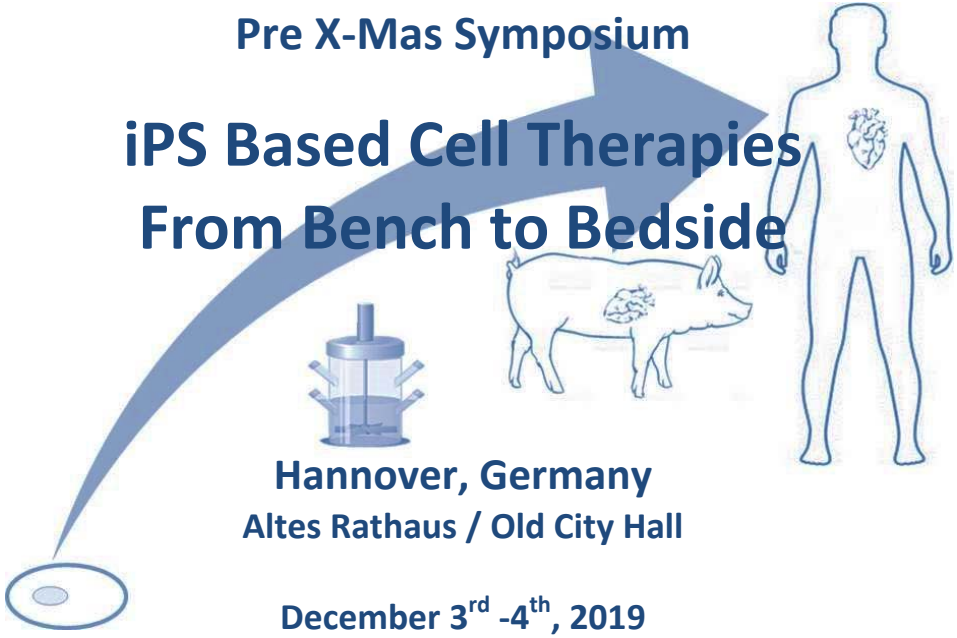
TECHN BEAT

Pre X-Mas Symposium

iPS Based Cell Therapies
From Bench to Bedside



Hannover, Germany

Altes Rathaus / Old City Hall

December 3rd -4th, 2019

Symposium booklet

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Welcome Note

Dear colleagues, partners and friends,

It's our pleasure to welcome you to the Pre X-Mas Symposium iPS Based Cell Therapies-From Bench to Bedside in Hannover!
Regenerative medicine is a fascinating topic inspiring both scientists and the general public.
The greater Hannover region including Göttingen and Braunschweig and in particular Hannover Medical School (MHH) is a leading internationally established hub in regenerative medicine with a strong focus on Human pluripotent stem cell (hPSC) research.
This research field has made a great leap forward in recent years and is now at the edge of clinical translation. This is true for a broad range of novel regenerative approaches across the whole human body including conditions such as heart failure, that affect a large number of patients. Although cardiac repair by hPSC- progenies is a key focus of this symposium, the international panel of renowned speakers covers numerous other exiting areas such as cell therapies for neurodegenerative disorders and diabetes.
Another aim of this meeting is to showcase the current progress and challenges that companies face during the development of hPSC-based treatments and their progress into marketable products.
The symposium is complemented by presenters that highlight the substantial ethical concerns related to this evolving field of biomedicine as well as regulators' view on the global situation for Advanced Therapeutic Medicinal Products (ATMPs).
Given the numerous alternative strategies that are currently under development for treating the loss of tissue and organ function, the meeting also showcases such alternative ideas, including the use of xenogenic organ replacement approaches and technical devices such as advanced artificial heart replacement technologies as well.
We are pleased that a broad range of participants followed our invitation, including junior scientists at the PhD stage, senior scientists and PIs from academia and industry, plus stakeholders with diverse backgrounds. We hope that this multidisciplinary community will not only provoke livy discussions during oral presentations but throughout the poster sessions, industrial exhibition, and coffe breaks as well.
In general, social events and networking opportunities are a key component of every successful meeting. Thanks to the enormous financial support by public agencies and industrial sources, the meeting invites all participants to join us at a casual get together. This will include dinner and – importantly - "dance floor opportunities" to promote networking and friendship beyond the business.
Moreover, we hope you will find some time to dive into the picturesque X-Mas market around the meeting venue, to get some inspirations for the upcoming holiday season.
We are wishing you an exciting, inspiring meeting including novel contacts and collaboration opportunities; have a great stay in Hannover and a safe trip back home.

Kind regards,

Robert Zweigerdt and Ulrich Martin on behalf of the Organsing Commitee

Organising Committee

TECHNOBEAT Consortium

Robert Zweigerdt	Joost Sluijter	Falk Schneider
Ulrich Martin	Kfir Molakandov	Katharina Kinast
Christine Mummery	Josef Itsokovitz Eldor	Serge Jooris
Peter Andrews	Dirk Strunk	

Conference Office

Hannah Arpke, TECHNOBEAT, LEBAO, Hannover Medical School

Programme

Pre X-Mas Symposium

iPS Based Cell Therapies- From Bench to Bedside

Hannover, Altes Rathaus / Old City Hall, December 3rd -4th, 2019

Tuesday, December 3rd	
08:30	**Registration**
09:00	**Opening** **Robert Zweigerdt and Ulrich Martin**
Session topic I: hPSC progeny production and the regulatory environment for cell therapies **Chairs: Sebastian Knöbel and Dirk Strunk**	
09:15	**Key Note: Christine Mummery (NL)** Intercellular dialogue in the heart modelled using differentiated hPSC
09:50	**Paul Burridge (US)** hPSC cardiomyocytes: Evolution of differentiation and application processes
10:15	**Coffee break**
10:45	**Robert Zweigerdt (GER)** Upscaling hPSC and progeny production including CMs, ECs and macrophages
11:10	**Henrik Semb (DK)** Pluripotent stem cells for cell therapy in T1D
11:35	**Gerald Schumann (GER)** The current regulatory environment for iPS cell-based medicinal products
12:00	**Lunch with poster session** Committee: Henning Kempf, Katharina Kinast, Ruth Olmer, Valeria Orlova
Session topic II: Sharing companies' perspective and experience **Chairs: Ina Gruh and Joseph Itskovitz-Eldor**	
13:30	**Sebastian Knöbel, Miltenyi (GER)** Making GMP-compliant media and reagents for cellular therapeutics
14:00	**Philipp Nold, DASGIP-Eppendorf (GER) and Serge Jooris, Ovizio (BEL)** Innovations of Materials and Technologies for process development and monitoring of cell therapeutics
14:30	**Michal Izrael, Kadimastem (ISR)** From hPSCs Research to Cell Therapies for Neurodegenerative Disorders
14:55	**Allan Karlsen, Novo Nordisk (DK)** From hPSCs Research to Cell Therapies
15:20	**Michael Harder, Corlife (GER)** Cell-free allografts: the long journey of a therapeutic idea
15:45	**Coffee Break**
Session topic III: (Genetic) Safety risks and ethics in cell therapies **Chairs: Ivana Barbaric and Yoji Sato**	
16:15	**Key note**: **Nissim Benvenisty** Genomic variants in pluripotent stem
16:50	**Peter Andrews (UK)** The Origin, Mechanisms and Consequences of Acquired Genetic Variants in Human Pluripotent Stem Cells

17:15	**Ulrich Martin** Enrichment of Small Scale Genomic Variants during Reprogramming
17:40	**Dirk Strunk (AT)** Progress and risks in Mesenchymal Stromal Cell therapies
18:05	**Nils Hoppe** Stem Cells and Gene Editing: Legal and Ethical Issues
18:30 Closing	
19:30 Evening Get-Together Gartensaal, Neues Rathaus	

Wednesday, December 4th	
Session topic IV: Current State in the Art of iPS cell based heart repair: large animal models and clinical translation **Chairs: Christine Mummery and Paul Burridge**	
09:30	**Key Note: Yoji Sato (JP)** Updates to Japan's regulation and/or quality issues of PSCs for cell therapies
10:05	**Joost Sluijter (NL)** Optimisation of cellular retention
10:30	**Ina Gruh (GER)** hPSC-derived cardiac aggregate transplantation in non-human primates
10:55	**Coffee Break**
11:30	**Wolfram Zimmermann (GER)** Translation of Engineered Heart Repair
11:55	**Philippe Menasche (FR)** hPSC-derived cardiomyocyte: clinical transplantation for heart repair
12:20	**Poster session and lunch**
Session topic V: Current State in the Art of heart support or replacement and beyond: Technical devices, human organ transplants **Chairs: Philippe Menasché and Wolfram Zimmermann**	
13:20	**Key Note Johann Bauersachs and Axel Haverich (GER)** - Cardiologist's view on the current clinical practice for treating heart failure - Surgeon's view on the current clinical practice for treating heart failure / Organ care system
14:10	**Gregor Warnecke (GER)** Clinical heart transplantation: Current State, Limitations and Perspectives
14:30	**Coffee Break**
Session V continued **Chairs: Robert Zweigerdt and Ulrich Martin**	
15:00	**Key Note Bruno Reichart (GER)** Xenotransplantation: pig hearts for human heart replacement **TBC**
15:35	**Heiner Niemann (GER)** Gene editing and porcine pluripotent stem cells: Novel tools for the production of multi-transgenic pigs for xenotransplantation
16:00	**Samir Sarikouch (GER)** Challenges in choosing heart failure patients for first-in-man iPS cell-therapies
16:25	**Thomas Thum (GER)** Supportive strategies to foster cardiac hPS cell therapies
16:50	**Meeting closing and poster prize winner announcement** **Ulrich Martin and Robert Zweigerdt**

Orientation

Registration

The welcome and registration desk is located in the lobby area of the first floor of the Altes Rathaus. There you will be given your welcome packs, name tags and further information.

Symposium Venue

Altes Rathaus

This historic landmark of Hannover lies in the heart of the old city. Dating from the early 15th century it was used as town hall until the 1860s. It now serves as event location and houses several businesses.

Its central location is ideally suited for easy access by public transport, shopping opportunities, and of course, the annual X-mas market that is taking place at the same time as the Technobeat Symposium.

Address:

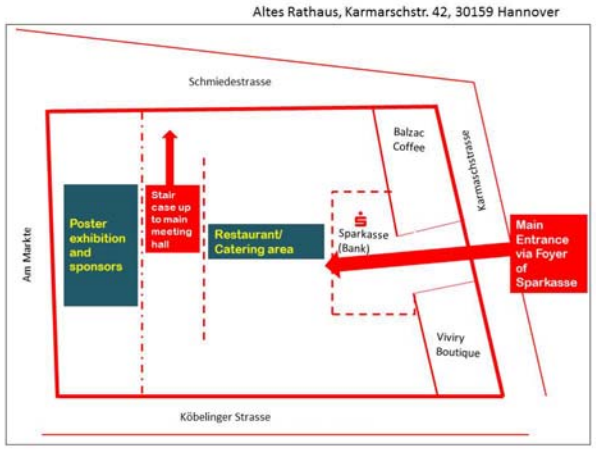
Karmarschstraße 42, 30159 Hannover, Germany

Directions

From Langenhagen Airport the S5 suburban train (S-Bahn) starts every 30 minutes running from 03:36 until 01:06. The S-Bahn station is located in Terminal C. The journey takes about 18 minutes and takes you straight to Hannover Central Train Station.

From Hannover Central Train Station you can either walk (approx 10 minutes) along the pedestrian zone or take the subway numbers 3,7,9 getting off at Markthalle/ Landtag. The journey in total takes about 5 minutes.

Orientation at the venue



Evening reception

Der Gartensaal at the Neues Rathaus

Restaurant Gartensaal is located in the back of the new town hall (Neues Rathaus). Built in 1913, it is the seat of the city administration, while it is also open to the public to admire the architecture or take a ride up the dome to view the city from above.

The Gartensaal faces the Maschpark- a small landscaped park with a large pond that invites for walks and nature watching.

Address:

Trammplatz 2, 30159 Hannover, Germany (about 700m from the meeting venue)

Directions

From Langenhagen Airport the S5 suburban train (S-Bahn) starts every 30 minutes running from 03:36 until 01:06. The S-Bahn station is located in Terminal C. The journey takes about 18 minutes and takes you straight to Hannover Central Train Station.

From Hannover Central Train Station you can either walk (approx 16 minutes), passing the Alte Rathaus along the pedestrain zone or take the subway numbers 1,2,3,7,8,9 getting off at Ägidientorplatz and walking 500m. The journey in total takes about 12 minutes.

