapy of melanoma (TRP2/ GMCSF/ IL4), leukemia (WT1/ GMCSF/ IL4), CMV (pp65/ GMCSF/ IFNa), HCV (NS3/ GMCSF/ IFNa).

- We developed a novel humanized mouse model with lymph node regeneration and adaptive human T and B cell responses.

- Clinical development under GMP ongoing for Phase 1 clinical trials: melanoma (Deutsche Krebshilfe), CMV (Else Kroener Fresenius Stiftung).

Planning

- Additional technological developments (lymphoid tissue engineering, 3D scaffolds with biomaterials, “printing”).

- Further mechanistic characterization and expansion of use of novel humanized mouse model.

- Development of clinical trials for leukemia patients after haematopoietic stem cell therapy and patients with metastatic melanoma.
Group members: A. Daenthanasanmak, B. Sundarasetty, A. Schneider, L. Macke, H. Schwarzer, R. Stripecke, G. Salguero (from left to right).

Publications


Cooperations

- T. Feuchtinger, University Children’s Hospital Tübingen, B. Eiz-Vesper and C. Figueiredo, Institute for Transfusion Medicine, MHH, CMV/ Stem Cell Transplantation.


- C. Muenz, Institute of Experimental Immunology, University of Zurich, C. Guzman, Helmholtz Centre for Infection Research, Braunschweig, HZI, C. v. Kalle, National Center for Tumor Diseases (NCT) Heidelberg, Immune humanized mice.

- A. Schambach, Institute of Experimental Haematology, MHH, T. Moritz, Institute of Farm Animal Genetics, Mariensee, FLI, Institute of Experimental Haematology, MHH, WT1 a a model antigen for tumors and iPS.

- U. Koehl, GMP-DU IFB-TX, H. v. d. Leyen (HCTC), B. Eiz-Vesper, Institute of Transfusion Medicine, MHH, A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy, M. Schmitt, University of Heidelberg, W. Herr, University of Regensburg, HCMV / Haematopoietic stem cell transplantation.

- Z. Li, Institute of Experimental Haematology, MHH, Arnold Ganser, Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, FLT3-ITD leukemia signature and immune dysfunctions.

Grants, awards, patents, outreach

- SFB 738 Innovative Approaches for Transplantation: Area A/ Project A6: Lentiviral vector programmed dendritic cells for enhancement of engraftment and expansion of donor T cells.

- Deutsche Krebshilfe (Project 109049): Pre-clinical evaluation of self-differentiated and self-eliminated dendritic cells for melanoma immunotherapy.

- Else Kroener Fresenius Stiftung (Project 2012_A238): Clinical development of lentivirus-induced DC vaccine against HCMV.

**Goals**

Our goal is to develop targeted interventions for enhanced local immune regeneration in (aging) hosts. For this we wish to apply e.g. self-differentiating DCs to enhance lung mucosal immunity. In addition, we aim to generate advanced \textit{in vitro} systems for DC differentiation.

**Achievements**

- We identified cell specific expression and novel functions of polysialic acid (PSA) on dendritic cells (DCs) for migration and chemokine sensing.
- We identified autophagy inhibition as a novel mechanism of HDAC inhibitor-induced apoptosis that selectively targets leukemic cells with low basal level autophagy. These data backup translational application of HDACi in a current clinical trail.
- We showed that immunization of mice with the synthetic TLR-antigen construct BPPcysOvamPEG significantly reduced airway eosinophilia and efficiently primes CTL immunity.
- We provide evidence that HOXB4-transduced lin(-) bone marrow cells can serve to generate fully functional DCs for scientific and therapeutic applications.
- We identified novel ways to use autophagy for enhanced MHC II presentation and how drug-related organ toxicity can result from autophagy inhibition.

**Planning**

- Targeted interventions for enhanced local immune regeneration in (aging) hosts (e.g. self-differentiating DCs to enhance lung mucosal immunity).
- Generation of advanced \textit{in vitro} systems for DC differentiation.
Publications


Cooperations

- F. Büttner, R. Gerardy-Schahn, Institute for Cellular Chemistry, MHH, Unit 1.4 Stem Cell Glycomics and Proteomics.
- R. Stripecke, Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.4 Regenerative Immune Therapies Applied.
- B. Sodeik, Institute of Virology, MHH, Unit 8.1 Automated and Quantitative Microscopy of Intracellular Trafficking.
- A. Hai Hovav, Jerusalem University.
- M. Leverkus, Heidelberg University.
- J.-H. Klusmann, Department of Paediatric Haematology and Oncology, MHH.
- F. Vondran, Clinic for General, Abdominal and Transplant Surgery, MHH.
- R. Bauerfeind, Institute for Cellular Biology in the Centre for Anatomy, MHH.

Grants, awards, patents, outreach

- KFO 250, TP1, Z1, 10/2009–09/2013 (DFG).
Goals

- To study the regeneration of haematopoiesis using nicotinamide to (vitamin B3), NAMPT and sirtuins.
- To identify substances capable to activate haematopoietic-specific Lyn substrate-1 protein (HCLS1) for the regeneration of myelopoiesis.
- To identify the tools for the correction of myeloid differentiation in patients with severe congenital neutropenia using patients-specific IPS cells.

Achievements

- Identification of the new mechanisms of NAMPT-triggered myelopoiesis by deacetylation of transcription facors LEF-1, C/EBPα.
- Evaluation of the downstream effects of SiRT2 on cell proliferation by Akt deacetylation and activation of GSK3β/β-catenin signalling.
- Identification of the haematopoietic-specific Lyn substrate 1 protein (HCLS1) as an essential player in the G-CSFR-triggered myeloid differentiation.

Planning

- To evaluate the effects of vitamin B3 (NA), NAMPT, NAD+ and SIRTs on the myeloid differentiation of mouse ES cells and human iPS cells.
- To analyse myeloid differentiation of human haematopoietic cells after inhibition of NAMPT, SiRT1 or SiRT2.
- To establish readout system for HCLS1 activation in haematopoietic cells for screening of small molecule libraries.
- To establish serum-free haematopoietic differentiation of human iPS cells.

Dose-dependent effects of HCLS1 protein in haematopoiesis.

SIRT2-triggered deacetylation of Akt activates Wnt/β-catenin signalling.

Mechanism of G-CSF/NAMPT-triggered granulopoiesis in healthy individuals and CN patients.
Cooperations

- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.
- D. Steinemann, Dept. Cell- and Molecular Pathology, MHH, Unit 9.6 Genomic Profiling.
- T. Moritz, Institute of Farm Animal Genetics, Mariensee, FLI; Institute of Experimental Haematology, MHH, Unit 1.3 iPSC based haematopoietic regeneration.
- R. Grosschedl, MPI of Immunobiology and Epigenetics, Freiburg.
- DC Dale, University of Washington, Seattle, WA, USA.
- J. Burkhardt, University of Pennsylvania, Philadelphia, USA.
- Q. Tong, Baylor College of Medicine, Houston, Texas, USA.

Publications


Grants, awards, patents, outreach

- Deutsche Krebshilfe e.V. “Mechanisms of STAT5 hyperphosphorylation and of subsequent LEF-1 protein degradation and their role for leukemic transformation in patients with severe congenital neutropenia”.
- Deutsche José Carreras Leukämie-Stiftung e.V. “The role of LEF-1 transcription factor and its interaction partner HCLS1 in the inhibition of cellular senescence of leukemic cells”.
- Deutsche José Carreras Leukämie-Stiftung e.V. “CSF3R and RUNX1 mutations in leukemogenic transformation”.
- DFG “G-CSF-triggered de-/acetylation of transcription factors in myelopoiesis and leukemogenesis”.
- M. Klimiankou, O. Klimenkova received a Young Researcher Achievements Awards on the ASH Meeting 2012.
- Abstract of J. Skokowa was selected as a best abstract for the Plenary Session on the EHA Meeting 2013.
- In 2013 Julia Skokowa was invited to be a Member of the Editorial Board of the BLOOD Journal.
Goals

- To understand molecular mechanisms regulating granulopoiesis for identification of therapeutic targets.
- Understanding the molecular mechanisms of myeloid differentiation for better modulation of granulopoiesis.
- Analysis of miR-125b on signals mediated by G-CSFR and its function in mature granulocytes.
- Search for new miR-125b targets involved in granulopoiesis.

Achievements

- miR-125b affects myelopoiesis by targeting multiple signalling pathways.
- Overexpression of miR-125b completely blocks G-CSF-induced differentiation of murine 32D myeloid precursor cells, but enhances myelopoiesis in mouse bone marrow chimeras.
- First identification and functional characterization of STAT3, c-JUN and JUND as miR-125b target genes in myeloid cells.
- Generation of miR-125b chimeric mice.

Planning

- Regulation of progenitor differentiation and granulocyte function by miR-125b.
- Improvement of miR-125b chimeric mice: kinetics of engraftment, lineage composition, granulocytic maturation, potential transformation.
- Functional analysis of miR-125b overexpressing granulocytes: response to inflammatory stimuli, ROS production, migration, phagocytosis.
- Comparison of G-CSF-, GM-CSF/IL-3- and vitamin B3-induced granulopoiesis in primary cells upon overexpression of miR-125b.
- Search for miR-125b targets using miRNA prediction programmes and biochemical validation.
miR-125b chimeric mice

- miR-q-RT-PCR: 180-fold miR-125b expression
- GFP expression in peripheral blood (week 10)
- Morphology of peripheral blood granulocytes (May-Grunwald-Giemsa staining)

Generation and analysis of miR-125b chimeric mice.

Group members: David Barzan, Matthias Eder, Michaela Scherr, Karin Battmer, Joanna Jagielska, Iris Dallmann.

Cooperations

- A. Krueger, Institute of Immunology, MHH, Unit 2.3 Regenerative Immunology.
- E. Gessner, Department of Immunology and Rheumatology, MHH.
- J. Skokowa, Department of Molecular Haematopoiesis, MHH, Unit 6.6 New Regulatory Mechanisms of Myelopoiesis.
- D. Hilfiker-Kleiner, Department of Cardiology and Angiology, MHH, Unit 5.2 Endogenous Regeneration Mechanisms of the Heart

Publications

Area B2 | Regenerative Technologies
Area B2
The Area

Area B2 can be subdivided into two sub-areas, ‘Regenerative Materials and Laser Engineering’ (Unit 7.1 to Unit 7.4) and the newly established ‘Imaging Platform’ (Unit 8.1 to 8.6). Within the ‘Regenerative Materials’ group, different tailor-made functionalized polymers and regenerative agents are synthesized for specific applications in tissue engineering. Within the first funding period of REBIRTH, a comprehensive tool kit for biocompatible functionalized polymers for use as a bio-artificial extracellular matrix was developed and applied for different purposes. In order to apply biohybrid devices to patients such that tissue-specific compatibility is ensured, general procedures for the functionalization of organic and inorganic surfaces are available. These techniques allow the functionalization of inert materials in such a way that certain cell types grow easily and efficiently on the surfaces. New synthetic tools were established for the synthesis of highly functional and highly active small molecules for application in stem cell differentiation, biofilm inhibition and antiproliferation.

The laser engineering activities can be subdivided into four different subunits: i. Laser printing; ii. Nanosurfaces; iii. Nanoparticles; and iv. Laser manipulation and cellular engineering. Several nanomaterials can be generated via laser technologies. Nanocarriers for directed and stimulus-induced ion, drug or gene delivery can be produced in a highly pure and productive manner. These laser techniques can also be used for the functionalization of biological devices and implants, and the nanomaterial itself can be used as nanosensors for biolabelling and bio-imaging. A major aspect of the laser engineering work within the research consortium is the generation of 3D scaffolds via laser printing using two-photon polymerization techniques. The fabrication of 3D scaffolds with microfluidic channels as a model system for vascularization is possible. This printing activity enables implantable tissues to be created by means of func-

(top) Lung segmentation using an adaptive watershed algorithm.
(middle) Schematic illustration of the rotor construction used for FAd measurements.
(bottom) Pattern of two transferred cell types; cells were stained with fluorescent dye prior to the transfer (green: epithelial cells, blue: fibroblasts).
tional vascularization. The laser printing device allows the printing of living cells and biomaterials in 3D structures. In tissue engineering, this system serves as a model for the (future) printing of complete organs. Additionally, the printing system can be used to arrange cells and biomaterials in this specific pattern for cell and cell-environment studies. This opens up a new area of pharmacological testing.

The laser engineering group developed a sophisticated optical-transfection platform with protocols for a variety of cell types. This optical-transfection system makes it possible to transfect single cells in mixed cultures or tissues (including primary and stem cells) with extremely high efficiency and without addition of chemicals or biological agents for the transfection process. This optical-transfection technique can also be used to transfect single cells within a cell consortium.