Once you get to Trammplatz, walk into the entrance hall of the new town hall, throughthe man hall past the stairs to the back.

Abstracts

Key Notes

Genomic variants in human pluripotent stem cells

Nissim Benvenisty

The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University, Israel

We have recently generated haploid human pluripotent stem cells (hPSCs) from unfertilized human oocytes, and demonstrated the utility of these cells is genetic screenings. To define the genes involved in the biology of hPSCs, we generated a genome-wide loss-of-function library utilizing CRISPR/Cas9 technology with 180,000 guide RNAs, targeting virtually all coding genes. We thus defined the essential genes in hPSCs, showing the relative role of each cellular compartment in promoting or restricting cell growth, and categorizing human genetic disorders according to their role in early embryogenesis. We have also identified all growth-restricting genes, highlighting the role of the p53 pathway in this context. This analysis enabled us to generate an atlas of all genomic variants in human pluripotent stem cells. We have also demonstrated that p53 is the most spontaneously mutated gene in hPSCs, and these mutations gradually take over the culture. We have recently examined the rate of culture-adapted mutations in other cancer-related genes, revealing recurrent mutations in over 20 verified tumorigenic genes other than p53. Importantly, naive hPSCs were found to harbor four-times more cancer-related mutations than their primed counterparts. Our results suggest that prolonged culturing and pluripotent cell state transition enhance hPSC cancer-related mutagenesis. These mutations should be taken into consideration in future applications, especially in clinical contexts.

Intercellular dialogue in the heart modelled using differentiated hPSC

Christine Mummery

Dept. of Anatomy & Embryology, Leiden University Medical Center, The Netherlands

Derivation of cardiovascular cell types from human pluripotent stem cells is an area of growing interest as a platform for drug discovery and toxicity. Most particularly, the recent availability of methods to introduce specific disease mutations into human pluripotent stem cells and/or to derive these cells as hiPS cells by reprogramming from any patient of choice, are creating unprecedented opportunities to create disease models “in a dish” and study ways to treat it or slow down its rate of development. Most recently our lab has been investigating organs on chip solutions in which multiple cardiac and vascular cell types into microtissue formats. Crucial has been methods to promote cardiomyocyte maturation and to quantify the outcomes of drug and disease mutation responses in situ. The use of isogenic pairs has proven very important since variability between “healthy control” hiPSC lines is often greater than the difference between a diseased cells and its isogenic control. We have shown that iPSC derived cardiomyocytes with mutations in ion channel genes can accurately predict changes in cardiac electrical properties and reveal drug sensitivities also observed in patients.

Xenotransplantation: pig hearts for human heart replacement

Bruno Reichart

Klinikum der Universität München, Herzchirurgische Klinik

Heart transplantation is the only cure for patients with terminal cardiac failure, but the supply of allogeneic donor organs falls far short of the clinical need1–3. Xenotransplantation of genetically modified pig hearts has been discussed as a potential alternative4. Genetically multi-modified pig hearts that lack galactose-α1,3-galactose epitopes (α1,3-galactosyltransferase knockout) and express a human membrane cofactor protein (CD46) and human thrombomodulin have survived for up to 945 days after heterotopic abdominal transplantation in baboons5. This model demonstrated long-term acceptance of discordant xenografts with safe immunosuppression but did not predict their life-supporting function. Despite 25 years of extensive research, the maximum survival of a baboon after heart replacement with a porcine xenograft was only 57 days and this was achieved, to our knowledge, only once6. Here we show that α1,3-galactosyltransferase-knockout pig hearts that express human CD46 and thrombomodulin require non-ischaemic preservation with continuous perfusion and control of post-transplantation growth to ensure long-term orthotopic function of the xenograft in baboons, the most stringent preclinical xenotransplantation model. Consistent life-supporting function of xenografted hearts for up to 195 days is a milestone on the way to clinical cardiac xenotransplantation.

Japan's regulation and quality issues of cell therapy products derived from human pluripotent stem cells

Yoji Sato

Division of Cell-Based Therapeutic Products, National Institute of Health Sciences, Kawasaki, Japan

Development of regenerative medicine and cell therapy (RM/CT) using cell-based therapeutic products (CTPs) is keenly anticipated in Japan, because of difficulties in securing human organs and tissues in our country.

There are two tracks for the development of RM/CTs in Japan. The first is that aims at manufacturing and marketing authorization of CTPs under the Pharmaceuticals and Medical Devices Act (PMD Act). This approach involves research and development by a commercial enterprise, followed by a stepwise process consisting of evaluation and approval of the product by the relevant regulatory authorities to confirm its safety, efficacy and quality. It is notable that the PMD Act introduced an independent product category called “regenerative medicine products” (RM products), which include CTPs and gene therapy products, and has a process for conditional and time-limited authorization of RM products, which allows approvals with the clinical data likely to predict clinical efficacy, not with the confirmatory trials.

The second approach is RM/CTs conducted for non-commercial purposes, according to the Act for the Safety of Regenerative Medicine (ASRM). The ASRM specifies the regulations, to which medical practitioners, review committees, and cell culture/processing facilities have to be compliant, when providing RM/CTs not only in clinical researches but also in other medical practices. ASRM is expected to enable not only flexible explorative clinical researches, but also nation-wide safety monitoring of RM/CTs.

The advent of human pluripotent stem cells as raw materials of CTPs is currently expected to become one of the major breakthroughs in RM/CTs. So, at the session, I would like to introduce not only the regulatory framework for RM/CT in Japan, but also the recently published guidance documents on points-to-consider for evaluation of tumorigenicity, the major and unique safety concern of CTPs derived from human pluripotent stem cells.

The current regulatory environment for iPS cell-based medicinal products

Gerald G. Schumann

Division of Medical Biotechnology, Paul-Ehrlich-Institut, Langen, Germany

Induced pluripotent stem cell (iPSC) derivatives provide a uniquely, scalable source of functional differentiated cells that can potentially repair or regenerate damaged or diseased tissues to treat a wide spectrum of disease and injuries, because they are highly expandable in culture and can be directed to form almost any celltype of the body. Currently, more than eleven interventional clinical trials (Phase I or Phase I/II) applying iPSC-derived differentiated cells are ongoing worldwide. However, gathering sound data on their biodistribution, longevity, function and mechanisms of action in host tissues is imperative to evaluate their clinical benefit. The general availability of therapies involving iPSCs is in the making and supported by regulatory pathways required to ensure their safety. Here we will address specific regulatory aspects of the establishment of iPSC banking systems, the characterization of the manufacturing process resulting in a final differentiated cell product and summarize regulatory guidance.

Abstracts

Talks

The Origin, Mechanisms and Consequences of Acquired Genetic Variants in Human Pluripotent Stem Cells

Peter W. Andrews

The Centre for Stem Cell Biology, The Department of Biomedical Science, The University of Sheffield, UK

Mutation in human pluripotent stem cells (hPSC) on prolonged passage in culture tends to be commonly associated with gains of all or parts of chromosomes 1, 12, 17 or 20, or losses of parts of chromosomes 10, 18 and 22, or point mutations in *TP53*. The non-random nature of these genomic changes largely reflects their selective growth advantage over wildtype cells. They are of concern, not only in the development of clinical applications for these cells in regenerative medicine, but also for applications in disease modelling and drug screening. There is, therefore, an imperative to appreciate the consequences of these common variants on the phenotypes of the undifferentiated cells and their derivatives, as well as to understand the mechanisms of mutation and selection that lead to their appearance. In this respect the mutation rate in hPSC seems to be comparable with that in somatic cells, but the mechanisms by which they maintain their genetic integrity appears to differ, perhaps reflecting the requirements of the early embryo.

hPSC cardiomyocytes: Evolution of differentiation processes and applications

Paul W. Burridge

Center for Pharmacogenomics, Northwestern University Feinberg School of Medicine, Chicago, USA

We will discuss the developments in media used to culture hiPSC, including recent negligible-cost formulation and development of weekend-free methodologies. We will also provide a summary of progress that has been made in hiPSC cardiac differentiation methodologies concentrating on the pathways involved, efficiency, and reproducibility. Finally, we will cover an example of the application of hiPSC-derived cardiomyocytes in modeling the genomic basis of drug-induced cardiotoxicity.

hPSC-derived cardiac aggregate transplantation in non-human primates

Ina Gruh[*1], Serghei Cebotari[*1], Caroline Halloin[1], Andreas Martens[1], Annette Schrod A[2], Wiebke Loebel[1],Paul Frank[1], Amir Moussavi[2], Kerstin Maetz-Rensing[2], Mark Kuehnel[1], Christopher Werlein[1], Jörg Eiringhaus[1], Stella Reamon-Buettner[3], Vanessa Neuhaus[3], Christian Veltmann[1], Danny Jonigk[1], Susann Boretius[2], Axel Haverich[1], Robert Zweigerdt[#1], Ulrich Martin[#1]

*[1]Hannover Medical School, Hanover, Germany [2]Leibniz Institute for Primate Research, Goettingen, Germany, [3]Fraunhofer ITEM, Hanover, Germany; *,# contributed equally*

Background – Induced pluripotent stem cells (iPSCs) and their progeny are promising sources for cell-based therapies. Human iPSC-derived cardiomyocytes (hiPSC-CMs) can be generated in vitro and have been proposed for myocardial repair in vivo, but so far, limited data is available from large animal models. We have previously established transplantation and tracking of human iPSCs in a pig model of myocardial infarction (Templin et al., Circulation 2012) as well as implantation of pulmonary, aortic and mitral valves in sheep (Tudorache et al., Eur J Cardiothorac Surg 2016 & Iablonskii et al., Eur J Cardiothorac Surg 2018).

Aim – In an ongoing study, it was our aim to establish a pre-clinical non-human primate model to investigate the regenerative potential of hiPSC-CMs after myocardial infarction.

Methods – Myocardial infarction (MI) was induced by coronary artery ligation in cynomolgus monkeys (Macaca fascicularis; n=6) under general anesthesia. Animal medication included analgesic, antibiotic, antiarrhythmic, and immunosuppressive drugs. Cardiac function was assessed via telemetric ECG recording, echocardiography and MRI at different time points. Human iPSC-CMs expressing a fluorescent reporter gene (Venus) were generated by targeted differentiation in large-scale suspension cultures. 5 - 7 x 10 7 hiPSC-CMs were injected directly into the myocardium 2 weeks after MI. After 2 weeks or 12 weeks, animals were sacrificed and graft survival was assessed histologically.

Conclusion – Both 2 weeks and 12 weeks after cell transplantation, large hiPSC-CM grafts (>1 mm2) were identified in myocardial tissue sections based on their reporter gene expression and after staining with a species-specific antibody targeting human cardiac troponin I. Human iPS-CMs expressed cardiac markers with visible cross striations reflecting sarcomeric structures and showing cellular alignment. Currently, the evaluation of functional cardiac data is ongoing. In conclusion, our pre-clinical non-human primate model allowed hiPSC-CMs

to engraft and survive in the infarcted heart for up to 12 weeks and will be further used to investigate the regenerative potential of hiPSC-CMs.

Cell-free allografts: The long journey of a therapeutic idea

Michael Harder

CEO, corlife oHG, Hanover, Germany

Congenital heart disease (CHD) accounts for almost one third of all congenital abnormalities. For Asia, Europe and North America, prevalence rates of 7-9 per 1,000 live births are reported. Every thousandth child needs heart surgery and sooner or later needs an aortic or pulmonary valve replacement. Conventional heart valve prostheses are disadvantageous for young people: patients take anticoagulant drugs for prostheses made of artificial materials. Valves made of biological material can degenerate rapidly. This also applies to heart valve transplants that have not been matched. None of the concepts has the potential for regeneration or even growth. Several working groups around the world began pursuing the concept of the cell-free heart valve in the 1990s. The concept followed ideas from musculoskeletal surgery. Physically and chemically prepared bone and tendon transplants have been used successfully in patients for quite some time. The transplants are largely cell-free and sterile and therefore safe and well tolerated. After countless experiments in vitro, only a few methods for decellularization of heart-valves appeared safe were verified in animal models. Around the turn of the millennium, the first cell-free pulmonary valves were implanted in humans. In 2006 corlife started to deal with this topic. The manufacturing protocols were standardized, quality assurance was developed and introduced, personnel was trained and a manufacturing site qualified. In 2013, corlife received approval for the cell-free pulmonary valve. This was followed in 2015 by approval for the cell-free aortic valve and in 2019 for large vessels close to the heart. The developments were accompanied by clinical studies. Since 2013, more than 500 cell-free heart valves have been implanted. The expectation that they would be better tolerated has been fulfilled. However, we are not yet able to conclusively answer the question of whether the cell-free heart valves will grow with the patient. The statistical basis for this is still missing. What have we learned? Logistics and regulation are very serious challenges that determine economic survival. Quality, seriousness, modesty and a lot of patience are the basis for the introduction of an innovation in the field of tissue medicine.

Stem cells and gene editing: Legal and ethical issues

Nils Hoppe

Centre for Ethics and Law in the Life Sciences (CELLS), Leibniz University Hannover, Hanover, Germany

Both the different sources and uses of stem cells, as well as novel biotechnologies such as CRISPR/Cas9, give rise to knotty legal and ethical questions. Under what circumstances have cells been procured and produced? What kind of genetic changes are legitimate, and which are not? Who benefits from the results of these technologies? Can we anticipate societal objections or legal obstacles? This talk will outline some of the most challenging legal and ethical issues and seek to identify analogies to inform a responsible reflection of normative aspects within the science of stem cells and gene editing.

The road to the clinic: Safety and efficacy of human astrocytes AstroRx® following intrathecal transplantation in hSOD1 and NSG animal model

Michal Izrael, Guy Slutsky, Arik Hasson, Graciela Kuperstein, Yehezkel Shiran, Leonardo Solmesky, Alina Zhuravlev, Judith Chebath and Michel Revel

Kadimastem LTD, Nes-Ziona, Israel

Background: ALS is a Motor Neuron (MN) disease characterized by the loss of MNs in the central nervous systems. As MNs die, patients progressively lose their ability to control voluntary movements, become paralyzed and eventually die from respiratory/deglutition failure. Despite the selective MN death in Amyotrophic lateral sclerosis (ALS), there is growing evidence that malfunctional astrocytes play a crucial role in disease progression. Thus, transplantation of healthy astrocytes may compensate for the diseased astrocytes.
Methods: We developed a GMP-grade protocol for generation of astrocytes from human embryonic stem cells (hESC). The first stage of our protocol is derivation of astrocyte progenitor cells (APC) from hESCs. These APC can be expanded in large quantities and stored frozen as cell banks. Further differentiation of the APC yields an enriched population of astrocytes with more than 90% GFAP expression (AstroRx®). AstroRx® were injected intrathecally to hSOD1^{G93A} transgenic mice and rats to evaluate their therapeutic potential. The safety and biodistribution of AstroRx® were evaluated in a nine-month study conducted in immunodeficient NSG mice under good laboratory practice (GLP) conditions.
Results: *In vitro*, AstroRx® possesses the activities of functional healthy astrocytes, including glutamate uptake, promotion of axon outgrowth and protection of MNs from oxidative stress. A secretome analysis shows that these AstroRx® also secrete several inhibitors of metalloproteases as well as variety of neuroprotective factors (e.g. TIMP-1&2, OPN, MIF and Midkine). Intrathecal injections of AstroRx® to transgenic hSOD1^{G93A} mice and rats significantly delayed disease onset and improved motor performance compared to sham-injected animals. Safety study in immunodeficient mice showed that intrathecal transplantation of AstroRx® is safe. Transplanted AstroRx® attached to the meninges along the neuroaxis and survived for the entire duration of the study without formation of tumors or teratomas. Cell-injected mice gained similar body weight as the sham injected group and did not exhibit clinical signs that could be related to the treatment. No differences from the vehicle control were observed in hematological parameters or blood chemistry.
Clinical study: Based on these results we were approved to initiate a phase I/IIa clinical trial (ClinicalTrials.gov Identifier: NCT03482050), a dose escalating study to evaluate the safety and efficacy of AstroRx in patients with ALS.

From hPSCs research to cell therapies

Allan Ertmann Karlsen

Professor, Stem Cell Research in Stem R&D, Novo Nordisk A/S, Denmark

In Novo Nordisk we have worked with stem cells for more than a decade, originally with the exclusive focus to differentiate human pluripotent stem cells to become islet like structures, to be encapsulated and transplanted into people with Type 1 Diabetes to repopulate their beta-cell population destroyed by autoimmunity.
During this process we have gained a significant amount of knowledge within the area of stem cells, from making the stem cell lines, protocol development to differentiate the cells, GMP production of cells as well as regulatory and clinical trial experience.
We have therefore recently established a Stem Cell R&D unit within Novo Nordisk, with the exclusive aim to develop new stem cell opportunities for the treatment of serious chronic diseases. To make this happen and expand our stem cell pipe-line our collaboration model is based on engaging with specialists within the different therapy areas and wok together bridging the translational gap to get into human trials and further towards commercialization Examples from our growing pipeline (e.g. T1D, Parkinson's disease, Chronic Heart Failure, Eye and Kidney diseases), our GMP production abilities as well as other technologies, considerations and challenges will be presented, hopefully inspiring new collaborations within the existing and new therapy areas to be formed.

Making GMP-compliant media and reagents for cellular therapeutics

Sebastian Knöbel

Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Translation of research protocols into GMP-compliant manufacturing constitutes a widely underestimated challenge. The regulatory assessment of an ATMP covers on the one hand the manufacturing process and on the other hand the reagents used for manufacturing.
Regulatory requirements define the quality aspects to be fulfilled by industry and among many other aspects imply the use of qualified raw materials, highly reproducible and validated manufacturing processes and quality control assays. MACS GMP Media and cytokines are currently used in clinical trials worldwide and thus have successfully undergone review by different regulatory agencies. MACS GMP Products are manufactured and tested under a quality management system (ISO13485) and are in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials. Manufacturing and testing of these products complies with the requirements laid down in the Ph.Eur. Chapter 5.2.12. Relevant quality criteria will be discussed as well as the implementation of reagents into manufacturing processes.

Reprogramming enriches for somatic cell clones with small scale mutations in cancer-related genes

Maike Kosanke[1]*, Katarzyna Osetek[1], Alexandra Haase[1], Lutz Wiehlmann[2], Philippe Chouvarine[2], Sylvia Merkert[1], Stephanie Wunderlich[1], Marie Dorda[2], Samira Mielke[2], Ulrich Martin[1]

[1]Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Department of Cardiothoracic-, Transplantation and Vascular Surgery, REBIRTH-Cluster of Excellence; German Centre for Lung Research; [2]Department of Paediatric Pneumology and Neonatology; [3]Institute of Human Genetics; [4]Institute of Experimental Haematology; Hannover Medical School; Hanover, Germany

Summary: Recent studies demonstrated that the observed high mutational load in induced pluripotent stem cells (iPSCs) is largely derived from their parental cells, but as yet it is unknown whether reprogramming may enrich for individual mutations from their parental cells.

We have derived 30 human iPSC lines from neonatal and aged individuals under comparable conditions. High accuracy exome and amplicon sequencing showed that all analyzed 'true' small scale variants pre-existed in their parental cells. We now provide first evidence that individual mutations present in small subpopulations of parental cells become highly enriched among iPSC clones during reprogramming. Among those, putatively actionable driver mutations affect genes related to cell death / survival, cell cycle control, and pluripotency, and may confer a selective advantage during reprogramming. Considering the various common characteristics of pluripotent stem cells and cancer cells, such mutations are likely to account for an increased tumor risk and may impact the clinical value of patient-derived iPSCs.

hPSC-derived cardiomyocyte: Clinical transplantation for heart repair

Philippe Menasché

Department of Cardiovascular Surgery, Université de Paris, France

The rationale for using pluripotent in patients with heart failure primarily stems from the assumption that regeneration of scarred myocardium likely requires the supply of cells phenotypically matched to the target tissue. This approach is made possible by the intrinsic pluripotentiality of embryonic and induced pluripotent stem cells (ESC and iPSC) which allows to drive their fate *in vitro* towards a cardiac lineage. Our program has targeted the generation of early cardiac progenitors from ESC through a stepwise approach including: (1) the expansion of hESC to generate cell banks under Good Manufacturing Practice conditions (GMP); (2) a growth factor-induced cardiac specification; (3) the purification of committed cells by immunomagnetic sorting to yield a SSEA-1-positive cell population; (4) the incorporation of these cells into a fibrin scaffold epicardially delivered onto the infarcted area; and (5) a safety assessment focused on (i) the loss of teratoma-forming cells and (ii) the absence of cytogenetic abnormalities and of microbiological contamination. We then completed the first-in-man clinical trial of transplantation of these SSEA-1+ progenitors in 6 patients with severe left ventricular dysfunction and otherwise requiring a coronary artery bypass operation. With a maximal follow-up which will reach 5 years in October, 2019, the primary safety end point has been met: no tumor has been detected (as assessed by computerized tomography and ^{18}F-FDG positron emission tomography scans) and none of the patients presented arrhythmias (detected by serial interrogations of the cardioverter-defibrillators implanted in all of them). One patient developed a new-onset clinically silent alloimmunization demonstrated by the presence of donor-specific antibodies. There has been a significant improvement in the regional wall motion of myocardial areas covered with the cell-laden patch (most of which were not simultaneously revascularized). This trial demonstrates the technical feasibility of producing clinical-grade hESC-derived cardiovascular progenitors and supports their short- and medium-term safety. In parallel, we found that the cardioprotective effects of these ESC-derived cardiovascular progenitors could be duplicated by the sole injection of the extracellular vesicles that they secrete, which opens the way to a new paradigm of cell-based a-cellular therapy whereby pluripotent stem cell-derived differentiated derivatives could be assigned the new role of exclusive *in vitro* producers of a purified secretome considered as the only therapeutics given to the patient.

Gene editors and pluripotent stem cells: The porcine biotechnological tool box becomes complete

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Because of their great genetic, anatomical and physiological similarities with humans, the domestic pig is considered to be an excellent model for human diseases, cell therapies and even as donor for porcine xenografts, However, the production of pigs with targeted genetic modifications has been extremely inefficient, mainly due to the lack of true pluripotent embryonic stem cells. This situation has changed with the introduction of gene editing tools and the recent availability of porcine pluripotent stem cells with expanded potential. Here, I discuss the use of gene editing for the production of multi-transgenic pigs for xenotransplantation and report on our recent discovery of porcine pluripotent stem cells with expanded potential.

Genetically modified pigs for xenotransplantation

The ability to genetically modify pigs significantly enhances their potential as organ donor. The discovery of new molecular tools to modify complex mammalian genomes such as ZFNs, TALENs and CRISPR/Cas, and the improved genomic maps of human and pigs have opened new avenues towards efficient, precise and fast modification of the porcine genome. Prior to clinical application of porcine xenografts, three major hurdles have to be overcome: (i) various immunological rejection responses, incl. HAR (hyperacute rejection response), AVR (acute vascular rejection), DXR (delayed cellular rejection), (ii) physiological incompatibilities between the porcine organ and the human recipient, incl. a severe dysfunction of the coagulation cascade, and (iii) the risk of transmitting zoonotic pathogens from pig to humans.

The current view is that long-term survival of xenografts after transplantation into primates requires the production and characterization of multi-transgenic pigs to control HAR, AVR and DXR and a specifically tailored immunosuppression regimen compliant with current clinical standards. The recent emergence of novel molecular tools such as Zinc-Finger nucleases (ZFN), Transcription-activator like endonucleases (TALENs), and the CRISPR/Cas9 system has significantly increased efficiency and precision of the production of genetically modified pigs for xenotransplantation. Several candidate genes, incl. hTM, hHO-1, hA20, CTLA4Ig, have been explored in their ability to improve long-term survival of porcine xenografts after transplantation into non-human primates and pigs with up to six genetic

modifications have been produced and characterized and their organs could now be used in pig-to-non-human primate experiments.

Derivation of porcine pluripotent stem cells

While various pluripotent cell lines and the different pluripotency statuses (primed, naïve) are well defined in the laboratory mouse model, little is known in large domestic animals, specifically the domestic pig. Numerous attempts have been made to arrive at well characterized porcine pluripotent stem cells, either by extracting cells from early embryos or by the iPSC approach, but met with little success. The porcine stem cells reported so far only met some of the pluripotency criteria, but usually failed in any in vivo proof putative pluripotency tests.

We have recently reported the derivation of porcine expanded potential stem cells (pEPSC) lines either directly from preimplantation embryos or by reprogramming porcine fetal fibroblasts. The pEPSCs expressed key pluripotency genes, permitted genome editing, differentiated to derivatives of the three germ layers in chimeras, and produced primordial germ cell-like cells (PGCLCs) in vitro. Under similar culture conditions, human ESCs and iPSCs could be converted, or somatic cells were directly reprogrammed to EPSCs (hEPSCs) that display the molecular and functional attributes reminiscent of pEPSCs. Significantly, trophoblast stem cell-like cells could be generated from both human and porcine EPSCs. The pathway-inhibition paradigm applied in our study opens a new avenue for isolating mammalian embryonic stem cells, and EPSCs present new opportunities for translational research in biotechnology and regenerative medicine.