A new imaging platform for all researchers within this REBIRTH consortium has been established. The complexity of regenerative processes, which take place at successively more complex levels (molecular, cellular, tissue, organ, organism), require visualization of structure and function by a comprehensive set of imaging tools. They are centralized here. These tools – both well-established and newly developed ones; all optimized for applications in regenerative medical research – range from the nanometre resolution scale of small samples (electron microscopy and tomography), cell imaging by advanced light microscopy (confocal and multiphoton fluorescence) and non-destructive micrometer resolution imaging of medium-sized objects (optical-coherence tomography and scanning laser optical tomography), to non-invasive live imaging of small animals (MRI, PET-CT, SPECT-CT) and humans (MRI). Thus, pathological and repair processes can be characterized morphologically (organ, tissue, cell (ultra)structure) and physiologically (motility, metabolism, perfusion). In addition, target structures (e.g. labelled cells or gene products) can be traced and analysed with regard to their localization and distribution. Important aspects are correlative (hybrid) approaches combining multiple imaging modes on one and the same sample, automated high-throughput techniques for screening purposes, and the use of imaging datasets as input for further analysis by 3D reconstruction and quantitation by stereology and image analysis.

CRUs and Units

CRU 7
Regenerative Materials and Laser Engineering

Unit 7.1 Functionalized Polymers and Regenerative Agents
Unit 7.2 Nanoengineering
Unit 7.3 Nanoparticles
Unit 7.4 Nanosurfaces
Unit 7.5 Laser Printing
Unit 7.6 Laser Manipulation and Cellular Engineering

CRU 8
Imaging Platform

Unit 8.1 Automated and Quantitative Microscopy of Intracellular Trafficking
Unit 8.2 Quantitative Microscopy in Regeneration
Unit 8.3 Radionuclide Molecular Imaging
Unit 8.4 Small Animal MRI
Unit 8.5 Functional and Molecular MRI
Unit 8.6 Computational Image Analysis
Unit 8.7 Functional Molecular Microscopy
Goals

Biocompatible biopolymer-based materials with adjustable mechanical and biological properties will be prepared and used to embed various cell types and serve as artificial extracellular matrix. Depending on the cell-type targeted, it is necessary to attach additional signal molecules such as RGD peptides. A similar decoration approach will be followed to attach signalling peptides or biofilm inhibiting drugs to implant surfaces. Here it is crucial to ensure a dense covalent linkage of the active molecules to metal and polymeric surfaces. Therefore, different attachment strategies will be tested and applied.

In addition, the initial screening and further development of small molecule drugs will be performed. These drugs should be used for cell expansion of human ES and iPS cells in chemically defined media.

Achievements

Together with A. Kirschning (Institute of Organic Chemistry, LUH), we realized the following milestones:

- Synthesis of an array of stable and self gelling biopolymer-based hydrogels (aldehydes / hydrazines) for cardiac tissue engineering (with I. Gruh).
- Cu-free „click“ decoration of biopolymers with cyclic RGD-peptides.
- Plasma assisted surface modification of polymethylpentene (TPX) for the endothelialization of blood oxygenators (with B. Wiegmann, U. Martin, Novalung® and Plasmatreat GmbH).

Planning / Objectives

- Set-up of a toolkit with biocompatible functionalized polymers for use as bioartificial ECM.
- Covalent functionalization of organic and inorganic surfaces of biomedical implants with signal molecules.
- Synthesis of drug coated magnetic nanoparticles for controlled delivery and release.
- Development of new drugs for stem cell culture, biofilm inhibition or antiproliferation.
## Publications


## Cooperations

- U. Martin, I. Gruh, B. Wiegmann, R. Zweigerdt, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH.
- L. Sajti, Nanotechnology, Laser Zentrum Hannover, Unit 7.3 Nanoparticles.
- M. Stiesch, Clinic for Dental Prosthetics, MHH, surface modification of dental implants.
- S. Chirachanchai, Thailand, chitosan based scaffold construction.
- Plasmatreat GmbH.

## Public relations

Goals

**Laser Printing**
- Investigating the cells behavior, arranged in 2D and 3D patterns and tissue.
- Scaffold-free tissue engineering.
- Generating thick, vascularized tissue.
- Studying tissue development.

**Nanosurfaces**
- Scaffold-based tissue engineering.
- Generation of scaffolds with microfluidic channels.
- Development and application of different biomaterials.

**Nanoparticles**
- Metallic, Inorganic oxide-, Ceramic-, Polymeric.
- Inorganic-organic-, Core-shell, Multimaterial alloy.
- Surface mono- or multifunctionalized nanoparticles.
- Polymer based nanocomposites.
- Nanocoatings.

Achievements / Planning

**Laser Printing**
Printing of...
- biomaterials, bioagents.
- different vital cells,
- extra-cellular matrix,
... in two and three-dimensional structures.

**Nanosurfaces**
- Generating thick, vascularized tissue.
- Studying tissue development.

**Nanoparticles**
- Local drug-delivery.
- Bioactive surfaces (antibacterial, proliferative).
- Nanomarkers, nanosensors.
- Cell targeting.
Cell Biology

Selective Cell Control
- Inhibition of fibrous encapsulation.
- Stimulation of cells that promote implant integration.
- Cell adhesion, morphology, orientation, proliferation, physiology, differentiation.

Basic Research
- Cell attachment dynamics, ECM-guidance, integrin specificity, outside-in signaling cascades, stem cell differentiation pathways.
- Explanation of selective cell control to facilitate material (properties).
- Search for future biomedical applications.

Topography-dependent inhibition of fibroblasts, but enhanced neuronal and osteogenic differentiation.
Goals

- Fabrication of novel nanomaterials for application in biomedical science and regenerative nanomedicine.
- Assembly of nanocarriers for directed and stimulus-induced ion-, drug-, gene-, etc. delivery.
- Functionalization of biocompatible nanocomposite, biological devices, implants.
- Synthesis of medical nanomarkers / nanosensors for bio-labeling and bio-imaging applications.

Achievements

- Design of ultrapure nanomarkers / nanosensors.
- Light-mediated release, proving near 100% integrity of released molecules (peptides, oligonucleotides).
- Magnetic-field induced time-controlled release, not affecting function of released agents (penicillins, sulfadiazine, oligonucleotides).
- Nanoactivated surfaces improves biocompatibility (antibacterial, cell-proliferative).

Planning

- Development of novel cell-specific (targeting) and highly magnetic nanoparticles for local drug-release.
- Nanoparticle-coupled and light-mediated local drug-delivery systems acting at the cellular level.
- Visualization of magnetic nanoparticles and nano-hybrids in deep tissue combined with local cellular effectivity.
- Nanoparticle-based biohybrids for single and multiphoton in vivo diagnostics.
- Nanoparticles for targeting and selection of cellular subpopulations (e.g. hepatocyte).
Cooperations

- A. Haverich, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Unit 4.1 Biohybrid lung.
- G. Dräger, Institute of Organic Chemistry, Leibniz University Hannover, Unit 7.1 Functionalized Polymers and Regenerative Agents.
- M. Bock, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.4 Hepatic Cell Therapy - Patient Liver Stem Cells.
- M. Stiesch, Clinic for Dental Prosthetics, MHH, surface modification of dental implants.
- F. Wacker, Institute for Radiology, MHH.
- H. M. Escobar, Univ. of Veterinary Medicine Hannover.
- H. Fischer, Dental Materials and Biomaterials Research Center at the Universitätsklinikum Aachen.
- W. Marine, Centre Interdisciplinaire de Nanoscience de Marseille CINaM-CNRS, France.
- F. Brandi, Italian Institute of Technology, Italy.

Publications


Grants, awards, patents, outreach

- SFB/TRR 123 PlanOS TP A4, Laser-active polymer materials.
- BMBF-Projekt LapoNano, Laser-generated nanocomposites for optical applications.
- 1st poster prize on the NanoMed Conference in Berlin.
- 2nd poster prize on ANGEL 2010 conference.
Goals

- Development of laser-based technology for the fabrication of implantable 3D tissue with functional vascular network is the main challenge and long-term goal.
- Establishment of 3D *in vitro* cell culture model, as an alternative to animal test studies.
- Development of novel hydrogel and protein based biomaterials for 2PP fabrication of tissue engineering scaffolds.

Achievements

- Chemical synthesis of photo-crosslinkable materials for 2PP fabrication of TE scaffolds based on natural polysaccharides like hyaluronic acid (HA), chitosan and alginate has been established.
- High throughput large scale 3D tube and vascular structuring by 2PP has been demonstrated.
- New method for the analysis of material-cell interactions has been developed.

Planning

- By the end of 2013, modified chitosan and alginate hydrogels suitable for 2PP structuring will be developed.
- Creation of microvascular channels in different hydrogels will be investigated. Functionality of the engineered channels will be studied under perfused bioreactor conditions.
- The specific role of the ECM (Extracellular Matrix) and integrin receptors in cell behaviour will be analysed. Material influences on stem cell differentiation will be investigated.
Cooperations

- G. Dräger, Institute of Organic Chemistry, Leibniz University Hannover, Unit 7.1 Functionalized Polymers and Regenerative Agents.
- L. Koch, Nanotechnologie, Laser Zentrum Hannover, Unit 7.5 Laser Printing.
- A. Hilfiker, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Unit 5.6 Tissue Engineered Valves.
- A. Loos, BioMedimplant, MHH, Unit 10.5 Biocompatibility.
- Institut für Biomedizinische Technik, Universität Rostock (IBMT).
- Department of Prosthetic Dentistry and Biomedical Materials Science in the Center for Oral and Maxillofacial Surgery (MHH).
- Joint Department of Biomedical Engineering NCSU and UNC, North Carolina, USA.

Publications

Goals

- Generation of fully functional autologous tissue (or later – complete organs) with integrated capillaries and blood vessels for supplying the cells. Therefore, different cells will be arranged in precise 3D patterns mimicking the cell distribution in native tissue.
- Arranging cells and biomaterials in specific 2D and 3D high-resolution patterns to allow detailed studies of cell-cell and cell-environment interactions.

Achievements

It has been demonstrated that nearly 100% of cells survive the printing process unharmed. The differentiation behavior of stem cells is not affected.

It has been demonstrated that printed cells form intercellular adherens junctions and functional gap junctions, which proves the tissue formation.

This technique has been used to arrange cells in specific patterns to study cell-cell and cell-environment interactions.

Planning

Printing of different multicellular 3D tissue models for studying ex vivo the behavior of targeted cells in defined 3D cell environments and for investigating cell-cell and cell-environment interactions.

Since so far only skin tissue was generated, the formation of tissue with different printed cell types will be tested.

For the generation of thick tissue, the integration of vascular capillaries is indispensable. Different strategies for the vascularization will be investigated.

Cells directly after printing (left). After 5 days (bottom).
Cooperations

- S. Korossis, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Unit 4.1 Biohybrid Lung.
- F. Limbourg, Department of Cardiology and Angiology, MHH, Unit 5.9 Regenerative Agents.
- A. Schambach, Institute of Experimental Haematology, MHH, Unit 6.2 Regenerative Gene Therapy.
- N. Hofmann, Institute for Multiphase Processes, LUH, Unit 10.4 Cell Protection Technology.
- A. Loos, BioMedImplant, MHH, Unit 10.5 Biocompatibility.
- P. Vogt, Department of Plastic, Hand and Reconstructive Surgery, MHH.
- G. Steinhoff, FKGO, University of Rostock.
- S. Jockenhövel, RWTH Aachen.

Grants, awards, patents, outreach

- Our work on laser printing has been highlighted in several newspapers, magazines, radio and TV (e.g. Deutschlandfunk, NDR Visite, Wirtschaftswoche, etc., and recently: HAZ, Spiegel Online, Neue Osnabrücker Zeitung, Audimax-IT).
Goals

- Development of a new laser transfection method.
- Construction of a sophisticated transfection platform.
- Investigation of the fundamental processes.
- Proof of principle for the transfection of hard to transfect cell types (primary and stem cells).
- Detailed investigation of the toxicity in respect to long term and genotoxic effects.

Achievements

- Construction of a transportable functional model for end users.
- 88% transfection efficiency in cell lines.
- 70% efficiency for molecular delivery into primary mouse neurons.
- Efficient siRNA and Morpholino oligomers mediated gene knockdown in cell lines and primary neurons.