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Innovations of materials and technologies for process development and monitoring of cell therapeutics

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Stem cell-based technologies lay the basis for pioneering approaches in drug screening, toxicology testing, and regenerative medicine. Their routine application needs a constant supply of high cell numbers of consistent quality. Unlike biologics production of mammalian cells, industry-scale stem cell expansion and differentiation are not yet routine. Stirred-tank bioreactors have great advantages to achieve this demand of high cell numbers of constant high quality.

In order to successfully cultivate your stem cells and perform your experiment, a variety of parameters can be controlled. Critical parameters like pH, DO, and temperature need to be constantly monitored and eventually adjusted immediately during the cultivation process. Agitation in combination with impeller type allows the control of aggregate sizes which has an impact on cell pluripotency, differentiation and proliferation potential. This tight control of parameters during the process lays the basis for successful process development.

Moreover, adding 4D microscopy to monitor and analyze aggregate development patterns and morphology over time brings significant added values while improving process-specific parameters. Eppendorf and Ovizio have collaborated to establish a seamless integrated and optimized system that allows the cultivation and in-depth monitoring of both, biological and morphological, parameters of HiPSCs aggregates without negatively impacting their size and cell viability in low cultivation volume settings.

Challenges in choosing heart failure patients for first-in-man iPS cell-therapies

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Choosing patients for early-phase clinical studies with cardiac iPS-cell applications can be challenging even without taking into account the complex regulatory aspects. Eligible patients will have to fulfill several criteria, which maybe even contradict each other. The underlying heart disease of course has to be severe to life-threatening. On the other hand patients severely ill often do have a number of co-morbidities, which per se may impose an exclusion criterion or which may not allow for specific follow-up examinations needed for the robust evaluation of clinical results such as CMR. Rapid progression of the cardiac disease may come in conflict with the lengthy cell processing. Emergency applications have to be avoided in any case as emergency patients will have to be excluded from most clinical studies. In this presentation we will focus on the evaluation process for our planned first-in-man applications, which may target another patient cohort than the originally planned patient cohort with ischemic heart disease.

Pluripotent stem cells for cell therapy in T1D

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Key challenges in stem cell-based therapy in diabetes include safe manufacturing of sufficient number of functional beta cells in vitro. Through systematic studies in mice and human pluripotent stem cells (hPSCs) we have addressed how key intermediate steps from the epiblast to the mature beta cell are regulated. Interestingly, many of the regulatory principles are conserved, which facilitated in depth mechanistic studies in the human system. By defining the mode of action of added growth factor/small molecule we have generated cost-effective robust protocol for coaxing hESCs into beta cells. To address the need for an expandable system we have identified unique cell surface markers in human multipotent pancreatic progenitors making it possible to purify and expand the progenitor population that normally is responsible for the growth of the pancreas. This system not only facilitates expansion of beta cell progenitors but it also removes undifferentiated hPSCs (and other cell types), which represents the major safety concerns in hPSC-based cell therapy. Currently, we are manufacturing beta cells for future clinical trials in diabetic patients.

Progress and risk of ‘MSC’ therapies

Dirk Strunk

Professor, Cell Therapy Institute, Paracelsus Medical University, Salzburg, Austria

Stromal cells are important components of all mammalian tissues contributing to organ integrity (as fibroblasts/stroma) and vascular stability (as pericytes), in addition to their enigmatic niche function. Their inherent immunomodulatory capacity attracted particular attention resulting in the initiation of hundreds of clinical trials, mainly testing their trophic regenerative and immunomodulatory potential. Key stromal immune functions are still not understood.

Studies using allogeneic ‘MSC’ have shown surprising results despite lack of engraftment of the transplanted cells. Evidence will be presented showing that heir efficacy can be mediated by extracellular vesicles (EVs) in addition to secreted trophic factors.

Human induced pluripotent stem cells (iPSC) are frequently used for ‘MSC’ differentiation in preclinical research (to generate virtually unlimited amounts of transplantable cells) and for mechanistic studies. Data will be presented for using mesodermal brachyury-expressing iPSC progeny to monitor their stepwise maturation while re-acquiring phenotype, clonogenicity, gene expression and function of their parental stromal cells.

Because the majority of ‘MSC’ are extra-hematopoietic cells expressing variable levels of tissue factor they are prone to elicit an instant blood-mediated inflammatory reaction (IBMIR) resulting in thrombotic complications and reduced engraftment. Examples will be presented for monitoring hemocompatibility of commonly used ‘MSC’. A possible strategy for safer systemic ‘MSC’ and iPSC transplantation will be devised.

Translation of engineered heart repair

Wolfram-Hubertus Zimmermann

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Our lab is developing Engineered Heart Muscle (EHM) from induced pluripotent stem cells (iPSCs) for applications in patients with end-stage heart failure. Previous data from our lab supported the general strategy of cardiac remuscularization by EHM implantation in rodent models. To simulate the clinical path and to further scrutinize the safety of EHM implantation, large animal allograft studies are highly relevant. We chose the Rhesus macaque model because of the close phylogenetic distance to the human and the successful reprogramming of Rhesus macaque fibroblasts to iPSCs. EHM allo-/autografts were retained for at least 6 months in healthy macaques under optimized immune suppression, augmented the target heart wall, and did not cause palpable side effects (i.e., no arrhythmia, no teratoma formation, no compromise of heart function). The obtained data together with the development of a GMP-process for human EHM production as well as completed GLP-toxicity, biodistribution, and tumorigenicity testing are key for the translation of our preclinical findings into the first-in-patient DZHK20-BioVAT-HF trial.

Upscaling hPSC and progeny production including CMs, ECs and macrophages

Emiliano Bolesani[1], Santoshi Biswanath[1], Dorothee Bornhorst[1], Lika Drakhlis[1], Michelle Coffee[1], Clara-Milena Farr[1] Annika Franke[1], Alexander Goedel[5], Caroline Halloin[1], Lavanya Iyer[6], Henning Kempf[1, 4], Wiebke Löbel[1], Felix Manstein[1], Heiko Meyer[3], Katharina Ritzenhoff[1], Salim Seyfried[1], Jana Sklarek[1], Jan Hegermann[2], Lena Nolte[3], Kevin Ullmann[1], Dorota Zawada[5], Laura Zelarayán-Behrend[6], Robert Zweigerdt[1]*

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The clinical translation of human pluripotent stem cell (hPSC) progenies has made substantial progress in recent years. However, routine cell therapies for solid organs such as the heart, pancreas, liver or brain will require large cell quantities estimated at several billions per patient. For hPSC-derived blood cells such as macrophages and erythrocytes even higher numbers are discussed.

Instrumented stirred-tank bioreactor (STBR) technology represents a universal tool for enabling the systematic development and upscaling of bioprocesses by equivalent reactor design of incremental dimensions. STBRs ensure the homogeneous distribution of cells, nutrients and gases in suspension culture and enable the continuous online monitoring and, importantly, feedback-based control of key process parameters such as pH and dissolved oxygen (DO).

This talk will present our most recent progress in applying STBR technology for boh advancing the high-density compatible mass production of hPSC at the pluripotent state and the subsequent transition towards lineage-directed differentiation into specific lineages including cardiomyocytes (CMs) endothelial cells (ECs) and functional macrophages.

Moreover, the talk will address specific aspects along the transition of pluripotency towards mesendodermal specification, maintenance of lineage-intermediate progenies and the formation, characterisationa and application of 3D mesendodermal organoids.

Abstracts

Posters

1. Modelling of LMNA-cardiomyopathy in human iPSC-derived engineered heart tissue for testing novel therapies

Charlotta S. Behrens[1,2], Anke J. M. Tijsen[3], Bärbel Ulmer[1,2], Malte Loos[1,2], Ingke Braren[4], Aya Shibamiya[1,2], Arne Hansen[1,2], Gisèle Bonne[5], Yigal Pinto[3], Thomas Eschenhagen[1,2]

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Introduction: Inherited dilated cardiomyopathy (DCM) are caused in 6 to 8% of the cases by mutations in the gene LMNA encoding for lamin A/C. Lamin A/C makes up the nuclear envelope and therefore has several functions, however, the underlying molecular pathological mechanisms remain to be elucidated. Evidence has been provided both for haploinsufficiency and a dominant negative, toxic effect. Recapitulating the disease in a human 3D in vitro model could give valuable insights with regards to designing and testing novel therapies.

Methods and Results: Patient-specific human induced pluripotent stem cells (hiPSC) were generated from skin biopsies of two laminopathy patients heterozygous for either the mutation p.H222P or p.Q493X. At the same time an isogenic control cell line of p.H222P, p.H222P Rep, was generated with CRISPR/Cas9. HiPSC of these patients, the isogenic control cell line and an unrelated control cell line were differentiated into cardiomyocytes (CM) with an efficiency ranging from 75% to 95%. Engineered heart tissues (EHTs) were generated from dissociated CM and their force development was tracked over time via video optical recording. Both patient cell lines showed much lower capacity than control cell lines to remodel in the first two batches. The EHTs that remodelled showed lower force of contraction than the ones from unrelated control cell line. In particular, the p.H222P Rep showed a 3-fold higher force than p.H222P. Furthermore, 3D reconstructed nuclei were measured in a blinded manner and showed significant elongation for p.H222P EHTs compared to the unrelated control cell line.

Conclusion: Taken together, our preliminary results suggest reduced force of contraction and impaired capability of remodelling as a first phenotype in our model. Identifying a phenotype in the model is the first important step to determine disease mechanisms and test novel therapies.

2. In vitro stabilization of cardiac neural crest progenitor cells from hPSCs in defined culture conditions display multi-lineage and proliferative potential

Emiliano Bolesani[1]*, Dorota Zawada[2], Dorothee Bornhorst[1], Lavanya Iyer[3], Annika Franke[1], Alexander Goedel[2], Laura Zelarayán-Behrend[3], Salim Seyfried[1], Robert Zweigerdt[1]

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Cardiovascular progenitor cells (CPCs) may represent a favourable alternative for cell-based heart repair and provide a valuable model to investigate heart development. Histone Acetyl Transferases (HATs) are regulators of gene transcription, thereby defining progress of cell differentiation and specific developmental stages. In this study we focused on the role of the β-catenin binding HATs CBP and p300 during human pluripotent stem cells (hPSCs)-derived cardiac progenitors' development.

In our differentiation protocol the second heart field (SHF) multipotent progenitors' marker ISL1 is upregulated shortly before the expression of NKX2.5, which marks the onset of functional cardiomyocytes. The overarching hypothesis in this study is that the pharmacological modulation of CBP and p300 in hPSC-derived CPCs by the small molecule inhibitors IQ-1 would allow control of the downstream fate of CPCs. Mechanistically, this was enabled by favouring the IQ1-directed binding of CBP-to-βCAT at the expense of p300-βCAT interaction. Consequently, this directs the binding of cardiac transcription factors only to specific enhancers regions, regulates differential gene patterns and, importantly, allows the previously unfeasible maintenance of specific differentiation stages.

In this study, the stabilization of CPCs has been achieved by adding IQ-1 together with the WNT pathway activator CHIR (a chemical GSK3β inhibitor) at specific stages of hPSC differentiation. Stabilized CPCs were characterized by the co-expression of established SHF markers ISL1/GATA4 in conjunction with KI-67 and SIRPA. CPCs retain both their proliferative potential and multi-lineage plasticity resembling cardiac neural crest progenitors demonstrated by RNA-seq analysis. In vivo studies applying IQ1 in respective stages of zebrafish development closely recapitulated these observations; including maintenance of the CPC state in specific cardiac cell progenies, by blocking their progression into functional cardiomyocytes, supporting the hypothesised effect of IQ-1. Together, this is the first study presenting the in vitro stabilization of a cardiac neural crest progenitor-like population by defined culture conditions.

3. Higher throughput production and characterisation of 3D cardiac microtissues

Michelle Coffee, Santoshi Biswanath, Annika Franke, Robert Zweigerdt

Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Department of Cardiothoracic-, Transplantation and Vascular Surgery, Hannover Medical School, Hanover, Germany

In recent years there has been a surge in the generation and application of 3D human pluripotent stem cell (hPSC)-derived cardiac microtissues, which has proven to be an invaluable tool for cardiac research. However, as with all emerging technologies, there are still a number of challenges, two of them being (i) a need for higher throughput produced cardiac microtissues and (ii) a lack of suitable technologies for the thorough, yet routine analysis of such microtissues. This study aimed to overcome these two issues associated with current 3D microtissues based systems, specifically for the application to cardiovascular research. Here we compared several microcavity array platforms, namely the Sphericalplate 5D plates (Kugelmeiers AG), Statarrays© microcavity array (MCA) plates (300MICRONS) and the AggreWell™ 400 Plates (Stemcell Technologies™). The Statarrays© MCA plates are compatible with high throughput screening technologies and could produce 16224 microtissues per plate, while the latter allowed for the generation of up to 28800 cardiac microtissues per platform, as opposed to conventional approaches producing less than 1000 microtissues per platform thus far. Within these platforms, microtissues of various cell type compositions were generated to determine suitability of the platforms for multicellular cardiac microtissues. Given the large number of available microtissues, flow cytometry as well as real-time metabolic analysis could be applied to quantitively assess the cell composition within these microtissues as well as the effect of different cell compositions. Analysis of cell populations via flow cytometry indicated a change in the composition of multicellular microtissues, with an increase in the ratio of CMs over a period of up to 3 weeks and a decrease in the ratios of the initially added endothelial cells (ECs) and fibroblasts (FBs). Metabolic analysis revealed variations in mitochondrial respiration during stress response based on cell type composition of the microtissues. This study has therefore demonstrated an established method for higher throughput production and maintenance of cardiac microtissues as well as methods for the reliable, routine analysis thereof.

4. Generation of human induced pluripotent stem cell-derived lung basal cells using an in vivo teratoma model

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Chronic lung diseases characterized by pathological remodeling of the airway epithelium range among the leading causes of death worldwide. Cell therapeutic approaches are a promising novel attempt to match the growing need for curative treatment options. In healthy tissue, lung basal cells (BCs), located underneath the respiratory epithelium, is the main cell population responsible for homeostasis and tissue regeneration. To evaluate the regenerative potential of these cells for future clinical application, detailed studies of their stem cell capacity and niche-interactions have to be performed. Here, we established a human induced pluripotent stem cell (hiPSC)-derived teratoma model for the generation and characterization of the BC niche both in teratoma and in vitro.

We identified organized lung structures within the hiPSC-derived teratomas composed of basal cells, ciliated cells and club cells as confirmed by immunohistochemistry. We established a refined protocol for gentle teratoma dissociation and FACS mediated BC isolation using the surface markers NGFR (nerve growth factor receptor) and CD49f (Integrin A6). Final yield in NGFR+/CD49f+ cells was 1.1 % ± 0.25 % (MEAN ± SEM) and BC identity could be further assessed by immunocytochemistry staining of BC markers p63 and cytokeratin 5. Furthermore, we generated hiPSCs conditionally expressing different lung transcription factors upon doxycycline-mediated induction. When induced in vivo, these cells rendered a yield of up to 3% NGFR+/CD49f+ cells.
In summary, we have developed a sophisticated protocol for in teratoma BC generation from hiPSCs. This model gives us the opportunity to evaluate BC regenerative capacity in subsequent in vitro assays. Furthermore, the complex cellular environment within the teratoma enables us to analyse the conditions most suited for proper lung development and regeneration. These findings will certainly be useful for future cell therapeutic approaches with regards to beneficially influencing factors.

5. Immune suppression for xeno-cell transplantation strategies in the porcine animal model

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Background: Preclinical cell-replacement therapies are frequently based on the use of xenogeneic cells. Hence, immunosuppression is required to prevent rejection. Despite large differences in pharmacokinetics and -dynamics among species, standard immunosuppressive protocols are often applied without ensuring efficacy in the chosen model organism, thereby potentially resulting in translational failure.
Purpose: We aimed to design an immune suppression regimen that can be employed in the porcine model to allow xenograft cell transplantation.
Methods: Pigs (n=3) were subjected to a weekly increasing combination therapy of two different immunosuppressive drugs, Tacrolimus and Azathioprine, and a fixed dose of Methyl-Prednisolone. Careful biochemical evaluation of circulating levels of immunosuppressants, immune cell profile (haematology counter and flow cytometric analysis) and in vitro stimulation assays were performed until an adequate immune suppression. Subsequently, human iPS-derived cardiomyocytes alone or together with human mesenchymal stromal cells were subcutaneously transplanted in a Matrigel carrier in the pig abdomen. Two week after implantation the Matrigel and surrounding tissue were harvested for further analysis. Animals (n=2) without immunosuppressive drugs administration served as controls.
Results: All treated animals reached levels within human therapeutic range for Tacrolimus after increasing the dosage to 1mg/kg/day. In contrast, levels of Azathioprine (6-TGN) only reached half fold of the therapeutic range for humans when administering 7.0 mg/kg/day, while 6-MMP did increase. Stimulation assays with PBMCs, isolated from the pig's blood, showed reduced leucocyte activation in vitro. After explantation, detailed histological analyses demonstrated massive cell infiltrate in Matrigel plugs of control treated animals,

positive for CD3+ T cells, CD4+ Th cells, CD8+ TC cells, NK cells, plasma cells and macrophages. In contrast, upon immune suppression we observed a 77% reduction in total number of infiltrated cells in Matrigel plugs compared to control treated animals.

Conclusion: Our combination therapy of three different immunosuppressive drugs significantly reduced immune cell infiltration after xeno-transplantation. Together, these results suggest this combination as an effective protocol for future xenograft studies in preclinical pig models.

6. New dual reporter pluripotent stem cell lines for the purification of ins-positive pancreatic islet cells

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Background and aims: Although in vitro differentiation of pluripotent stem cells towards pancreatic islets is promising, it is also challenging because of the heterogeneity of the cells that are generated during differentiation. In order to facilitate the characterization of insulin producing cells derived in vitro, we seeked to establish new reporter cell lines through CRISPR/Cas9 nickases (CRISPR/Cas9n) mediated homology directed repair. This reporter cell lines should give information about the developmental processes during human pancreatic organogenesis, prepare for therapeutic use of surrogate insulin-producing cells and enable the isolation of insulin-producing cells by magnetic or fluorescent activated cell sorting.

Materials and methods: To create a staggered cut around the stop codon of the INS locus, two pairs of sgRNAs were cloned using the Cas9 D10A nickase in PAM-out configuration and a distance of 46-66 bp between the nick sites. Then HES3 embryonic stem cells and human iPS MHHi006-A (Phoenix) cells were nucleofected with the pairs of CRISPR/Cas9n and a targeting vector comprising either a P2A-H-2KK-F2A-GFP2 (HES3) or a P2A-mCherry (Phoenix) gene cassette flanked by 500 bp 5' and 3' homology arms. Either a floxed hygromycin or blasticidin gene was used for clonal selection of targeted cells. Cell clones were screened by PCR and DNA sequencing and then the functionality of the knock-in was tested by differentiation into insulin producing cells using a new differentiation protocol.

Results: Nucleofection of pluripotent stem cells with both designed CRISPR/Cas9n pairs and the HDR vectors lead effectively to integration. In total, 18 HES3 clones were obtained, 8 with homozygous integration, 9 with heterozygous integration and 1 only with hygromycin resistance without any integration. Nucleofection of Phoenix cells lead to 12 Phoenix clones with homozygous integration. The tested clones activated GFP2 or mCherry upon differentiation. The HES3 clone ICN2 and the Phoenix clone PICNC1 were then used for further characterization. GFP2-positive cells also co-expressed the surface antigen H-2KK, which allowed MACS-assisted cell purification. MACS-sorted H-2KK-positive cells were positively tested for insulin gene expression, whereas H-2KK-negative cells remained mainly negative for insulin.

Conclusion: In summary, this study reports the derivation of new pluripotent reporter cell lines which comprises the knock-in of the fluorescence reporter mCherry or GFP2 and the surface antigen H-2KK into the INS locus. Due to the design of the targeting vector, the INS locus remained unharmed so that mCherry, GFP2 and H-2KK are expressed along with INS under the control of the endogenous gene promoter. The analysis of fluorescent protein-

expressing cells showed that they co-express insulin. The duality of the reporter gene knock-in allows the purification by magnetic or fluorescence-activated cell sorting. Thus, we conclude that this cell lines are powerful tools to study the developmental processes during human pancreatic organogenesis. Furthermore, these cell lines could be used to purify insulin-producing cells for cell replacement therapy of diabetes.

7. Recapitulating early embryonic heart development with human pluripotent stem cells

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In response to proper stimuli, human pluripotent stem cells (hPSCs) self-organize into embryo-like tissue and organ patterns in vitro. To trigger tissue-specific spatiotemporal differentiation, (bio-)chemical stimuli are typically combined with either two-dimensional (2D) geometric confinement or matrix encapsulation of hPSCs to form 3D "organoids". Despite substantial progress in other organs, including small intestine, kidney and brain, proper in vitro models of cardiogenesis do not exist. Here, we direct hPSC differentiation into complex, highly structured "heart-forming organoids" (HFOs). These organoids are composed of an epi-, myo- and endocardial cell layer framing an inner core of endodermal origin. The inner core is further pervaded by a vessel-like network lined with endothelial cells. This architecture closely resembles embryonic heart anlagen prior to heart tube formation, which occurs in an established interplay with foregut endoderm and dorsal aorta development. We subsequently show the utility of HFOs to model aspects of congenital heart disease. Compared to heterozygous controls, NKX2.5 knock-out HFOs showed a myocardial noncompaction and hypertrophy phenotype, which reflects cardiac malformations known from in vivo studies in mice and observations of human patients. Together, an advanced model of early human cardiogenesis is presented opening new perspectives to study mechanisms of human heart development and disease in a dish.

8. Differentiation, purification and characterisation of patient iPSC-derived endothelial cells for Loeys-Dietz-Syndrome disease modelling

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Background: Loeys-Dietz-Syndrome (LDS) is a rare connective tissue disorder characterised by a familial predisposition for thoracic aortic aneurysms often leading to acute aortic dissections. LDS associates with a signature of high transforming growth factor β (TGFβ) signalling in vascular smooth muscle cells (vSMCs), although caused by heterozygous mutations in ligand, receptor or downstream mediators of TGFβ-signalling. Besides vSMC pathobiology, vascular endothelial cell (EC) dysfunction may contribute to LDS, but a comprehensive characterisation of endothelial function in LDS has not been performed. Hence we generated LDS-patient specific induced pluripotent stem cells (iPSC) from three related LDS patients with the same heterozygous point mutation in exon 4 of the TGFβ1-receptor (TGFBR1) gene (p.M253I; c.759G>A). LDS-hiPSC derived ECs were generated to study their contribution to LDS-pathophysiology in vitro.

Methods and results: CD34 + cells from peripheral blood samples of LDS patients were reprogrammed using integration free Sendai viruses. These expressed typical markers like Oct3/4, Nanog, SSEA-3, SSEA-4 and Tra-1-60, detected via flow cytometry. The presence of the TGFBR1-mutation in LDS iPSCs was confirmed via Sanger sequencing. LDS-hiPSCs were then analysed concerning their proliferation and differentiation potential and karyotype. To generate and purify CD31+ ECs, we used a scalable suspension culture protocol developed in our lab followed by magnetic cell separation (MACS) based on positive selection of CD31 (PECAM-1) surface expression. As control we used hiPSC-derived ECs from three different wildtype cell lines.

ECs from LDS- and wildtype hiPSCs differentiated with comparable efficiencies of 43.6 ± 4.0 % and 49.3% ± 3.9 of CD31+, respectively. Magnetic cell sorting of cells for CD31, resulted in a mean purity of cells of 96.43 ± 0.47% and 96.21 ± 0.73%for LDS and wildtype, respectively. Sorted ECs expressed typical endothelial markers (VECadherin and von Willebrandt Factor) and were still proliferative over ten passages with decreasing proliferation capacity, which indicates the maturation of the cells. Matrigel assays resulted in tube formation which shows the functionality of the hiPSC derived EC, yet results need to be analysed and compared.

Conclusion and Outlook: LDS iPSCs are an unlimited source of ECs for in vitro disease modelling to study the effects of endothelial function on the disease. We could show that LDS-iPSCs can be successfully differentiated into EC which express typical endothelial markers and are proliferative over ten passages. Functional analysis of the cells is to be carried out. We are planning to further analyse our iPSC-derived ECs using functional assays like scratch assays, sheer stress assays and telomerase activity and endothelial Nitrogen monoxide synthetase assays. To evaluate how the cells are reacting under stress in comparison to wildtype derived ECs.