Planning

- Fundamental experiments concerning the perforation effect and theoretical modelling of laser-particle interactions.
- Selective transfection of murine neurons.
- Molecular delivery in tissue.
- Cell type specific manipulation by targeted gold Nanoparticles.

Transfection of primary murine neurons (left). Functional model (bottom right).
**Group members:** Sabine Donner, Heiko Meyer, Tammo Ripken, Markus Schomaker, Nadine Tinne, Stefan Kalies, Dag Heinemann (from left to right).

**Publications**


**Grants, awards, patents, outreach**

- Exhibition at the Compamed, Düsseldorf 2012.
- International Symposium on Cell and Gene-Based Therapies, Granada 2012.
Unit 8.1 | Automated and Quantitative Microscopy of Intracellular Trafficking

Goals

- Eliminate Host Barriers against Herpes Simplex Vector Transduction.
- Establish Infrastructure for Assay Development with a Pipetting Robot and an Automated Microscope to Standardize Cell Biology Assays.
- Finish Genome-wide RNAi Screen on Host Factors for HSV1 Vector Expression; Validate Potential of 3 Hits to Improve Vector Efficacy.

Achievements

- 2° RNAi Screen done, 3° Screen ongoing.
- Implemented Automated Microscopy at MHH.
- Implemented Quantitative Image Analysis.

Planning

- Implement Pipetting Robot at MHH.
- Further Automate Microscopy Assays.
- Further Automate Image Analysis.
- Implement Quantitative Electron Microscopy.
- Further Publications.
Cooperations

- L. Pelkmans, University of Zürich (Ch), RNAi screen for viral gene expression; Automated microscopy; Object segmentation; Quantitative Image Analysis Algorithms based on Cell Profiler.
- K. Grünewald, Oxford University (U.K.), Analysis of HSV1 vector cell entry by cryoelectron tomography of frozen native samples.
- M. Kann, Bordeaux University (F), Nuclear targeting of viral vectors - role of dynein and nuclear pores.
- I. Christae, Princeton University (USA), Quantitative Mass Spectrometry of vector - host protein complexes.

Grants, awards, patents, outreach

- Member of MHH Forschungskommission; Steering Committee of SFB900 and SFB1125; Beirat Gesellschaft für Virologie.

Publications

**Goals**

We provide advanced microscopy (in particular EM) methods in combination with unbiased methods for quantitative assessment of micro-structure of cells, tissues, and organs (stereology).

We aim at a comprehensive quantitative assessment of lung micro-structure in animal models of lung disease using a correlative approach from non-destructive imaging of the whole lung to high-resolution electron microscopy.

**Achievements**

- Dissemination of stereological methods for quantification of organ, tissue, and cell (ultra)structure.
- Quantitative non-destructive ex vivo imaging of the mouse lung using microCT and scanning laser optical tomography (SLOT).
- Assessment of exogenous surfactant therapy in experimental ischemia / reperfusion injury of the lung.
- Analysis of heart regeneration in the newt.
- Assessment of silk protein fibroin as scaffold for cardiac tissue engineering.

**Planning**

- Further development and application of non-destructive imaging techniques combined with stereology and image analysis for quantitative lung imaging based on microCT and SLOT.
- Extension of EM spectrum by establishing volume-EM of the lung (based on serial block face SEM, focused ion beam SEM, and electron tomography).
- Develop and submit (to American Thoracic Society) guidelines for quantitative microscopic assessment of emphysema in mice.
Publications


Cooperations


- T. Ripken H. Meyer, Biomedical Optics, Laser Zentrum Hannover, Unit 7.6 Laser Manipulation and Cellular Engineering, SLOT.

- B. Rosenhahn, Institute for information processing, LUH, Unit 8.6 Computational Image Analysis, image analysis.

- E. Hoffman, University of Iowa, microCT.

- A. Günther, University of Gießen, T. Geiser, University of Bern, lung fibrosis.

- T. Wahlers, T. Wittwer, University of Cologne, lung I/R injury.

- F. Engel, T. Braun, Max-Planck-Institute Bad Nauheim, heart regeneration.

Grants, awards, patents, outreach

- DFG OC 23/10-1 and MU 3118/2-1.

- BMBF (DZL) Imaging Platform.

- Adjunct Professorship (University of Saskatchewan, Canada) (M.O.).

- Co-chair of the American Thoracic Society Project “Guidelines for proper quantification of structural changes in mouse models of emphysema” (M.O.).

- REBIRTH News 1.2013: Introduction of Central Research Unit EM at MHH.

Group members: C. Mühlfeld, M. Kellner, M. Kühnel, J. Hegermann, C. Wrede, R. Grothausmann, M. Ochs (from left to right).
Goals

Developing a Platform for Noninvasive Imaging of Regenerative Biology

In Unit 8.3, we seek to establish a translational molecular in vivo imaging environment, which facilitates

- effective guidance of regenerative therapies based on image-defined individual disease biology, and
- hand-in-hand development of novel imaging techniques and novel therapies, from basic science to clinical practice.

Achievements / Planning

Novel in vivo Imaging Protocols Using Dedicated Small Animal Cameras

Using the new preclinical molecular imaging lab and its high-resolution PET-CT and SPECT-CT systems, various imaging protocols have been developed, including: Lung and heart perfusion, bone metabolism, liver metabolism / hepatobiliary clearance, neurotransmission (SPECT); Glucose utilization, oxidative metabolism, somatostatin-receptor density (PET); Contrast-enhancement, respiratory & electrocardiogram gating (CT)

In parallel, protocols for direct radionuclide labeling of therapeutic cells, and for reporter-gene based imaging techniques are being prepared.

Towards Integration of Molecular Imaging and Therapy

The newly available imaging techniques are employed to study regenerative mechanisms (first target: infarct healing), and – in a next step and through close collaboration with other units – to determine the effect of novel therapeutic interventions developed in REBIRTH.
Cooperations

- K. Wollert, Department of Cardiology and Angiology, MHH, Unit 5.1 Secreted factors and non-cell-based strategies for cardiac regeneration.
- D. Hilfiker-Kleiner, Department of Cardiology and Angiology, MHH, Unit 5.2 Endogenous Regeneration Mechanisms of the Heart.
- F. Limbourg, Clinic for Nephrology, MHH, Unit 5.9 Regenerative Agents.
- T. Moritz, Institute of Farm Animal Genetics, Mariensee, FLI; Institute of Experimental Haematology, MHH, Unit 6.1 Enhanced and Synthetic Cells for Regeneration.
- M.R. Abraham, R. Wahl, Johns Hopkins University, Cardiac stem cell imaging.
- J. Knuuti, Turku University PET Center, Metabolic imaging.

Grants, awards, patents, outreach

- Subaward to NIH R01 HL092985 (“Imaging of the Myocardial Micro-environment to Facilitate Cardiac Stem Cell Engraftment”), PI: F. Bengel (MHH); M.R. Abraham, R. Wahl (Johns Hopkins University, Baltimore, MD).
- EU FP7 Reintegration Grant PIRG 08-2010-276889 (“Stem Cell Imaging”), PI: F. Bengel.
- CIHR Fellowship Grant (“Tracking of Endogenous Progenitor Cells and Inflammation Following Myocardial Infarction”), PI: J. Thackeray.

Publications

Goals

- Improve Image accuracy / Improve Image quality.
- Functional and anatomical imaging in disease models.
- Noninvasive characterization of tissue physiology.
- Longitudinal monitoring of systemic and/or organic function and anatomy.
- Multiparametric studies: combining several MR acquisitions, to probe several parameters, are of particular interest, e.g., MRI and MRS sequences offer a variety of contrast. A fast acquisition modulus, offering short acquisition but weak contrast is combined with a modulus that sensitizes to local value of parameter such T1, T2, T2*, perfusion, diffusion, local tissue displacement.

Achievements

- Imaging of all in disease models relevant small animals as well as aquatic animals Cell imaging / tracking in mice and rat.
- Noninvasive phenotyping of mice.
- Preclinical diagnostic imaging in disease models.

Planning

- Multimodality Image Data acquisition as part of the joined imaging center.
- Validation of new Imaging Methods.
Exemplary images of special imaging techniques.

Publications


Cooperations

- Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH.
- Department of Plastic, Hand and Reconstructive Surgery, MHH.
- Clinic for Nephrology, MHH.
- Department of Nuclear Medicine, MHH.
- Institute for Neuroradiology, MHH.
- Institute for Radiology, MHH.

Grants, awards, patents, outreach

- Helmholtz VH-VI-523 (5 years from 2012).
- Translation of ASL imaging technique as a clinical diagnostic tool in Ischemia reperfusion injury - AKI with Radiology, Nephrology, Institute of Animal Science, Hannover Medical School.
Goals

- Establishment of a truly non-invasive and radiation exposure free imaging platform for translational imaging that utilizes morphologic as well as functional imaging to monitor regenerative therapies from bench to bedside.
- Application of inflammation imaging cell tracking techniques using MRI.
- Monitoring of transplants and treatment using MRI.
- Development of advanced algorithms for post-processing of imaging data.

Achievements / Planning

- In vivo monitoring of bioimplants and transplants using MRI techniques determining morphology, organ function, perfusion and tissue characterization.
- Characterization of stability, functional properties and potential problems of regenerative tissue grafts with MRI in large animal models and humans.
- Establishment of inflammation imaging and cell tracking using iron labeled granulocytes and macrophages and testing of other labeling technologies.
- Implementation of new MRI techniques with alternate signal sources such as 19F or hyperpolarized Xenon.
- Establishment of functional MRI of the lung.

Map of quantitative pulmonary microvascular perfusion measured with MRI in a control patient (left) and a patient with mild COPD (right).

Morphologic image (a), ADC map (b), FA map (c) and tractography (d) in a normal rat kidney measured by MRI (1.5 T). On morphologic image (a) the 4 anatomical layers of the rat kidney can be differentiated (CO; OS; IS; IM).
Magnetom Avanto 1.5 Tesla Siemens.

Publications


Cooperations

- Siemens Healthcare provided advanced MRI acquisition sequences such as 4D flow MRI, Modified look-locker inversion-recovery (Molli) for quantification of fibrosis, T2 mapping technique for quantitative estimation of myocardial edema.

- A. Haverich, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Noninvasive monitoring of morphology, function, and tissue characteristics after transplantation and implantation of bioimplants in experimental animal studies.

- Institute of Toxicology and Experimental Medicine (ITEM), Establishment of functional MRI of the lung.

- Johns Hopkins University, Functional MRI of the lung and heart in humans and quantitative imaging of inflammation in large animal models.

- P. Jakob, Department of Experimental Physics of the University of Würzburg, Germany, Functional MRI of the lung and development of new parallel imaging techniques to accelerate MRI.

Grants, awards, patents, outreach

- Dr. med. K. Hüper; IFBTx 2012, Functional magnetic resonance imaging for characterization of delayed renal graft function: comparison with biopsy results.
Goals

- In-depth understanding on processing medical images.
- Development of algorithms capable to process huge amount of data, fully automatic and being applicable to different imaging resolutions.
- Concentration on novel state-of-the art image acquisition devices: e.g. OCT, OPT/SLOT.
- Different semantic levels of image interpretation: e.g. Markov Random Fields, Boosting methods for detection or registration.

Achievements

LZH (Krüger):
OCT motion compensation: Development of a fully automatic motion compensation algorithm of 3D in vivo spectral-domain optical coherence tomographs (SD-OCT) of percutaneous implants in sedated mice using a Markov Random Field approach.

Planning

LZH (Krüger):
Motion estimation and compensation of swept-source Doppler OCT scans of a (vibrating) vocal fold and fusion of several OCT B-scans for increasing the signal-to-noise ratio of in vivo scans.

MHH (Rhode):
Analysis of future image sets. Furthermore, we will improve on the statistical optimization methods for more reliable image registration and want to develop a framework for parameter optimization for bioengineering.
Cooperations

- Laser Zentrum Hannover, OCT.
- R. Rohde, A. Haverich, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, RPT.
- Institute for Multiphase Processes, LUH, Cryo.

Grants, awards, patents, outreach

- Grant: AIF-ZIM KF2511004FR1.
- ERC-Starting Grant “Dynamic MinVIP”.
- ERC PoC-Grant “IIP” (Individualized Implant Placement).
- AIF-ZIM KF2511005DB1 (HyMo).
- 13171832.2-1906 Temporally consistent superpixels (Thomson Licensing).
- 13162354.8-1906 Method and apparatus for image segmentation (Thomson Licensing).
- 13305157.3-1906 Method for superpixel life cycle management (Thomson Licensing).

Publications

Goals

Proper differentiation of induced pluripotent stem cells (iPSCs) as well as maintenance of the physiological properties of engineered cells or tissues represents an essential pre-requisite for their successful clinical application. The main aim of our project is to conduct standardized multi-parametric protocols for the analysis of subcellular and cellular processes in modified cells and tissues by combination of non-invasive optical, biophysical and electrophysiological techniques. We also aim to adapt optical and biophysical techniques in order to study complex, functionally intact biological preparations (i.e. tissues and organs) and to analyse the metabolism-derived signals.

Achievements / Planning

Quantitative analysis of intracellular processes in living cell

The use of Förster resonance energy transfer (FRET) has become a pivotal technique for the analysis of intra-molecular interactions. However, there are serious limitations concerning the quantitative analysis of FRET signals. Therefore, we have recently developed a novel method for the spectral analysis of FRET-signals called linear unmixing FRET. To analyse the intracellular activation patterns of small GTPases (which regulate cellular morphology) in living cells, we combined confocal time-lapse microscopy with FRET techniques and developed FRET-based biosensors allowing for measurement of the RhoA activity. Upon activation, a conformational change of the biosensor occurs, which leads to changes in the FRET efficiency between donor (Cerulean) and acceptor (Venus) fluorophores (Fig. 1). By combination of FRET measurements with the time-resolved confocal microscopy, we were able to demonstrate receptor-mediated activation of RhoA in glioblastoma cells (Fig. 1) Moreover, we have established a set of FRET-based biosensors for detection of a broad ensemble of physiological parameters at the single-cell level including ion composition (Ca²⁺), concentration of second messengers (cAMP) and cytoskeleton dynamics. Such combined experimental strategies will be applied for the detailed characterization of the derived iPSCs and genetically modified cells on molecular and functional basis.

Uptake mechanisms for the miRNA enriched exosomal secreted by cardiac fibroblasts.

MicroRNAs (miRNAs) are known to be directly involved in many cardiovascular disease conditions, but recently also emerged as novel paracrine signalling mediators. In collaboration with Thomas Thum we investigated a potential paracrine miRNA cross-talk between cardiac fibroblasts and cardiomyocytes. In particular we analysed uptake mechanisms of miRNAs enriched exosomes secreted by cardiac fibroblasts. For that we labelled secreted fibroblast-derived exosomes with a fluorescent marker PKH67 and analysed exosomal uptake in the cardiomyocyte cell line HL-1 by confocal microscopy in combination with the spectral unmixing. These studies revealed an increased exosomal uptake over time (Fig. 2), which was further confirmed in cultures of primary cardiomyocytes. To confirm that exosomes are taken up into the cardiomyocytes and not simply attached to the cell surface, we also performed the 3D reconstructions of the confocal image stacks revealing the exact internalization of exosomes. These findings suggest that miRNA-enriched exosomes can represent paracrine signalling mediator in the crosstalk between cardiac fibroblasts and cardiomyocytes.
Cooperations

- I. Gruh, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 5.7 Myocardial Tissue Engineering, Structural and functional analysis of bioartificial cardiac tissue.
- T. Thum, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration, Distribution dynamics of exosomes enriched with miRNA.
- T. Moritz, Institute of Farm Animal Genetics, Mariensee, FL; Institute of Experimental Haematology, MHH, Unit 1.3 iPSC based haematopoietic regeneration, Development of automated in silico high throughput quantification of miRNA modulating generation of iPSCs.
- B. Chichkov, Laser Zentrum Hannover, Unit 7.4 Nanosurfaces, Functional integrity and physiological properties of tissues produced by the 3D laser printing.
- S. Sigrist, FU Berlin, Super-resolution microscopy of synaptic structures.
- A. Dityatev, DZNE, Magdeburg, Role of the extracellular matrix in regulation of cellular functions.
- D. Rusakov, UCL, London, UK, Calcium imaging.
- M. Niv, Hebrew University of Jerusalem, Computational analysis of protein-protein interaction.

Publications

Area C | Clinical Translation and Regenerative Products
Area C comprises Central Units (CRU) 9 ‘Regenerative Pathology and Pharmacotoxicology’ and CRU 10 ‘Regenerative Products, Clinical Trials, Ethics and Law’, with six and seven research groups, respectively. Groups in Area C have a dense established network of collaborations, both within the Cluster and with external institutions, and possess the expertise that is critical for the success of the planned experiments within REBIRTH. Members of Area C have published many papers in peer-reviewed journals, frequently as joint publications between several REBIRTH partners, some of which have appeared in prominent scientific journals. Furthermore, groups in Area C have received a large amount of competitive research funding and have been able to attract a considerable number of talented young scientists, indicating the excellent reputation that the Cluster enjoys in the outside world. The expertise provided in Area C should enable findings to be transferred from basic research into clinical application (translational approach).

CRU 9.1 is a new addition to REBIRTH. The goal is to provide new large-animal models. Functional protocols for somatic cell nuclear transfer (SCNT) and the recent availability of novel tools for precise genetic engineering, including zinc-finger nucleases (ZFNs), TALEN, CRISP-Cas and transposons, provide new options for effective production of transgenic animals. The main aim of this consortium consisting of various REBIRTH groups, led by ING / FLI, Mariensee, is...
to produce human cardiomyocytes and hepatocytes in the domestic pig for use in cell therapies with human patients. This will entail challenging experiments involving chimera formation between porcine and human cells. Another goal is the production of a useful disease model, specifically pigs showing symptoms of familial hypertrophic cardiomyopathy (FHC). This work has already been started and pregnancies from transgenic porcine fibroblasts carrying the FHC mutation induced by TALE-nuclease technology have been obtained. The group in Mariensee is closely associated with the recently established DFG-funded Transregio SFB ‘Biology of xenogenic cells - from bench to bedside’ that will move xenotransplantation closer to clinical application. This further strengthens the transplantation expertise of Hannover Medical School (MHH).

An important aspect of treatment with novel cellular therapies that will emerge from basic research in REBIRTH is to ensure the safety of the products. Units 9.2 and 9.3 have specific expertise in pathology of animal models and teratoma diagnosis, and will make this know-how available to the REBIRTH community. An important aspect of safety is genomic stability. This is specifically addressed in research Units 9.5 and 9.6, which provide technologies for cytogenetic and genomic profiling (including banding analysis, quantification of DNA repair, multicolour fluorescence in situ hybridization and telomere length measurement) to detect potential genetic instabilities. The chief goal will be to obtain insight into the underlying mechanisms of genetic instability and the factors triggering expansion of dominant clones after genetic modification of stem cells, ultimately to prevent malignant transformation. In addition, research Unit 9.4 looks at specific aspects of preclinical safety and toxicology associated with gene therapy trials (ITEM, Fraunhofer).

An important prerequisite for clinical application of novel cellular therapies is mass production of therapeutic cells, including bioactive macromolecules, pluripotent stem cells and their derivatives. REBIRTH has outstanding expertise in these areas, based on previous achievements such as the production of endotoxin-free cytokines and development of the prototype of a suspension culture system for human pluripotent stem cells in research Unit 10.2. Industrial collaborations with regard to process development and reactor technology have already been established. Clinical application of novel cellular therapies requires reliable technology
for banking of cells or at least to stabilize tissues and macromolecular assemblies. These aspects are addressed in research Units 10.3 and 10.4, located at the Leibniz University of Hannover. The research will include safety aspects, effects of long-term storage on the stability of cells and tissues, and the efficiency of the freezing process. An important prerequisite for clinical application of products derived from research in REBIRTH is to provide services assisting with all the required aspects of biological-safety evaluation of medical devices and novel therapies. Unit 10.5 specifically addresses these aspects, and the BioMedimplant facility provides the full range of methods for testing biological safety of medical products based on tissue engineering and nanomaterial. Unit 10.6 has in-depth experience in clinical trial management and will make this expertise available for all products / therapies emerging from REBIRTH activities. REBIRTH acknowledges the ethical and legal dimension of the work that is done within the Cluster. Unit 10.7 specifically explores these novel ethical questions; it will provide advice on legal aspects and stimulate the ethical and moral debate on cellular therapies.

In conclusion, Area C of REBIRTH provides the necessary infrastructure for translation of novel products or therapies derived from basic research by REBIRTH scientists into clinical settings. It offers a unique blend of know-how with regard to production of large-animal models, determination of (epi)genomic stability, pathology, upscaling of stem cell production, long-term storage of biological material, and clinical trial management, and even addresses the ethical and legal aspects of regenerative medicine. This should help to move regenerative medicine closer to clinical application, which is a major goal of REBIRTH.

CRUs and Units

**CRU 9**
**Regenerative Pathology and Pharmacotoxicology**

- **Unit 9.1** Large Animal Models
- **Unit 9.2** Pathology of Humanized Animal Models – Human Pathology
- **Unit 9.3** Histopathology of Animal Models and Teratoma – Veterinary Pathology
- **Unit 9.4** Preclinical Safety and Toxicology
- **Unit 9.5** Cytogenetic Profiling
- **Unit 9.6** Genomic Profiling

**CRU 10**
**Regenerative Products, Clinical Trials, Ethics and Law**

- **Unit 10.1** Production and Purification of Recombinant Proteins
- **Unit 10.2** Mass Production of Pluripotent Stem Cells and Derivatives
- **Unit 10.3** Biostabilization of Tissues and Macromolecular Assemblies
- **Unit 10.4** Cell Protection Technology
- **Unit 10.5** Biocompatibility
- **Unit 10.6** Clinical Trial Management
- **Unit 10.7** Ethical and Legal Dimensions
Goals

The Pig as a Large Animal Model:

Novel biotechnological tools such as Zinc-Finger Nucleases (ZFNs), Transcription-Activator like endonucleases (TALENs) or transposons such as the Sleeping Beauty System allow for effective knock outs of a specific genomic locus and / or insertion of transgenes into specific safe harbors of the genome. The domestic pig shares many genetic, physiological and anatomical features with humans, and can thus serve as a predictive model for human diseases. Besides, the limited availability of human donors for transplantation prompts research into alternatives, such as the production of humanized organs in pigs. Recently, a humanized porcine pancreas was generated in a pig in which the endogenous pancreas had been eliminated (Matsunary et al., 2013), indicating the feasibility of producing pigs with exogenous organs. Our goal is to produce pigs with internal organs (such as hearts and kidneys) with a significant contribution from human cells and generate pig models for human diseases such as familial hypertrophic cardiomyopathy (FHC).

The pig as model for human diseases

Familial hypertrophic cardiomyopathy (FHC):

FHC is a heart disease characterized by hypertrophied (thickened) myocardium, which can lead to sudden unexpected cardiac death in any age group. Point mutations in cardiac related target genes have shown to be responsible for this heart failure. Using new technologies, such as Transcription activator-like effector nucleases (TALENs), specific point mutations can be induced into the porcine genome. To obtain living piglets with the genetic predisposition for this disease, somatic cell nuclear transfer (SCNT) is conducted. This animal model can then be used to follow the onset and course of this disease.

Production of pigs with humanized organs

In order to evaluate the possibilities to incorporate human cells into porcine organs, we are currently working with non-human primate iPSC since they are similar to human pluripotent cells, while at the same time they not present ethical concerns. These cells will be injected into porcine embryos or fetuses at different stages of development and their contribution to heart or liver will be determined. Results will be used to assess the potential for using human pluripotent stem cells to produce humanized pigs, as well as the risks associated with such procedures.

**Fig. 1:** Transcription activator-like effector nucleases (TALENs) can be designed to bind to a specific gene of interest; after dimerization of the nucleases (FokI) a double strand break (DSB) is formed and base pairs can get lost; if a donor DNA with homologous ends suitable to the targeted site is available, specific point mutations or transgenes can be introduced into the targeted gene.

![Image of TALEN binding and DNA modification](image_url)
Preliminary Results

- Injection of non-human primate iPSC into parthenogenetic porcine blastocysts.

Cloning results using mutated wild-type-fibroblasts to generate pigs as a large animal model for FHC.

Publications


Project Partners

- B. Brenner, Institute for Molecular and Cell Physiology, MHH, Unit 9.1 Large Animal Models.

- U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung regeneration and repair.

- M. Ott, Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 3.5 Hepatic Cell Transplantation and Genetic Manipulation.

- T. Cantz, MPI-Cell and Developmental Biology; Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 1.1 Translational Hepatology & Stem Cell Biology.

Grants, Awards, etc.


- Martin-Lerche-Forschungspreis der DVG (Deutsche Veterinärmedizinische Gesellschaft), Oktober 2012.
Goals

**Animal Model of Familial Hypertrophic Cardiomyopathy**
- Generation of knock-in animal model for FHC with missense mutations in head domain of ventricular (β-cardiac) myosin heavy chain (e.g., R723G, R719W).
- Characterization of phenotype development in the transgenic pigs compared to FHC-patients.
- Identification and testing of novel therapeutic approaches, e.g., via changes in allelic imbalance (unequal expression of mutant / wild-type mRNA and protein).