9. GMP-compliant generation of universally applicable human iPS cell banks for allogenic cell therapy

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RheinCell is building a key resource for future iPS cell-based therapy. By exploiting GMP-compliant conditions in an A-in-B clean room setting with privileged access to well-characterized ATMP-grade cord blood material, HLA-homozygous CD34+ cells are converted to a pluripotent state by means of direct reprogramming. The key feature of the resulting HLAh hiPSCs is that they are immunocompatible with millions of patients thereby avoiding immune rejections upon transplantation. Reflecting its origin, the GMP-grade HLAh hiPSC library is enriched for caucasian haplotypes mostly representing Europe and North America. Targeted genetic manipulation, however, will increase collective immunocompatibility of the resource to approximately 90% of the world's population.

10. GMP-compatible manufacturing of three iPS cell lines from human peripheral blood

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The utilization of human induced pluripotent stem cells (hiPSCs) for disease modeling and drug discovery is already reality, and several first-in-man-applications as cellular therapeutics have been initiated. Implementation of good manufacturing practice (GMP)-compliant protocols for the generation of hiPSC lines is crucial to further increase the application safety as well as to fulfil the legal requirements for clinical trials approval. In this study we describe the development of a GMP-compatible protocol for the reprogramming of CD34+ hematopoietic stem cells from human peripheral blood (CD34+ PBHSC) into hiPSCs using non-integrating Sendai virus-based reprogramming vectors. Three GMP-compatible hiPSC (GMP-hiPSC) lines were manufactured and characterized under these conditions.

Haase, A., Glienke, W., Engels, L., Gohring, G., Esser, R., Arseniev, L., and Martin, U. 2019. GMP-compatible manufacturing of three iPS cell lines from human peripheral blood. Stem Cell Res 35:101394.

11. Perturbation of replication dynamics in human pluripotent stem cells links structural and numerical chromosomal instability

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Human pluripotent stem cells (hPSC) acquire large karyotypic changes through a process of mutation and selection that occurs over prolonged periods of in vitro expansion. It is currently unknown how these karyotypic abnormalities will affect the behaviour of their differentiated derivatives should they be transplanted in vivo, this uncertainty threatens the translational promise of hPSC in regenerative medicine. For this reason, it is crucial that we minimise the mutations that occur during culture to reduce the appearance of genetic changes during expansion and differentiation of hPSC intended for clinical applications.

hPSC divide rapidly with a cell cycle time shorter than many somatic cell types. The truncated cell cycle of hPSC is thought to be driven by high expression of cyclin E that is responsible for the progression of cells through G1 and into S phase. Cyclin E has been well characterised as an oncogene in cancer and has been shown to cause genetic instability and enhance tumorigenesis through a variety of mechanisms. These include the perturbation of replication fork dynamics, depletion of metabolites required for successful DNA replication and collision between the replication and transcription machinery as a consequence of deregulated S phase entry. These events can lead to the formation of double strand breaks and the formation of under-replicated regions that, if repaired incorrectly or carried into mitosis, can lead to the appearance of karyotypically variant cells. We have found that hPSC express high levels of gH2A.X, a marker of DNA damage, and extended comet assay tail moments, both indicating a high frequency of DNA double strand breaks. Additionally, we have shown that hPSC display altered replication dynamics, consistent with cyclin E oncogenic activity. These results provide evidence that hPSC show characteristics of genetic instability that is consistent with high cyclin E expression. Based upon our findings, we have also developed new culture conditions for hPSC self-renewal that result in improved replication dynamics and a reduction in markers of DNA damage and double strand breaks, and potentially decreases rates of mutation.

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12. Labeling cardiac mitochondria: novel fluorescence dyes with high photothermal stability

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The heart is the most energy-consuming organ in the human body and derives all of its energy from mitochondrial oxidative phosphorylation [1] mainly. Mitochondria are extremally crucial for cardiac development and healthy function. Human-induced pluripotent stem cell (hiPSC) have been widely utilized to generate human cardiomyocytes *in vitro* models for disease modelling, drug screening and novel drug discovery, as well, as preclinical research for cardiac regeneration in models of myocardial infarction (MI) and chronic heart failure (HF)[2].

Recent studies have demonstrated that there is a strong connection between mitochondrial function and pluripotency. Also, it has been suggested that the morphology, localization, abundance and function of mitochondria could be used as a marker of pluripotency[3]. In this sense, fluorescent probes are powerful tools for monitoring morphological changes and studying theses processes.

Commercial mitochondrial cell markers have been high efficiently for several assays. However, these present some disadvantage, which can interfere with the tests; for instance, they can change the membrane potential, present weak fluorescent signal and be thermo- and photo-labiles, therefore, they require the special condition of storage (<10 °C) and protection from light [4]. In this sense, new compounds with more chemical- and photostability are required for the scientific community.

Herein, we are introducing two novel fluorescence dyes based benzothiadiazole; both are chemically stable, do not have evidence of photobleaching at room temperature and have a natural mitochondrial-targeting ability in living hipsc-cm. Stained hipsc-cm continue beating after 48 h incubation with the fluorescence dyes. Confocal images exhibited not internal cell diffusion, high photostability under repeat irradiation and almost unseen damage of photobleaching. The results look promising for tracking mitochondria during cellular differentiation.

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13. The long non-coding RNA Cyrano is dispensable for self-renewal and pluripotency of murine and human iPSCs

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Non-coding RNAs (ncRNAs) make up the vast majority of the human transcriptome (98%), but were considered evolutionary junk for a long time. Within the last two decades it became clear that many cellular processes are regulated by ncRNAs. Thus, ncRNAs are now studied for their high potential as diagnostic and prognostic biomarkers as well as therapeutic targets in various diseases.

A large subgroup of ncRNAs, long non-coding RNAs (lncRNAs) are transcripts longer than 200 nt. They can regulate protein expression transcriptionally and posttranscriptionally, thereby influencing virtually all cellular processes including for example cellular proliferation, differentiation, stress responses and apoptosis.

By screening publicly available datasets for lncRNAs in cardiac regeneration we identified Cyrano, a highly conserved lncRNA. We showed that Cyrano is enriched in cardiomyocytes and dysregulated in different cardiovascular diseases in mice and humans, suggesting a role of Cyrano in cardiomyocyte function. In order to study a potential functional involvement of Cyrano in cardiac disease processes we started to generate knockout and knockdown murine and human induced pluripotent stem cells (iPSCs), respectively, aiming to differentiate them into Cyrano null cardiomyocytes.

Interestingly, during this work Cyrano was reported to be important for the maintenance of pluripotency in murine embryonic stem cells (ESCs) as demonstrated by means of siRNA knockdown of Cyrano.

This was in marked contrast to our observation of genetically Cyrano deficient murine iPSC by using a dual CRISPR/Cas9 knockout approach or by an inducible CRISPR interference (CRISPRi) approach in human iPSCs resulting in the targeted repression of Cyrano expression. In both instances, pluripotency of iPSCs was not affected by Cyrano-deficiency as analysed by qPCR, immunofluorescence staining and flow cytometry for key pluripotency factors (NANOG, SOX2, OCT4, TRA1-60, SSEA4) as well as by alkaline phosphatase staining. To further corroborate our results we are currently investigating the effects of Cyrano knockdown in human ESCs.

In further studies the knockout and knockdown iPSCs will allow us to investigate the role of Cyrano during the differentiation from pluripotency to the mesodermal lineage on one hand and its role in differentiated cardiomyocytes on the other hand.

14. In-vivo grafting of large engineered heart tissue patches for cardiac repair

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Introduction: Engineered heart tissue (EHT) strategies, by combining cells within a hydrogel matrix may overcome the limitations of intracoronary/myocardial cell delivery routes. EHTs regenerate heart muscle in small animal models but data regarding clinically relevant engineered heart tissue (EHT) patches large enough for first-in-human studies are lacking.

Methods: An upscaled EHT patch (approx. 3cm x 2cm x 1.5mm) consisting of up to 50 million human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) embedded in a fibrin based hydrogel was developed. A rabbit myocardial infarction model was then developed to test for feasibility and efficacy of EHT grafting.

Results: The patches began to beat spontaneously within 3 days of fabrication and after 28 days of dynamic culture (late EHTs) showed the development of several mature characteristics when compared to early patches (<14 days from fabrication). Late EHTs contained hiPSC-CMs which were more aligned; showed better contraction kinetics, and faster calcium transients. We then tested the EHT patch in-vivo using a rabbit model. Patches were applied to infarcted hearts (n=14 [n=7 EHT vs n=7 sham]). Sham operations used non-cellular fibrin patches. Blinded echocardiographic analysis revealed a significant improvement in function in infarcted hearts that underwent EHT patch grafting (n=7; absolute difference of 10.04 ± 3.1% over sham group; fractional area change, $P<0.01$). In-vivo telemetry recordings (n=5 MI/sham vs n=7 MI/EHT) indicated that no clinically relevant arrhythmia was seen in the MI / EHT group and arrhythmia provocation protocols (ex vivo

n=5 MI/sham vs n=6 MI/EHT) confirmed that the patch was not pro-arrhythmic (arrhythmia inducibility score 5.6 ± 1.0 [MI/patch] vs 5.0 ± 0.6 [MI/sham]; p=ns).
Conclusion: An upscaled clinically relevant EHT patch was developed and improved function in infarcted hearts without causing arrhythmia. Therefore EHT may have specific advantages over the direct intramyocardial injection of cells.

15. Intronic CRISPR repair in *LZTR1*-deficient iPSC-cardiomyocytes from Noonan syndrome patients rescues disease phenotype

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Noonan syndrome (NS) is a multisystemic developmental disorder characterized by its clinical variability with common symptoms such as typical facial dysmorphism, short stature, developmental delay and intellectual disability as well as congenital heart disease. The disease is causally linked to gain-of-function mutations in a number of genes leading to an increased signal transduction along the RAS-MAP kinase (MAPK) signaling pathway. However, our understanding of the pathophysiological alterations and mechanisms, especially of the associated cardiomyopathy, remains limited and effective therapeutic options are lacking. In this study, we present a family with two siblings displaying an autosomal recessive form of NS with severe hypertrophic cardiomyopathy caused by biallelic mutations within leucine zipper like transcription regulator 1 (*LZTR1*). Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) of the affected siblings recapitulated the hypertrophic phenotype and uncovered a causal link between LZTR1 dysfunction, RAS accumulation, RAS-MAPK signaling hyperactivity and cellular hypertrophy. Intronic CRISPR repair in the patients' iPSCs normalized RAS-MAPK signaling activity and cellular hypertrophy paving the way for personalized medical treatment.

16. Reprogramming enriches for somatic cell clones with small scale mutations in cancer-related genes

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Recent studies observed high mutational load in iPSCs, which is largely derived from their parental cells, but as yet it is unknown whether reprogramming may enrich for individual mutations that pre-exist in the parental cell population. We have derived 30 human iPSC clones from neonatal and aged individuals under comparable conditions. High accuracy exome and amplicon sequencing showed that all analysed small scale variants pre-existed in their parental cell population. We demonstrate that individual mutations present in small subpopulations of parental cells become enriched among iPSC clones during reprogramming. Evaluation of the variant impact and gene function in cellular processes and in cancer development imply a potential role of some of those mutations as putatively actionable driver mutations. Especially as some of the enriched, putatively actionable mutations affect genes of cell death / survival, cell cycle control, and pluripotency, somatic cells carrying such a mutation might experience a selective advantage during reprogramming. In view of the various common characteristics of (pluripotent) stem cells and cancer stem cells, the same mutations are likely to account for an increased tumor risk. Notable, on average, iPSCs of aged donors carry only an insignificantly higher number of these putatively actionable mutations. The reprogramming-associated selection for individual potentially pathogenic or carcinogenic mutations that have been acquired during lifetime may impact the clinical value of patient-derived iPSCs.

17. Bioprocess development for GMP compliant production of human pluripotent stem cell-derived cardiomyocytes

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The development of regenerative cell therapies for heart failure requires the production of billions of human cardiomyocytes (CMs). In order to produce sufficient numbers of CMs, human pluripotent stem cells (hPSCs) can be used as the initial cell source. Recent work enabled the expansion and differentiation of hPSCs as cell-only aggregates in suspension culture, thus facilitating the process scale-up into instrumented stirred tank bioreactors (STBR). However, to apply hPSC-derived CMs for cell therapy in humans, several regulations need to be followed to ensure safety and efficacy of the treatment. One part of this regulation is the so-called “Good manufacturing practice” (GMP) for the production of drugs and therapies.