**Achievements**
- Transgenic porcine fibroblasts successfully generated via TALE-nuclease technology.
- Successful somatic cell nuclear transfer (SCNT).
- Embryos successfully transferred to synchronized recipients.
- Expression of mutant myosin in muscle tissue demonstrated at mRNA level.
- First functional studies.

**Planning**
- Generation of transgenic breeding lines of 4 different FHC mutations in the head domain of β-cardiac myosin.
- Structural / functional analysis of myocardium and skeletal muscle for effects of FHC-related mutations (comparison with effects in humans).
- Study development of FHC-phenotype vs. development of allelic imbalance (unequal ratio of mutant / wildtype mRNA and protein).

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(A) Scheme of TALE-nuclease action (top); generation of transgenic fibroblasts (bottom). (B) SCNT and reproductive cloning.

(A) Force-pCa relation of isolated left ventricular cardiomyocytes (maximum force, Ca++-sensitivity of force generation before and after PKA treatment). (B) Fraction of mutant mRNA relative to total β-myosin-mRNA in left ventricular tissue of transgenic piglets compared to tissue samples of patients with same mutation (R723G).
Related projects

In collaboration with U. Martin und R. Zweigerdt (HTTG) we currently establish a model system of FHC at the cellular level, which is based on iPSC-derived cardiomyocytes, generated from dermal fibroblasts of FHC-patients. Such FHC-patient-specific cardiomyocytes, which co-express wildtype and mutated β-cardiac myosin allow us to further study mechanisms of unequal allelic expression of mutated and wildtype myosin. We found this “allelic imbalance” in all patients for which we so far analyzed β-cardiac myosin expression at mRNA and protein level (Tripathi et al., BRC 2011). We hypothesize that unequal expression of mutated and wildtype myosin in individual cardiomyocytes could be a major initial trigger for disease development.

Thus, by longitudinal studies in the FHC-pig model we will be able to analyze whether, e.g., the allelic imbalance changes over time and correlates with disease progression. In addition, with a model system of human cardiomyocytes expressing different β-myosin mutations, we will investigate mechanisms of gene regulation and test whether the expression of the mutated myosin can be specifically suppressed by modifying allelic imbalance. Here the large animal model will be essential to test such techniques in vivo. Particularly for severely affected FHC-patients, it would be highly desirable to restore normal sarcomere function with such an approach.

Grants, awards, patents, outreach

- DFG Grant KR1187/19-1,2, PI: T. Kraft.

Cooperations

- B. Petersen, H. Niemann, Institute of Farm Animal Genetics, Mariensee, (Animal model).
- Sangamo BioSciences Inc., Richmond, CA, USA (TALE-nucleases).
- C. Mühlfeld, M. Ochs, Institute of Functional and Applied Anatomy, MHH, Unit 8.2 Quantitative Microscopy in Regeneration.
- S. Sarikouch, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, (Echocardiography, MRI).

Publications

Goals

- Professional patho-anatomical analysis of genetically manipulated animals.
- Platform for information (modes of fixation and tissue banking), technical support (tissue sections and stain, immunohistochemistry, laser microdissection, RNA analysis).
- Standardized Tissue Processing (fixation and embedding with clinical standards).
- Training (microscopical analysis).

Studies on stem cells and regenerative mechanisms depend to a large extent on experimental models in animals. The phenotypic analysis of animal models is a prerequisite for the generation and verification of hypotheses. It was in order to establish a set-up for professional analysis of animal organisms and tissues that the Service Unit was established.

The Service Unit provides guidance for REBIRTH groups with regard to handling, processing and storing of animal tissues from the experimental-design stage onwards. Advice is provided on macroscopic analysis and microscopy, including special techniques such as immunohistochemistry. Organ specialists at the Institute of Pathology are involved in the diagnosis and interpretation of particular organ-specific phenotypic changes.
Cooperations

- A. Schambach, Institute of Experimental Haematology, MHH & T. Moritz, Institute of Farm Animal Genetics, Mariensee, FL; Institute of Experimental Haematology, MHH, Unit 6.1 Enhanced and Synthetic Cells for Regeneration.

- M. Eder, M. Scherr, Department of Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.7 Molecular Control of Granulocytic Differentiation.

- R. Stripecke, Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.4 Regenerative Immune Therapies.

- U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung Regeneration and Repair.

- C. Guzman, Helmholtz Centre for Infection Research, Braunschweig, HZI.

- AG Jehn, AG Rudolph, AG Kotlarz / Klein.

Publications


Unit 9.3 | Histopathology of Animal Models and Teratoma – Veterinary Pathology

Goals

The Experimental Pathology is located in the Institute for Laboratory Animal Science, MHH. With our collaboration partner Fraunhofer ITEM we are able to provide a competent platform for histopathological analyses of animal models and stem cell research (teratoma assay) and have access to a broad network with scientific expertise. We work closely with various groups from REBIRTH as well as others.

Besides contributing expertise to histological analysis of stem cell research, project-specific processing and histological scoring has been accomplished for various animal models (e.g., asthma models, lung infection models, colitis models, GvH model, embryonic development disorders, tumour models, immunodeficiency models). Expertise is offered for pathomorphology / histology, immunohistochemistry, haematology and clinical chemistry.

Achievements / Planning

**Project I, Glage / Bleich:**
- Characterization of the pluripotency of stem cells by subcutaneous and subcapsular kidney injection in immunodeficient mice for collaborating research groups.
- Whole body trimming in accordance with international guidelines for phenotyping animal models.
- Tailored advice for histopathological questions, developing of project-specific methods and scoring.

**Project II, Rittinghausen / Glage:**
- Establishing an antibody panel for the characterization of the pluripotency of stem cells of different species origin.
- PhD student Sandra Kunz.

**Project III, Glage / Bleich:**
- Characterization of the Rag1-ko rat, a new immunocompromised animal model.
- PhD student Katharina Schulz.
Publications


Grants, awards, patents, outreach

- 2010–2013 DFG, Role of CD14 in intestinal barrier and immune function (Bleich).

- 2009–2013 DFG SFB621 Pathobiology of the intestinal mucosa, Z1 Gnotobiology (Dorsch / Hedrich / Bleich).


- 2013–2016 DFG Priority programme 1656, A gnotobiology Unit within the SPP 1656 (Bleich / Autenrieth / Haller).
Goals

Gene therapy
- Short and long term toxicity *in vivo* and *in vitro*.
- Stability of therapy.

Service Unit
- GLP studies according to ICH, EMA & FDA guidelines incl. inhalation.
- γ-irradiation of cells and mice.
- (immuno)histopathology & morphometry guidelines.

Achievements / Planning

GLP-studies:
- Ongoing (GLP) mouse study with gene-modified haematopoietic cells (for Unit 6.2).
- Further studies with a dose of 8 Gy will be started soon.

Service γ-irradiation:
- A Gamma radiation source (Gamma Service Recycling GmbH) with a highly radioactive source (HRQ) was obtained and installed at Fraunhofer-ITEM.
- Dosimetry measurements using a thermo-luminescence dosimeter (MPA.NRW) were undertaken to determine the dose level at various positions within the irradiation chamber.
- The HRQ can be used to inactivate cells, which thereafter can be taken as feeder cells. In addition, the bone marrow of mice was successfully depleted at 10 Gy.

GLP toxicology course for PhD students at Fh-ITEM planned.
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<td>Group Leader</td>
<td>Dr. Roman Halter</td>
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<th>Head of Department</th>
<th>Prof. Dr. Clemens Dasenbrock</th>
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**Group members:** Susanne Rittinghausen, Roman Halter.

Irradiation Chamber (carousel for mice).

Dosimetry of the HRQ using calibrated TLD dosimeter (rods) for γ-irradiation (MPA.NRW).

**Publications**


**Cooperations**

- Institute of Experimental Haematology, MHH.
- Institute for Laboratory Animal Science, MHH.
- Weizmann Institute of Science, Israel.
- Consejo Superior de Investigaciones Científicas CSIC, Spain.

**Grants, awards, patents, outreach**

- EU-CELL-PID, Advanced Cell-based Therapies for the treatment of Primary ImmunoDeficiency (Institute of Experimental Haematology; Fraunhofer-ITEM subcontractor).
Methods we offer all REBIRTH groups to detect genetic instability.

### Goals
- Determine the karyotypic stability of modified human and murine stem cells.
- Perform cytogenetic monitoring within gene therapy trials.
- Determine culture conditions and reprogramming protocols that increase (or hopefully prevent) the development of chromosome aberrations.

### Achievements / Planning
During the last few years it has become more and more important to show that the reprogramming procedure does not lead to DNA damage or to the selection of clones that have growing advantages due to acquired genetic changes.

We will provide cytogenetic technologies to all REBIRTH groups and will build up a Core Facility (see also group of Doris Steinemann).

Different technologies depending on the scientific questions and on the source of cells will be applied, to guarantee a high quality of cells in the sense of genomic stability.
Group members: Gudrun Göhring, Kathrin Thomay, Beate Vajen, Marwa Farid, Azam Salari, Maike Hagedorn, Andrea Schienke.

Publications


Cooperations

- C. Niemeyer, M. Wlodarski, Universitätsklinikum Freiburg, Germany.
- S. Nimer, H. Xu, Memorial Sloan-Kettering Cancer Center, New York, USA.
- K. Döhner, R. Schlenk, Universitätsklinikum Ulm, Germany.

Grants, awards, patents, outreach

Goals

Before advanced stem cell products can be applied in the clinic, it will be important to confirm the genetic integrity of advanced stem cell products. We propose to use array-based comparative genomic hybridization (array-CGH), a method already implemented in routine cytogenetics, to provide a most detailed knowledge on genetic imbalances.

We will make available the modern molecular cytogenetic technique of array-CGH, both for human as for murine cells, to cooperating REBIRTH groups. In this context, we also offer the bioinformatic analysis of array-CGH data and provide consulting with regard to scientific questions on cytogenetic results.

Achievements / Planning

Genome-wide profiling of advanced stem cell products, i.e. reprogrammed and gene corrected human or murine cells, by array-CGH.

Decipher conditions and factors associated with an increased genetic instability, particularly small genomic deletions and duplications.

Compare genetic instability of modified haematopoietic stem cells and iPS cells with that observed in human tumours to assign different risk categories to defined genomic changes.
Cooperations

- C. Baum, O. Kustikova, Institute of Experimental Haematology, MHH.
- Z. Li, A. Schwarzer, Institute of Experimental Haematology, MHH.
- A. Schambach, Institute of Experimental Haematology, MHH & T. Moritz, Institute of Farm Animal Genetics, Mariensee, FLI; Institute of Experimental Haematology, MHH, Unit 6.1 Enhanced and Synthetic Cells for Regeneration.
- T. Cantz, MPI-Cell and Developmental Biology; Department of Gastroenterology, Hepatology and Endocrinology, MHH, Unit 1.1 Translational Hepatology & Stem Cell Biology.
- R. Zweigerdt, Institute of Experimental Haematology, MHH, Unit 10.2 Mass production of Pluripotent Stem Cells and their Derivatives.
- U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung regeneration and repair.

Grants, awards, patents, outreach

Production of thioredoxin fused murine stem cell factor (TRX–mSCF).

Biological testing of purified mSCF (stimulation of M-07e cells).

Production of thioredoxin fused human leukemia inhibitory factor (TRX-hLIF).

Biological testing of purified hLIF (stimulation of Brachyury ES cells).

Efficient production of cytokines, which can bind to specific receptors of target cells for triggering cell-specific responses, is of crucial importance for advancements in tissue engineering and stem cell culture techniques. Here we describe new production methods for cytokines, e.g. leukemia inhibitory factor (LIF), stem cell factor (SCF) and others, using the thioredoxin fusion approach which leads in many cases (e.g. LIF, SCF) to a soluble fused cytokine which can be directly applied in cell culture experiments.

**E. coli based production of cytokines**

**Stem cell factor (SCF)**

Known as the c-kit ligand, plays important roles in spermatogenesis, melanogenesis and early stages of haematopoiesis. As for the latter, SCF is essential for growth and expansion of haematopoietic stem and progenitor cells. We herein describe the production of recombinant murine SCF from E. coli as soluble thioredoxin-fusion protein. Bioactivities of mSCF and the uncleaved fusion protein (trx-mSCF) were proven by different tests applying a human megakaryocytic cell line.

**Leukemia inhibitory factor (LIF)**

Is a polyfunctional cytokine with numerous regulatory effects. In stem cell cultures it is the essential media supplement for the maintenance of pluripotency of embryonic and induced pluripotent stem cells. We describe the production and purification process for human LIF (hLIF) from recombinant E. coli. Both of the uncleaved hLIF (trx-hLIF) and cleaved hLIF were active in maintaining the proliferation potential of murine embryonic and induced pluripotent stem cells and retained their pluripotency.
Next generation bioreactor

**Mist-chamber bioreactor**

is designed to meet today's demands of gentle and cost-efficient cultivations. This bioreactor is basically a gas-phase bioreactor where immobilized microorganisms, cells and tissues are cultured in vaporized media. It provides three major advantages: a high oxygen uptake rate, reduction of hydrodynamic sheer stress and a limited use of media.

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**Biocompatibility analysis**

**High-throughput screening system**

is established to examine the effects of the cytokines on different mammalian cell lines, to control the production and purification of cytokines, and to detect any potential toxic contaminants. With this screening system different biological processes, such as active metabolism, proliferation, production of reactive oxygen species, and gene expression can be measured.

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**Conclusion and outlook**

- The novel protocol for the production of recombinant cytokines is very suitable and effective for the production of poorly soluble proteins through expression as fusion with the solubilizing partner thioredoxin. Based on this fusion technology, murine protein Fam163a, which belongs to proteins of the FAM163 family and is secreted by cells of neuroblastoma, is also successfully produced in the soluble form and purified as biological active protein.

- The mist-chamber bioreactor shows a great potential to provide a high oxygen transfer rate, low sheer stress and reduced medium consumption for cell culture applications.

- A high-throughput screening system is successfully established for bioactivity and biocompatibility evaluation studies for the production of cytokines.
Unit 10.2 | Mass Production of Pluripotent Stem Cells and Derivatives

Introduction and Goals

The routine application of human pluripotent stem cells (hPSC; particularly human induced pluripotent stem cells hiPSC) and their derivatives in regenerative medicine will require large cell numbers in consistent high quality. Stirred, well monitored and controlled tank bioreactor systems represents a suitable technology to establish the required cell production in suspension culture. This is a straightforward strategy for process optimization and subsequent up-scaling.

- Establish hPSC culture in clinical scale / quality.
- Perform process development and optimization in a parallel tank bioreactor platform followed by process scale up.
- Establish efficient cardiomyogenic differentiation of hPSCs for heart repair.
- In vitro modelling of congenital cardiomyopathies.

Achievements

- Scalable hPSC suspension cultures as cell-only aggregates in 100ml scale, stirred bioreactors.
- Efficient cardiomyocyte differentiation in chemically defined media with 80–90% cardiomyocyte purity.
- Routine cardiomyocyte production for tissue engineering and in vitro disease modelling.

Planning

- Combining hPSC expansion and cardiac differentiation into an „one-step“ process followed by process up-scaling (see StemBANCC project in „Grants“).
- Collaborative development of novel monitoring technologies to assess hPSC’ pluripotency & differentiation directly in native aggregates (see TOMOsphere project in „Grants“).

Robust expansion and maintenance of pluripotency (right panel showing homogeneous expression of pluripotency-related markers SSEA3 and OCT4 on cell aggregate sections) of single cell-inoculated hPSCs cultured as aggregates in suspension in fully controllable, stirred bioreactors.
Efficient differentiation of hiPSC lines in suspension culture. After cell seeding immunofluorescence staining for cardiac markers cardiac TroponinT and sarcomeric Actinin revealed the induction of ~60% cardiomyocytes.

Publications


Grants, awards, patents, outreach

- Industrial collaborations on process development & bioreactor technology: STEMCELL Technologies DASGIP / Eppendorf.
- StemBANCC: STEM cells for Biological Assays of Novel drugs and predictive toxicology. (http://stembancc.org): Large-scale, 5 year academic-industry partnership that received support from EFPIA companies and the European Union. Aims: generate patient specific hiPSC lines for in vitro models of chronic diseases and drug testing.
- TOMOSphere: Tomographisches Monitoring von 3D Zellkulturen aus pluripotenten Stammzellen; BMF funded Network Projekt in collaboration with the Lazer Zentrum Hannover and numerous industrial partners.

Cooperations

- F. Büttner, Institute for Cellular Chemistry, MHH, Unit 1.4 Stem Cell Glycomics and Proteomics, Proteomics of hPSC culture platforms.
- S. Cowley, Oxford University, StemBANCC, Single cell suspension culture.
- T. Kraft B. Brenner, Institute for Molecular and Cell Physiology, MHH, Unit 9.1 Large Animal Models; U. Martin, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 4.2 Lung regeneration and repair; Familial Hypertrophic Cardiomyopathy in vitro modelling.
- P. Sasse, B. Fleischmann. Life & Brain Center, Bonn, Cardiomyocyte electrophysiological assessment.
- I. Gruh, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), MHH, Unit 5.7 Myocardial Tissue Engineering; A. Haverich, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG); Cardiac Tissue Engineering & Animal Models.
- H. Meyer, Biomedical Optics, Laser Zentrum Hannover, Unit 7.6 Laser Manipulation and Cellular Engineering; Tomography for monitoring of hPSC 3D-cultures.
Unit 10.3 | Biostabilization of Tissues and Macromolecular Assemblies

Goals

The central aim of the group is to investigate biostabilization of biomolecules, cells, and tissues, during biopreservation strategies (cryopreservation and freeze-drying). Biopreservation encompasses both cryobiology and anhydrous biology in order to prolong the "shelf life" of fragile biomaterials in the chilled, frozen, or dried state. During biopreservation processing, biological structures are exposed to conditions that are usually not encountered under normal physiological conditions. The research is focused to elucidate the biophysical mechanisms that are involved in stabilization of macromolecular structures during biopreservation strategies. Transport processes, heat transfer, and macromolecular stability in cells and tissues, are investigated by temperature-dependent spectroscopy and thermal analysis.

Achievements

- Showed cell stability can be increased using liposome treatment.
- Demonstrated feasibility of freeze-dried heart valve matrices stabilized by sucrose.
- Established spectroscopic methods to investigate transport processes in cells and tissues: water, protective agents.
- Simulated cell volume behavior during freezing: predicted optimal cooling rates.

Planning

- Subcellular imaging of subzero transport processes and macromolecular stability: CARS, multiphoton imaging, FLIM (Research Unit).
- Tm-mediated uptake of trehalose, DNA, nanoparticles: used for stabilization, image-enhancement, cell sorting.
- Preservation of cells in the dry state: i.e. platelets for topical wound healing.

Freeze-dried heart valve scaffolds hold promise as heart valve replacements.

Temperature-dependent spectroscopic imaging of cells during cryopreservation.
The ultimate goal: preservation of living cells in the dry state

**Publications**

**Grants, awards, patents, outreach**
- BMWi: ZIM – Kooperationen – Biochemical and biophysical characterization of matrix implants.
- Canadian Blood Services - Liposomes in Transfusion Medicine.

**Cooperations**
- J. Acker, University of Alberta, Canada.
- D. Fioretto, University of Perugia, Italy.
- J. Bischof, University of Minnesota, USA.
- H. Zimmermann, Fraunhofer Institute Biomedical Engineering, St. Ingbert.
- J. Schiller, University of Leipzig.
- K. Müller, LIZWR, Berlin.
- G. Jung, Saarland University.
- C. Mallidis, University Clinics Münster.
- H. Sieme, University of Veterinary Medicine Hannover (TiHo).
- H. Oldenhof, University of Veterinary Medicine Hannover (TiHo).
- A. Hilfiker, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), MHH, Unit 5.6 Tissue Engineered Valves.
- D. Rath Institute of Farm Animal Genetics, Mariensee, FLI.
- L. Sajti, Laser Zentrum Hannover, Unit 7.3 Nanoparticles for durable live-staining of stem cells in tissue culture.
- N. Hofmann, Institute for Multiphase Processes, LUH, Unit 10.4 Cell Protection Technology.
Goals

Cell Protection Technology for

- Storage of therapeutic valuable cells for off-the-shelf application in regenerative therapy.
- Verify the safety of current routine cryopreservation protocols.
- Long-term storage of tissue and organs.

Offer to other groups:

- To develop or optimize cryopreservation protocols for valuable cells (e.g., iPS cells).

Achievements / Planning

What we did:

- Development of a cryopreservation strategy for a 3D human hemi cornea construct (HCC).
- Systematic optimisation of cryopreservation parameters for various cell types (including stem cells of different origin).
- Encapsulation of cells by using high voltage electrospraying.

What we will do:

- Establishment of alternative cryopreservation strategies with new CPAs.
- Investigation of epigenetic alterations due to cryopreservation procedures.
- Development of freezing protocols for 3D tissue.

Top: Construction of a 3D human hemi cornea (top). Encapsulated non-human primate MSCs; human epithelial cells (bottom).
Bottom: Epigenetic modifications: different histone acetylation level due to variable DMSO concentration.
Publications


Grants, awards, patents, outreach

- Grants submitted with ASKION GmbH (ZIM), NCO GmbH (KMU), Prof. Niemann, FLI (DFG), follow-up project with Dr. Reichl, TU-BS (BFR / BMBF).
- Grants in preparation with NCO GmbH (KMW), follow-up project with D. Reiche, Tu-BS / BFR / BMBF).
- Representation of REBIRTH in 2013 at IdeenExpo and Biotechnica.

Cooperations

- W. Wolters, Institute for Multiphase Processes, LUH, Unit 10.3 Biostabilization of Tissues and Macromolecular Assemblies.
- T. Müller, Institute for Transfusion Medicine, MHH, Former REBIRTH Units Embryonic Stem Cells.
- M. Dorsch, Laboratory Animal Science, MHH.
- H. Niemann, Institute of Farm Animal Genetics, Mariensee, FLI, Unit 9.1 Large Animal Models.
- R. Striepeke, Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.4 Regenerative Immune Therapies.
- T. Illig, HUB, MHH.
- H. Niemann, Institute of Farm Animal Genetics, Mariensee, FLI, Unit 9.1 Large Animal Models.
- B. Schlegelberger, Institute of Cell and Molecular Pathology, MHH.
- H. Murua-Escobar, University of Veterinary Medicine Hannover (TiHo).
- S. Reichl, TU Braunschweig.
- NKR e.V., Hannover, ASKION GmbH, Gera, NCO GmbH, Hannover.
- I. Goltsev, Ukraine, I. Braslavsky, Israel, J. Acker, Canada, X. He, USA.
- T. Zang, United Kingdom, C. Legallais, France, J. Bischof, USA.
Goals

Preclinical biological testing of medical devices

The biocompatibility laboratory BioMedimplant offers preclinical biological evaluation of medical devices according to international standards required for market access within the European Union. Our goals are to offer all the required tests for the biological safety evaluation of medical devices and methods for testing the general biological safety requirements of medical products based on tissue engineering. We are planning to fuse with the Medimplant Tierlabor GmbH within the next five years.

Achievements / Planning

Significant increase of turnover

Based on the quality of our testing service and the previous effort in terms of public relations BioMedimplant could achieve a 6-fold increase of turnover in the area of preclinical biological medical device testing. For 2013 further increase of turnover is expected. This is partly due to the fact that BioMedimplant has been officially listed by B. Braun Melsungen AG as a supplier for biocompatibility and microbiological testing service. But for being successful in the long run, our service has to cover all tests required for the market access of medial devices. BioMedimplant also needs a unique selling point.

Expansion and Fusion

Currently we are preparing a fusion with the Medimplant Tierlabor GmbH. In future we will focus on preclinical biological testing of Advanced Therapy Medical Products (ATMP) and nanomaterials. We are planning to set up a screening method for toxicity testing and localisation studies of nanoparticles in tissues.
Publications


Cooperations

- Medimplant Tierlabor GmbH.
- HIK Hannover.
- Biopior.
- S. Barcikowski, University of Duisburg-Essen.
- M. Wilhelmi, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTg), MHH.

Grants, awards, patents, outreach

- ISO technical committee 150 “Implants for surgery” subcommittee 2 working group 7 “cardiovascular absorbable implants” and subcommittee 7 “tissue engineered medicinal products”.
- ISO technical committee 194 “biological evaluation of medical devices” working group 17 “nanomaterials” and working group 8 “irritation, sensitization”.
- DIN NA 027-02-12 AA Arbeitsausschuss Biologische Beurteilung von Medizinprodukten.
- DIN NA 027-02-21 AA Arbeitsausschuss Medizinische Produkte auf Basis des Tissue Engineering.
Goals / Milestones

- REBIRTH project translated into clinical trial.
- Completed quality management system (QMS) and SOPs for all areas of clinical trial management.
- Established sponsorship for clinical trials (according to German Drug Law).
- Participation / management of at least 5 international clinical trial projects funded by public bodies.
- Internationally established academic clinical research center.
- Leadership in the design and conduct of ground-breaking clinical trials.
- Development of knowledge that improves the care of patients through innovative clinical research.
- Self-sustainable financing and growth.