In comparison to 2D culture, bioreactors show the advantage of scalability and GMP-compliance. In industrial biotechnology, STBR are already well-established to produce high quantity and quality of bacterial, mammalian or yeast cells.

In this project, process modification and optimization is performed to enable GMP-compliant production of cardiomyocytes in STBR. After expansion of hPSCs as cell-only aggregates, directed cardiomyogenic and chemically defined differentiation using Wnt-modulators (CHIR99201 and IWP-2) is initiated. These modulators induce mesoderm formation and commitment to the cardiac lineage.

After the successful CM differentiation in a 150 ml process volume (DASBox®), we were able to up-scale the production process to a 400 ml scale (Bioblock®).

The successful up-scaling proves the robustness of our cardiomyogenic differentiation protocol. Although further process and handling optimization is necessary to ensure full GMP-compliance and to eventually develop a safe and efficient cell therapy for heart-failure patients, we here provide the first step towards a GMP-compatible platform.

18. Primary carnitine deficiency: Disease modelling with CRISPR/Cas9-edited hiPSC-CM in engineered heart tissues

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Primary carnitine deficiency (PCD) is an autosomal recessive monogenic disease due to mutations in the plasmalemmal carnitine transporter gene SLC22A5, encoding for the protein OCTN2. Untreated PCD patients suffer from muscular weakness, urinary carnitine loss and dilated cardiomyopathy and life-long carnitine supplementation is the treatment of choice. The cardiac phenotype is related to the alteration of energy substrate metabolism: OCTN2 mutations lead to reduced cellular uptake carnitine and in consequence generation and mitochondrial transfer of acylcarnitine is reduced.

The mechanism of cardiomyocyte toxicity mediated by OCTN2 mutations in the initial phase of the disease is not clear. Two aspects are considered relevant: Reduced cytoplasmic and thereby mitochondrial transport of acylcarnitine leads to decreased energy reserve (mitochondrial lipid deficiency) and a reduced fatty acid metabolism results in cytosolic lipid excess. The aim of this study is to establish a predictive PCD model utilizing human induced pluripotent stem cell- derived cardiomyocytes (hiPSC-CMs).

To achieve this, control hiPSC lines were genome edited by CRISPR/Cas9 technology. The missense mutation c.95A>G (p.N32S) was introduced homozygously and heterozygously (OCTN2 p.N32Shet,hom), OCTN2 was knocked out by deleting a 17kb large fragment in the SLC22A5 gene (OCTN2-/-).

Cardiomyocytes were differentiated with an embryoid body- and growth factor-based three-stage protocol. The human iPSC-CM-based engineered heart tissue (EHT) technology served as a three-dimensional disease model to demonstrate a PCD phenotype in vitro. Here, both PCD disease lines, OCTN2-/- and OCTN2 p.N32Shom, showed significantly lower forces and significant higher relaxation time after 3 weeks compared to the isogenic OCTN2+/+ control.

Further experiments are necessary to improve mechanistic understanding. To this end defined fatty acid-based media conditions will be evaluated to provoke a PCD phenotype. Furthermore analysis of mitochondrial status, ceramide accumulation, autophagy and apoptosis in hiPSC-CM will be employed.

19. Rescue of drug-induced long QT syndrome type 2 using a HERG channel activator in human pluripotent stem cell-derived cardiomyocytes

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Introduction: An important step in drug development is the evaluation of cardiac toxicity. Candidate drugs must have minimal effects on the HERG current that is essential for cardiac repolarization. Drugs that block the HERG channel can prolong the QT interval leading to lethal ventricular arrhythmias. The HERG channel is prone to promiscuous interactions with drugs due to easy access to the channel pore. HERG channel activators can shorten repolarization in human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs). One such activator, Ginsenoside, was recently shown to interact with the voltage-sensing domain of the HERG channel to stabilize the activated state. Ginsenoside may change the conformation of the open channel and potentially limit subsequent drug blockade. Here we report that Ginsenoside can rescue long QT Syndrome type 2 (LQTS2) induced by E-4031 in hPSC-CMs.

Hypothesis: E-4031-induced LQTS2 can be rescued by Ginsenoside in hPSC-CMs.

Methods: A hPSC line was differentiated into cardiomyocytes using an optimized cardiomyocyte differentiation media and protocol. Excitability of hPSC-CMs was assayed between days 22 and 25 in the presence of 10 nM E-4031 with and without 10 µM Ginsenoside.

Results: Differentiation of the hPSC line produced a population of beating cardiomyocytes with >80% cTnT-positive cells. A shift in gene expression to ventricle-like cardiomyocytes from day 15 to day 30 was observed (*Myl2*/*Myl7* = 14.2 at day 30). This level of expression was similar to that in adult human primary ventricular cardiomyocytes (*Myl2*/*Myl7* = 9.8). Electrophysiological experiments were performed between days 22 and 25 on hPSC-CMs. Application of 10 nM E-4031 for 10 minutes prolonged the field potential duration (FPD) and reduced the repolarization signal amplitude. The sequential addition of 10 µM Ginsenoside for 10 minutes shortened the FPD and increased the repolarization signal amplitude. Ginsenoside restored cardiomyocyte excitability similar to pre-treatment with E-4031.

Conclusion: Ginsenoside rescued drug-induced LQTS2 in hPSC-CMs, suggesting that HERG activators which target the voltage-sensing domain may be used to offset cardiac safety issues of promising candidate drugs.

20. hPSCreg's clinical study database for hPSC-derived cell therapies

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Due to the generation of human pluripotent stem cell (hPSC) lines at multiple sites, including core facilities, individual research laboratories, biobanks, etc., there is a high degree of variability in the availability of information on these hPSC lines, e.g. origin and degree of characterization. To make information on hPSC lines FAIR (Findable, Accessible, Interoperable and Re-usable), the Human Pluripotent Stem Cell Registry (hPSCreg; https://hpscreg.eu) collects and provides a wide range of data on hPSC lines in a standardized format, including ethical provenance, evidence of pluripotency, derivation conditions and characterization details, and genetic constitution. To also monitor the increasing use of hPSC lines as a starting point for cell replacement therapies, hPSCreg has created a clinical study database specifically for clinical applications of hPSC-derived cells (https://hpscreg.eu/browse/trials). This registry aims at providing an overview on hPSC based clinical trials performed worldwide. It is based on actual information collected from a large number of sources and is updated on a regular basis. The hPSCreg clinical study registry is unique with respect to comprehensiveness and actuality of information on hPSC-based clinical studie

21. Advanced high density cultivation of human pluripotent stem cells by feedback-control in stirred bioreactors

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Human pluripotent stem cells (hPSCs) present a unique cell source for the production of functional human cell types, fueling the development of advanced *in vitro* disease models and future regenerative therapies. Most applications will require the constant supply of billions of cells generated by high density culture strategies via robust and economically viable bioprocesses. Here we describe a rational and model-based process development that enables hPSC expansion at high density in matrix free cell-only aggregate suspension culture in stirred tank bioreactors (STBRs).

Firstly, we performed systematic analysis of process bottlenecks based on the online monitoring technologies implemented in the bioreactor platform. Next, building on the previously established perfusion-based cell feeding, feedback-based control of defined pH and dissolved oxygen (DO) levels was established.

To tackle another issue that is the viable cell loss after single cell-based process inoculation, preculture conditions were improved and standardized. Other important aspects include aggregate size control combined with shear-protective agents. Moreover, new feeding strategies were established including extra glucose supplementation to comply with the rampant metabolic requirement of exponentially proliferating pluripotent cells.

Combination of our knowledge on all the bottlenecks mentioned above enabled us to build an *in silico* model providing the basis for more rational process optimization at reduced experimental costs.

By taking full advantage of the parallel, multifactorial process control abilities of the STBR system and the model based *in silico* process development, an unmatched cell density of 3.5 x 107 cells/mL was achieved, enabling the production of 5.25 billion pluripotent hPSCs within 7 days of process duration in 150 mL culture scale.

Together, the work confirms the role of hypothesized and reveals previously unknown parameters limiting hPSC cultivation. Moreover, the study provides systematic strategies to overcome the identified hurdles. In consequence, a new level of high density cultivation of hPSC is achieved, allowing for more reproducible and efficient cell production in smaller culture scale and at lower costs, thereby fueling industrial and clinical applications of hPSCs and their progenies.

22. Caring for your cardiomyocytes: methods for hPSC-CM handling and functional analysis

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Human pluripotent stem cell (hPSC)-cardiomyocytes (CMs) are becoming accepted as one of the available tools for disease modelling, drug screening and toxicology studies. In these applications, hPSC-CMs serve as the best available *in vitro* model, however they are still limited in several aspects. They do not completely recapitulate adult human CMs in terms of maturity and function, plus they are low proliferating, functionally delicate, and in some respects difficult to engraft or even handle in culture.

Current standards and techniques for hPSC-CM application are advancing. Simple workflow, quality control, and banking of hPSC-CMs are crucial for their usability. We highlight some key aspects relating to these. Furthermore, for both manipulation and analysis of hPSC-CMs, we investigate mRNA as a gentle and efficient means to deliver genetic tools. We show CRISPR/Cas9 InDel formation at several loci, as well as bright optical readout from voltage and calcium sensors, following mRNA transfection. With this combinatorial approach we hope to facilitate the application of hPSC-CMs towards translational research.

23. Enhancement of human pluripotent stem cell differentiation by environmental cues

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A multiplicity of bio-/chemical signals and physical cues guide the spatio-temporal orchestration of induced pluripotent stem cell (iPSCs) proliferation and lineage specification. Here, we investigated specific environmental cues during iPSC cultivation and differentiation into cardiomyocytes (iPS-CM), comparing the culture substrates matrigel and laminin as well as 2D vs. 3D culture conditions.

Human iPSCs were propagated under serum-free conditions on laminin vs. matrigel. Embryonic bodies were generated under low attachment conditions before cardiosphere specification. During differentiation no morphological differences were observed at 3D, while monolayer CM specification resulted in a “spider-web”-like structure on laminin and circlular structured islands on matrigel. At day 9, the cardiac lineage phenotype identity was determined by flow cytometry, showing troponin I+ and actinin+ cell populations with a purity of up to 75 %. Visual monitoring showed significantly enhanced beat frequencies ($p<0.05$; $n=3$) in 3D cardiospheres initially expanded on matrigel compared to laminin. Moreover, the beating rates of 3D cardiospheres were significantly increased compared to monolayer iPS-CM ($p<0.05$; $n=3$). In order to predict the transplantability, the procoagulatory function was determined by using a clotting assay. Results indicated that 3D cardiospheres, reveal an increased hemocompatibility capacity when initially expanded on laminin compared to matrigel.

The present data show effects of matrix instructions during iPSC proliferation resulting in functional changes during subsequent 3D cardiosphere formation despite absence of the respective matrix. Future studies will focus on the identification of the role of matrix components on additional stem cell properties.

24. Preparation and banking of universal donor human iPSC lines as a starting material for the manufacturing of ATMP

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The use of human induced pluripotent stem cells (iPSCs) as a starting material for the manufacture of novel Advanced Therapy Medicinal Products (ATMPs) available for clinical applications, such as pancreatic islet beta cells, necessitates the creation of human iPSC lines manufactured in compliance with governmental directives and regulations. Defining the processes of iPSC manufacturing will furthermore create the basis for production of patient-derived iPSCs for autologous cell therapies. The goal of our project is to create standardized processes and a well-qualified collection of cGMP-grade human iPSC lines derived from healthy donors that harbor a unique repertoire of immunoregulatory molecules. Based on more than 70.000 donors from the German Red Cross Blutspendedienst Nord-Ost, we calculated that iPSC lines from only 3 donors with most prevalent homozygous HLA-class-I haplotypes, termed “universal donors”, will be sufficient to provide transplantation tolerance in more than 30% of the German population and around 50-75 millions of individuals of Western Europe decent. We developed a process that begins with the derivation of primary fibroblasts from skin punch biopsies of the most suitable, healthy “universal donors”. The reprogramming process is based on transient transfection of synthetic RNA transcripts (mmRNA) that encode for pluripotency factors, which we believe represents the safest current methodology to derive iPSC lines. We aim to produce human iPSC lines in compliance with current regulations of medicinal products and GMP standards, in order to use them as starting material for the production of ATMPs in a variety of medical applications.