Achievements

- Initial organization of sponsor duties at MHH.
- Adjustment of team size to handle larger international clinical trials.
- Initiation of collaboration with Duke Clinical Research Institute.

Planning

- Support of ATMP projects (Prof. Stripecke SMART DCs).
- Participation in translational medicine group (with Prof. Blasczyk and Dr. Zweigert).
- Set-up of MARVIN as standard EDC software at MHH.
- Completion of the data management SOPs.
- Implementation of the recent changes of the German Drug Law.

Seminar of the HCTC-Team.
Publications


Cooperations

- R. Stripecke, Haematology, Haemostasis, Oncology and Stem Cell Transplantation, MHH, Unit 6.4 Regenerative Immune Therapies.

- T. Thum, Institute of Molecular and Translational Therapeutic Strategies, MHH, Unit 5.5 miRNA in Myocardial Regeneration.

- M. Manns, Department of Gastroenterology, Hepatology and Endocrinology, MHH.

- J. Bauersachs, K. Wollert, Department of Cardiology and Angiology, MHH, Unit 5.1 Secreted factors and non-cell-based strategies for cardiac regeneration.

- HepNet, CapNetz, GPOH (MHH).

- Duke Clinical Research Institute (NIH-sponsored clinical trial).

- University of California, San Francisco, (UCSF).

- University of Florida (UFL).

- Sandoz.

- Steno, Kopenhagen (PRIORITY FP7 project).

- University of Albacete, Spain (PROHEARING, FP 7 project).

- ALL-Pediatric Consortium, University Debrecen, Ungarn.

Grants, awards, patents, outreach

- EU FP7 Network (Univ Albacete) 07/2012–12/2015 Lenarz / MHH PROHEARING.

- NIAID Network (DCRI) 12/2012–12/2017 Drusano / UFL VABP.

- CapNetz Single 10/2012–09/2015 Welte / MHH ABACOPD.

Goals

- Systematize normative dimensions and regulatory frameworks in REBIRTH, particularly in the fields of human body materials, informed consent, enhancement/anthropotechnology and research involving hybrids/chimeras.

- Contact research groups within REBIRTH to collaborate with and to advise concerning the normative dimensions of their research, develop support tools for responsible research and innovation.

- Pursue training activities for PhD and masters students on regulatory issues as well as on basic legal and ethical aspects of their work.

Achievements

- Collaboration started with REBIRTH groups involved in chimera research (Feb ‘13).

- Discourse project and symposium on “Ethics of Regenerative Medicine” (Feb & Mar ‘13).

- Expert contributions to new German policies for biobanking.

Planning

- International conference on Health, Law and Ethics as part of the LUH Grand Challenges scheme.

- International conference on “Research Ethics” (BMBF funded, see below).

- Intensify collaboration with other REBIRTH groups.

Publications


Cooperations

- HeLEX, InSIS, University of Oxford.
- Mason Institute for Medicine, Life Sciences and the Law, University of Edinburgh.
- European School of Molecular Medicine, University of Milan.
- IFB-TX, ESPOIR (MHH).
- Institute of Science and Ethics, University of Bonn.
- German Reference Centre for Ethics in the Life Sciences (DRZE).
- European PhD programme “Law, Science and Technology”.
- Working Group “Biobanking” of the German Network of Research Ethics Committees.
- StemBANCC, University of Oxford, Hoffmann-La Roche.

Grants, awards, patents, outreach

- CHIP-ME (COST) – Hoppe [Direct-to-consumer genetic testing].
- Public Involvement and Ethics in Regenerative Medicine (BMBF) – Strech.
- Fair access to samples in biobank research (BMBF) – Strech.
- EUCelLex (FP7) – Hoppe [Cell research governance].
- EBiSC (IMI) – Hoppe [European Stem Cell Bank setup].
Area M | Business Management
Area M
Administration and Organization of the REBIRTH Cluster of Excellence

The REBIRTH Cluster of Excellence is a central interdisciplinary scientific institution of Hannover Medical School (MHH). The seven partner institutions are represented on the various boards by their respective scientists.

REBIRTH’s official governing bodies and functions are as follows:

- the Coordinator of the Cluster of Excellence and his two Deputies,
- the Executive Board,
- the Steering Committee,
- the General Assembly,
- the Scientific and Economic Advisory Board.

The coordination of the REBIRTH Cluster of Excellence, with its nearly 60 work groups, calls for a wide range of management expertise. The administration area M consists of the three sections ‘Administration and Evaluation’, ‘Exploration and Communication’, and ‘Education and Gender (Human Resources)’. 

[Diagram of governance structure]
The Business Management (BM) team is specifically responsible for running day-to-day business. Its remit primarily involves planning and organizational activities, as well as control and management functions. These include financial controlling, provision of funds (resources) and the planning, control and monitoring of business processes. Management tasks in REBIRTH also include preparing the meetings of all governing bodies mentioned above, the preparation of draft proposals for resolution at board meetings (and their subsequent follow-up), drawing up plans for quality-assurance measures, and public-relations work; they also cover new actions to support communication. To promote sustainability, we combine financial and strategic measures to implement a sustainable ‘corporate identity’, thus establishing the Cluster as a key player in regenerative medicine.

Administration and Evaluation (Central Management)

As well as assisting the boards, the business manager assists individual scientists or pilot projects by performing managerial functions in response to well-defined REBIRTH-related requests.

Together with the financial administration of the partner institutions, the financial management makes sure that all budgetary actions are performed according to the decisions of the boards (e.g. allocation of flexible funds) and the rules and regulations. It will ensure that the funds received are correctly distributed and accounted for in the event of potential external auditing. Administrative experience is shared with the business managers of the other clusters of excellence.

The BM team processes the periodic reporting of all REBIRTH research groups following a structured format to ensure a consistent flow of information. It contains the following key points: a financial report including new grants, an updated project implementation plan, an activity report, and thoughts on career development, gender equality, interdisciplinarity, translation, dissemination, communication and sustainability. The BM team will provide ongoing guidance to the decision-making bodies in the structured evaluation of all Units in 2014/2015. The team also carries out data acquisition that is requested by the German Research Foundation (DFG) for statistical needs.

Business Manager:
Dr.-Ing. Tilman Fabian

Tilman Fabian coordinates and organizes REBIRTH. A graduate engineer with a background in materials sciences and implant materials, he has been on MHH’s staff since February 2007. He previously worked at the Leibniz University of Hannover’s Centre for Biomedical Engineering. In 2001 he established initial contact with MHH, taking on the coordination of the SFB 599 collaborative research centre run by Professor Thomas Lenarz (Head of the School’s Department of Otolaryngology) and, subsequently, that of the SFB / Transregio 37 (under Professor Axel Haverich). From 2005 he was also in charge of business management at the BiomeTI initiative which, in terms of its activities, was developing along the lines of an inter-university ‘Lower Saxony Centre for Biomedical Engineering’. The work involved at REBIRTH is a logical continuation of the direction he took in past years: being active in project management in an interdisciplinary environment at the interface between progressive medicine and biomedical engineering.
Assistant to the Business Manager: Sandra Siedler

Since May 2011 she is working as an assistant in the office of the REBIRTH Business Management. Before she joined the team, she gained her experience in the Secretary Office at Hannover Medical School’s Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG). She supports the Business Manager in everyday business and arranges meetings of all governing bodies.

Finance and public relations: Dipl.-Ök. Yvonne Stöber

After completing her economics degree in 2005, she worked for some years as a research assistant at the chair of Professor Schulenburg at the Leibniz University of Hannover. Her academic functions included research on issues relating to health economics and insurance business management. In September 2008 she moved to MHH, since when she has been responsible for finance and public relations within the REBIRTH Cluster of Excellence.

Media relations: Dipl.-Biol. Camilla Krause

Camilla Krause studied biology at Leibniz University in Hannover and graduated in 2005. Before joining REBIRTH, she did a journalistic traineeship at lower saxony’s location campaign Innovatives Niedersachsen reporting on scientific and technological innovation. She came to the REBIRTH management team in September 2009 and has been responsible for media and public relations within the REBIRTH Cluster of Excellence ever since.
Personnel development and coordination of the Ph.D. programme Regenerative Sciences:
Dr. Daniela Pelz

Daniela Pelz studied biology at Rheinische Friedrich-Wilhelms University in Bonn and received her doctorate at the Free University of Berlin’s Institute of Neurobiology. She has been part of the REBIRTH management team since August 2008. Her twin functions within the Cluster are both related to Area M: the coordination of the Ph.D. programme in Regenerative Sciences, and personnel development.

Daniela Pelz will be on maternity leave presumably until September 2014. In the meantime Annette Broll and Steffi Gomm are jointly coordinating the Ph.D. Programme.

Ph.D. programme Regenerative Sciences:
Dipl.-Informationswirtin Annette Broll

Annette Broll studied ‘Information and Documentation’ at the University of Applied Sciences in Darmstadt. Prior to joining REBIRTH she has been on MHH’s staff since February 2000 as documentalist in several institutes. She has been part of the REBIRTH Management team since April 2010.

Annette Broll especially sees to (new) students, organizes the application process, the intermediate exams and the student retreat. She also is in contact with the Ph.D. committee and the alumni.

Ph.D. programme Regenerative Science and Assistant to the Business Manager:
Wirtschaftsfachwirtin Steffi Gomm

After her vocational education as an office management assistant in 2005, she completed an advanced training for business administration in 2008. Since April 2011 she has been part of the REBIRTH management team. Prior to joining MHH, she worked for medium-sized companies, where she held a position in the marketing division and supervised apprentices.

Steffi Gomm particularly takes care of financial issues, acquisition of new Ph.D. projects, co-supervisors, final exams and the programme’s website.
The long-term success of the Cluster will greatly depend upon the lively interaction and critical contribution of its members and the ‘corporate identity’ formed over the years of successful joint work. In order to continuously improve the interconnectedness of the groups and Units in the Cluster, we have developed a communication system involving the local integration of the groups, our periodic reports, newsletters, scientific seminars, special lectures and regular REBIRTH colloquia with both internal and external invited scientists, co-supervisor meetings and annual retreats for those involved with the Ph.D. programme, the General Assembly, and our website. Our project ‘Internal and External Communication’ with the Department of Media, Information and Design at Hannover’s University of Applied Science and Arts has enabled us to develop our communication strategy.

In order to raise REBIRTH’s profile at both national and international level, one of the fundamental goals of the REBIRTH management is to enhance the external and internal perception of this integrated research project as a whole. As well as operative day-to-day business, communications platforms are increasingly being established with a view to safeguarding and strengthening REBIRTH’s position in this highly competitive and diverse field. These include the ‘REBIRTH News’ newsletter, the aim of which is to keep both the staff at the Cluster and interested ‘outsiders’ informed about research findings and developments. Frequent media exposure, trade shows, events and conventions, as well as informational material and presentations of papers by REBIRTH scientists – all of these activities serve the overall goal, namely to raise awareness about the Cluster of Excellence.

Finally, communication is also crucial in conveying the success of the Cluster to its surrounding institutions and the wider scientific and social environment, which is why we organize presentations to the lay public. It is our aim that – depending, of course, on our scientific and clinical successes – these measures will make REBIRTH increasingly known in the wider community.
To highlight one activity, this was also one of the main reasons why REBIRTH was present with its own stand at Hannover’s World Exposition Site in 2009, 2011 and again in 2013 for the ‘IdeenExpo’ (‘Ideas Expo’) event. The exhibit’s aim is to reach pupils and young students, as well as teachers and parents. The main goal of the ‘IdeenExpo’ is to counter the skills shortage in the science and technology sector by seeking to target young people, engaging with them to seek to encourage their enthusiasm for careers in these areas. For REBIRTH, the ‘IdeenExpo’ provides an outstanding platform for presenting its research and development activities to the general public, with a total of more than 342,000 young people coming to the Expo Plaza in 2013 to find out more: to visit exhibits and ‘hands-on stations’, to attend talks, workshops and seminars, and to take home ideas that will stimulate them to explore career options.
Quite rightly, the primary goal being pursued by the work groups portrayed in the previous chapters is that of performing excellent scientific work. However, a second general and overarching objective of the Excellence Initiative is to enhance awareness in Germany of the importance of scientific advances and help the general public embrace them more wholeheartedly, and thus get the up-and-coming generation interested in science. Concerted action of this kind (such as trade show presentations and the like) clearly shows that, in order to properly communicate the scientific activities of a multi-faceted Cluster of Excellence (with its many work groups) to the outside world, a ‘nerve centre’ is required. It was through the work of the REBIRTH management that the idea for REBIRTH to have a presence at events such as the ‘IdeenExpo’ was initiated and implemented.

Another event which has been centrally conceived and organized by the REBIRTH Cluster of Excellence’s business management team is the ‘Autumn Academy for Teachers’, which is – after 2009 and 2011 – to be held for the third time in 2013 in conjunction with the second Hannover-based Cluster of Excellence, QUEST (Quantum Engineering and Space-Time Research) at the Leibniz University of Hannover. The idea is to give education professionals an inside look at the research topics being investigated. At the Autumn Academy, upper-school teachers in various subjects – biology, chemistry, physics, mathematics and computer science – will have the opportunity to augment their theoretical knowledge with recent research findings, approaches and methods. At the same time, we hope that participating teachers will carry their enthusiasm for research – and ours – into the schools, which may inspire pupils to become researchers themselves in the long term.
Ph.D. Programme ‘Regenerative Sciences’

To promote human resources, we are continuing the International Ph.D. programme in Regenerative Sciences (REGSCI) with a few targeted improvements. Inaugurated in October 2007, REGSCI starts its seventh year in October 2013.

The programme is integrated into the Hannover Biomedical Research School (HBRS), benefiting from its experience in structured doctoral education. The aim of the Ph.D. programme is to enable students previously educated in different subjects related to regenerative sciences, to communicate and receive scientific training across disciplines. The curriculum includes compulsory and individual elements. The compulsory elements are spread over four semesters with weekly two-hour seminars followed by a one-hour tutorial on foundational topics in regenerative sciences and regeneration of the four organ systems covered in REBIRTH, as well as other organ systems to complete the picture. Additional topics look at enabling technologies, regulatory items and processes involved in translation from bench to bedside. The third year has no curricular obligations, thus maximizing the time available to complete the doctoral project. All lectures are evaluated, with remarkably positive feedback from the students over the past three years.

Individual curricula are tailored to the research project and include 80 hours of project-oriented seminars and courses such as guest lectures, master classes, and ‘summer schools’. Additionally, students have to cover 40 hours of soft skills (e.g. statistics, presentation & writing courses, time management and intercultural training). Students are also expected to regularly attend departmental meetings and journal clubs and to give their own presentations. They have to present their work at least once at an international meeting and annually during the programme’s all-student retreat. Students have to pass an intermediate exam after 18 months and the final exam after 3–4 years.
Each year the REGSCI programme attracts a large number of national and international applicants (up to 400 applications per year from more than 60 countries). The international dimension of the programme is indicated by its high proportion of foreign students and the fact that the entire curriculum is in English. At present, 30 foreign students are enrolled, originating from 19 different countries (Austria, Belarus, China, Egypt, Greece, Hungary, India, Indonesia, Iran, Italy, Jordan, Malaysia, Mexico, Poland, Russia, Taiwan, Tanzania, Turkey and the Ukraine). Altogether, 105 students have been enrolled in the programme. Up to now, 37 students have successfully defended their thesis, with another seven aiming to do so in June 2013. The programme’s high standard is highlighted by the fact that twelve students have dropped out as the programme proved too ambitious for them.

The Ph.D. programme has a governing committee that is supported by a Ph.D. office. Members of the committee comprise experienced senior and junior faculty as well as student representatives of each year. For REGSCI the committee members represent all major institutions involved in the Ph.D. programme as well as all relevant disciplines. The committee elects a chair from among its members.

The committee's most important functions in ensuring the quality of the programme include:

**Evaluation of submitted Ph.D. projects.** Ph.D. projects submitted by prospective supervisors are reviewed as to whether the topic fits with the focus of the Ph.D. programme, whether its scope is appropriate for a Ph.D. project, whether its aims are sufficiently specified, and whether they can be clearly distinguished from other projects of the hosting research group. The Ph.D. office also verifies the availability of sufficient financial resources.

**Selection of Ph.D. students.** A three-step selection process is required by the HBRS rules and requirements. Eligible applicants have to fulfil the following criteria:

- An academic degree (such as M.Sc., Diploma, MBBS, Staatsexamen) in the life or natural sciences including pharmacy, in dentistry, human or veterinary medicine, or in an engineering discipline.
- Excellent grades in academic exams.
- At least six months of research experience documented by a detailed project description of, for example, a Master’s thesis or medical doctoral project.
- High motivation for research.
- Excellent knowledge of English to be documented either by a test such as TOEFL or IELTS or by furnishing proof that instruction has been received in English at school or university.

The application is further supported by two letters of recommendation. After evaluation of the written application, the most promising applicants are invited to a personal interview before the Ph.D. committee. Successful applicants are chosen for admission on this basis. The committee is especially keen to admit ambitious and gifted young scientists with a strong desire to pursue a career in research.
Design of the curriculum. Depending on the research focus of each Ph.D. programme, the relevant committee establishes the curriculum. Each compulsory lecture is evaluated by the Ph.D. students and, if necessary, appropriate measures (such as changing topics) are taken by the committee.

Communication between students, supervisors, and lecturers. During the annual all-student retreat a plenary discussion on the Ph.D. programme is held which ensures that necessary adjustments and welcome improvements are implemented in a timely manner.

The Ph.D. committee is also strongly involved in quality control of individual projects.

Thesis advisory group. The committee appoints two co-supervisors for each Ph.D. student. Together with the supervisor they form the thesis advisory group (TAG). Co-supervisors should be senior scientists (minimum qualification: Habilitation post-doctoral lecturing qualification or equivalent) whose research is related to the Ph.D. project. In order to ensure the supervisor’s independence, they should come from a different department. The Ph.D. student should meet the TAG and report on his or her progress at least on an annual basis. Minutes of these meetings are to be submitted to the Ph.D. office.

Supervision agreement. The committee has set up a supervision agreement. The purpose of this agreement is to place the relationship between the Ph.D. student and the supervisor on a transparent basis, so that the doctoral project can be completed to a high standard within a period generally lasting three years. This agreement includes the ‘Principles of Hannover Medical School on Safeguarding Good Scientific Practice’.

Composition of students enrolled in the REGSCI programme (May 2013).
Intermediate exam. The Ph.D. committee appoints the examiners and monitors adherence to the requirements during the examination process. A committee member has to be present in each examiner group. As the exam is strongly project-oriented, this exam serves as a useful ‘external quality control’ at the midway point, taken as it is after 18 months.

Final exam. If all requirements for the final exam are met, the Ph.D. committee appoints an external and an internal reviewer for the Ph.D. thesis. Based on the written evaluations, the committee decides on the Ph.D. student’s admission to the final exam which is chaired by a committee member.

Additional measures to ensure quality control of individual projects are the following:

- The annual all-student retreat at which students have to present their project in front of all members of the Ph.D. programme, i.e. students, supervisors, lecturers and committee members, as well as submitting a two-page project report.
- On at least an annual basis, project presentations in the host department and at international conferences (where possible).

Flexible curriculum. To increase the freedom of learning in comparison with the fixed curricula for Bachelor’s and Master’s programmes, we have introduced two new teaching formats which can replace up to 30 hours of the compulsory curriculum. We have already established method courses, which were in great demand. The second teaching format recently introduced is called ‘Meet the Investigator’: this involves a small group of students meeting a junior or senior group leader for personal discussions not only on their research but also on career aspects, as we believe that role models play an important part in shaping career plans. We also consider e-learning tools to increase flexibility.
Promotion of early career researchers

We also actively support the career development of Ph.D. students and postdoctoral researchers within the Cluster, and include our former members in an alumni programme.

In 2011 we introduced an innovative scheme, the Research Gap Year, to attract school graduates to science. Graduates can apply for either a 12-month or two six-month research projects, which will give them initial technical experience as well as insights into scientific methods. The goal is to motivate graduates for university study, encourage them to take up science and provide direct contact with inspiring role models. This initiative perfectly complements our previous and continued activities of the Autumn Academy for Teachers (see above), and our exhibitions for pupils (Ideen-Expo). Furthermore, we implement numerous supportive measures motivating and persuading female academics to pursue a career in science. We have introduced childcare support as well as mentoring programmes for female scientists.

In REBIRTH, we also provide funds for positions enabling a replacement to be hired for a clinician going into research for a set period of time (‘Gerok positions’).

Alumni Programme. Another central task of the growing and maturing Cluster is the implementation of an alumni programme. Here, we benefit from the alumni programme of the HBRS, which keeps track of the career development of junior scientists, in some cases already leading to successful re-recruitment after a period of external training.

Promotion of Gender Equality

REBIRTH considers equal opportunities to be crucial for its success. Requirements to promote gender equality are in place at all levels of the Cluster’s management, in collaboration with the institutional equal opportunities officers. The major academic partners of REBIRTH (MHH and LUH) have received much acclaim for their achievements in the promotion of gender equality. Sound statistical data on REBIRTH staff has proven important for identifying inequalities as well as designing measures to eliminate these, and will be continuously updated. REBIRTH has already participated in the project entitled ‘Frauen in der Spitzenforschung’ (‘Women in Cutting-Edge Research’), and has taken part in the workshops held in Frankfurt and Hamburg.

Equal opportunity issues apply to male and female scientists as well as to parents and non-parents. The REBIRTH partner universities use numerous childcare measures to give scientists who are parents the freedom to concentrate on their research. REBIRTH is funding one nurse in the childcare facility “Die Hirtenkinder”.

Conclusion

In fact, the entire design of the Cluster, in all aspects of its research plan and management, has the goal of establishing an innovative and sustainable, internationally visible centre for regenerative medicine. The following points summarize our actions to promote sustainability:

(1) Strict continuation of our successful research plan, continuously reshaped through communication, allowing the evolution of new strengths and avoiding the perpetuation of weaknesses;

(2) Balanced support of basic science and translation in a field that is of great interest for public health, ensuring a long-term scientific and societal impact; and

(3) Flexible and transparent management of finances and career plans, supported by sustained core support of the Land of Lower Saxony, our home institutions and our own drive for excellence, thus generating competitiveness in grant acquisition, as well as the recruitment and retention of high-performing staff.
Pilot Project | REBIRTH-active / REBIRTH-active woman
The REBIRTH-active study group is an initiative of the REBIRTH Cluster of Excellence and comprises scientists from several departments including:

- Cardiothoracic, Transplantation and Vascular Surgery,
- Cardiology and Angiology,
- Paediatrics,
- Dental Prosthetics,
- Sports Medicine,
- Gynaecology,
- Clinical Pharmacology,
- Biometrics,
- Hannover Medical School’s Human Resources,
- REBIRTH Business Management.

The major aim of REBIRTH-active is improvement of daily activity and physical exercise to increase endogenous cellular regeneration, work ability and exercise capacity. In the first REBIRTH-active study, 67 inactive middle-aged employees of MHH underwent a six-month exercise programme involving 30 minutes of physical exertion per day.

Exercise training was individually organized, and the employees achieved an average of 220 minutes per week during the summer and 160 minutes per week in the winter.

Peak oxygen consumption – the most important marker of functional exercise capacity – rose by more than 20% after six months, corresponding to normal values in men 15 years younger. In addition, telomere length in peripheral blood mononuclear cells – a marker of cellular regeneration – increased, and the improvement of the work ability index matched normal values of men six years younger. Psychosocial indicators of burnout were reduced. The number of sick days taken by the active employees was reduced by 40% compared with the six-month period before the intervention. Cardiovascular and echocardiographic parameters were enhanced as well. Dental health was also assessed before and after the trial period. Severe periodontitis could reduce the benefits of long-term exercise training, and this condition is associated with cardiovascular diseases. Therefore, physical exercise and dental prevention should both be included in worksite interventions to boost each individual’s general health and work capability.

In the second REBIRTH-active study, the effects of a comparable worksite intervention on work ability, cellular regeneration, oxidative stress, cardiovascular function and dental health will be investigated in 300 women. The effect of the hormonal status with regard to the pre- and postmenopausal stage will be evaluated as well.

The REBIRTH-active group is a scientific partner in the German government-funded ‘Electromobility Showcase’ initiative. In this project, the effects of the increased use of pedal-assist e-bikes in Hannover on general health and work ability will be investigated between 2013 and 2016.
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