25. Human induced pluripotent stem cell-derived cardiomyocytes integration and maturation studied using living myocardial slices

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Pluripotent stem cell-derived cardiomyocytes can remuscularize the heart and integrate in the host myocardium; however, their immature phenotype and autonomous pacemaker activity can lead to undesired events of premature ventricular contractions, ventricular tachycardia or arrhythmias. The mechanisms by which injected cells integrate and synchronize within the host myocardium are unknown. A better understanding is essential to improve this translational approach and advance cardiac cell therapy research. Current in vivo studies can provide a general insight into cell integration, however a direct visual and functional assessment at a cellular/subcellular level is technically challenging. Here living myocardial slices were used to investigate hiPSC-CMs integration and synchronization in rabbit myocardium. hiPSC-CMs, cultured onto myocardial slices for 48 hours, could adhere, integrate and beat synchronously with the surrounding myocardium. The cells also started to acquire the orientation of the host muscle fibres (2 fold coherence increase, $p<.001$). Alterations in structural, functional and energetic gene sets were observed in association with hiPSC-CMs improved calcium handling, suggesting cellular maturation. Our data demonstrate that hiPSC-CMs integration can be investigated at cellular/subcellular level using living myocardial slices and that the environment and the relentless beating of the surrounding myocardium can alter cell behaviour and promote integration, maturation and synchronization.

26. Utilizing TGFBR1 mutated and corrected hiPSC lines for disease modelling of Loeys-Dietz Syndrome

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Loeys-Dietz Syndrome (LDS) is a connective tissue disorder that predisposes patients to premature death due to aggressive aneurysms, dissections, and rupture of vasculature. There is no known cure and a lack of full understanding of etiology. The emergence of hiPSCs for disease modelling provides a virtually limitless source of autologous cells, which allows insight into the pathophysiology of human diseases. This research project is set out to model LDS. It aims to recapitulate the disease phenotype in vitro through the differentiation of LDS patient-derived iPSCs into endothelial cells (ECs), smooth muscle cells (SMCs), and fibroblasts, which are key players of the vascular network.

CD34+ cells from three LDS Type I patients were reprogrammed into iPSCs and characterized concerning pluripotency marker expression, in vitro differentiation potential, and genomic stability. LDS-Patient iPSCs have been successfully differentiated into ECs through a mesodermal intermediate stage, with high differentiation efficiencies. In addition, LDS-Patient ECs express typical EC markers, form networks on a matrix, and exhibits sheet migration. With a few modifications, this protocol was able to generate SMC-like cells as shown by expression of known SMC genes. These cells can support ECs in the formation of networks on a matrix and contract slightly upon stimulation with carbachol. LDS-Patient iPSCs were differentiated into fibroblasts by activation of epithelial-to-mesenchymal transition during mesoderm induction. The current protocol generates CD44+/CD90+/CD73+ fibroblastlike cells that are capable of expansion on plastic.

Utilization of LDS-hiPSC derived vascular cell types can serve as a basis for disease modelling purposes. Recapitulating the vasculature in vitro can offer a robust platform for developing LDS-patient specific stem cell based therapies. Furthermore, the mutation in the TGFBR1 gene will be corrected via CRISPR-Cas9 to further understanding of the disease mechanism.

27. Generation, cultivation and characterization of a large scale stem cell-derived bio-artificial cardiac tissue

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Damages to cardiac tissue due to myocardial infarction (MI) result in massive loss of cardiomyocytes (CMs) and generation of a non-contractile scar tissue that seriously compromises the contractile strength and functionality of the heart.

Bio-artificial cardiac tissue (BCT) represents a promising therapeutic option to replace damaged myocardium. BCT is a 3D structure resembling the cardiac microarchitecture by combining human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) with supporting cell types and matrices such as fibroblasts and hydrogels, respectively. Here we describe a protocol to generate and characterize BCTs of clinically relevant size implemented in a custom-made bioreactor. The system aims to recapitulate the complex interplay of biochemical, mechanical and electrical stimuli necessary for proper tissue development and maturation.

The protocol for small BCT formation1 was adapted for a custom-made tissue chamber to generate a 43 mm diameter patterned tissue using optimized parameters such as cell/matrix ratio and cell density. The active remodeling of the matrix by the resident cells to increase intercellular connection was optically monitored during the tissue culture, revealing a progressive reduction up to ~27% of the initial area after 15 days. After 15 days of culture the BCT exhibited spontaneous macroscopic contractions, which were video-optically recorded and evaluated, showing an estimated beating rate of 102 bpm.

Maturation of CMs within the tissue was confirmed by immunofluorescence staining showing cross-striations (sarcomeric α-actinin, cTnT, titin), gap junctions (Cx 43) and adherent junctions (N-Cadherin).

The integration of the tissue chamber in the novel custom-made bioreactor allowed investigating the initial parameters for electromechanical stimulation (such as pressure, stress amplitude, frequency) necessary for proper tissue maturation in a more physiological manner.

The next steps include the optimization of the tissue culture conditions within the bioreactor, as well as evaluation the electrophysiological properties of the tissue and possible vascularization strategies for future therapeutic purposes.

1. Kensah G, et al. 10.1093/eurheartj/ehs349.

28. Strategies for the expansion of human induced pluripotent stem cells as aggregates in single-use Vertical-WheelTM bioreactors

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Human induced pluripotent stem cells (hiPSCs) have been regarded as an enormous breakthrough for medicine, due to their ability to generate virtually all types of cells in the human body. One of the great bottlenecks in the usage of these cells for Regenerative Medicine applications is their expansion to clinically-relevant quantities. The Vertical-Wheel bioreactors, by PBS Biotech, present a novel configuration, whose vertical agitation allows for homogeneous mixing conditions inside the vessel, while conveying less shear stress to the cells when compared to traditional alternatives.

This work reports the expansion of hiPSCs as aggregates in the Vertical-Wheel bioreactors, while also demonstrating different strategies to increase the performance of the culture and allowing for the harvest of higher cell numbers. Cultures were performed in the PBS MINI 0.1 bioreactor with 60 mL of working volume. Two different culture media were tested, mTeSR1 and mTeSR3D, in a repeated batch or fed-batch mode, respectively, as well as dextran sulfate (DS) supplementation. mTeSR3D was shown to sustain hiPSC expansion, although with lower maximum cell density than mTeSR1. DS supplementation led to an increase in 97% and 106% in maximum cell number when using mTeSR1 or mTeSR3D, respectively. A maximum cell density of (2.3±0.2)×106 cells·mL–1 and a volumetric productivity of (4.6±0.3)×105 cells·mL–1·d–1 were obtained after 5 days with mTeSR1+DS, resulting in aggregates with an average diameter of 346±11 µm. The generated hiPSCs were analysed by flow cytometry and qRT-PCR and their differentiation potential was assayed, revealing the maintenance of their pluripotency after expansion.

The results here described present the Vertical-Wheel bioreactor as a promising technology for hiPSC bioprocessing. The specific characteristics of this bioreactor, namely in terms of the innovative agitation mechanism, can make it an important system in the development of hiPSC-derived products under current Good Manufacturing Practices.

29. A roadmap to manufacturing immuneoptimized clinical grade induced pluripotent stem cells as a starting material for advanced therapy medicinal products

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The manufacturing of human induced pluripotent stem (iPS) cell lines according to clinical standards is important for autologous and allogeneic regenerative therapies globally. The cellular therapy sector is growing rapidly and only in the last quarter received an investment of over one billion US for company creation specialized in ATMP development. Despite the hype, there is little information regarding suitability of iPS cell lines that are being manufactured in GMP facilities in order to receive authorizations to be used as a “starting material” in production of differentiated cells for human therapies.
We have created a roadmap, prototyped and are awating regulatory approvals to manufacture autologous and allogeneic iPS cells as a starting material for manufacturing of differentiated cells for clinical applications. The unique features of our manufacturing process are 1. the use of fibroblasts as a starting material, as opposed to blood mononuclear cells by other programs, meaning that our cells will fit better neuronal differentiation; 2. the use of mRNAs to transiently express reprogramming factors, as opposed to use of viruses by other programs, which creates high risk; and 3. we selected several donors that are collectively immunologically matched to several millions of European individuals.
Within our talk, we will explain the principles of risk assessment that guide the qualification of reagents, instruments, and processes for the manufacturing of clinical grade human iPS cells under GMP standards. We will furthermore illustrate the criteria that underlie healthy donor selection and tissue procurement for the generation of human iPS cells that can be used as starting material in cellular therapies.

30. Chemically-defined, xeno-free, scalable production of hPSC-derived definitive endoderm aggregates with multi-lineage differentiation potential

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For the production and bio-banking of differentiated derivatives from human pluripotent stem cells (hPSCs) in large quantities for drug screening and cellular therapies, well-defined and robust procedures for differentiation and cryopreservation are required. Definitive endoderm (DE) gives rise to respiratory and digestive epithelium, as well as thyroid, thymus, liver and pancreas. Here we present a scalable, universal process for the generation of DE from human induced pluripotent stem cells (hiPSCs) and embryonic stem cells (hESCs). Optimal control during the differentiation process was attained in chemically-defined and xeno-free suspension culture and high flexibility of the workflow was achieved by the introduction of an efficient cryopreservation step at the end DE differentiation. DE aggregates were capable of differentiating into hepatic-like, pancreatic, intestinal, and lung progenitor cells. Scale-up of the differentiation process using stirred-tank bioreactors enabled production of large quantities of DE aggregates. This process provides a useful advance for versatile applications of DE lineages, in particular for cell therapies and drug screening.

31. Generation of a blood brain barrier spheroid model using human induced pluripotent stem cell derived endothelial cells

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Introduction: A variety of blood-brain barrier (BBB) *in vitro* models have been developed over the years (Avdeef, Deli, Neuhaus, 2015). Until now, in human BBB models, based on primary or immortalized human cells, the desired barrier properties can´t be achieved. The differentiation of human induced pluripotent stem cells (hiPSC) into brain capillary endothelial cells (BCEC) is a promising approach to achieve a BBB model with *in vivo* like barrier properties (Lippmann et al., 2012, 2014). Transwell BBB models are in use because of the simplicity in generation. However, a major drawback of these transwell co culture models is the lack of direct cellular interactions, only indirect effect of astrocytes and pericytes on BCECs can be explored. It is known, that in 2D models, endothelial cells undergo a phenotypic drift, including loss of functionality. Urich et al. and Cho et al. have shown that a 3D BBB spheroid model can be generated spontaneously using immortalized or primary cells (Urich et al., 2017, Cho et al., 2013). Thus, we focus on an advanced 3D BBB model to reflect the physiological interplay and cellular interaction of cells from the neurovascular unit.

Methods: We previously established a protocol to generate a 2D transwell BBB model with hiPSCderived BCECs, primary pericytes and astrocytes (Appelt-Menzel et al., 2017). Here, we explore the possibility of using hiPSC derived BCECs, primary astrocytes and primary pericytes for the development of an advanced 3D spheroid model of the BBB. We characterized the structure and spheroid formation via optimal medium selection, histology, immunofluorescence and gene expression.

Results: The three cell types can self-assemble and form spheroids with an outer layer of BCECs.

Conclusions: The generation of hiPSC-based BBB spheroids is a technology characterized by a high novelty grade and could offer the potential for disease and infection modelling as well as pharmaceutical studies.

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32. Development of novel treatment strategies for SGCE-myoclonus dystonia using patient-derived induced pluripotent stem cells (iPSCs)

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Myoclonus dystonia is a combined dystonia characterized by myoclonic-like appearance of fast dystonic movements. Bilateral, alcohol-sensitive myoclonic jerks predominate in arms and axial muscles and can be accompanied by psychiatric disorders. Myoclonus dystonia (DYT-SGCE, formerly DYT11) is an autosomal dominant disorder caused by mutations in the gene encoding ε-sarcoglycan (*SGCE)* leading to a dysfunction of a widely expressed transmembrane protein. Generally, it is suggested that alterations in the GABAergic system lead to dysfunction of the cerebello-thalamic pathway and impaired striatal plasticity. There is currently no specific treatment for myoclonus dystonia and symptomatic medications usually have limited efficacy. To investigate pathophysiological mechanisms, iPSC-derived medium spiny neurons from two patients carrying a heterozygous mutation in the gene *SGCE* (SGCE,c.298T>G or SGCE,c.304C>T) are functionally investigated to explore an associated neuronal phenotype. The directed differentiation of striatal medium spiny neurons (MSNs) is accomplished using double SMAD and WNT inhibition. The expression of β-III tubulin (TUJ1), gamma-aminobutyric acid (GABA), dopamine- and cAMP-regulated neuronal phosphoprotein 32 (DARPP32) and transcription factor BAF chromatin remodeling complex subunit BCL11B (CTIP2) is analyzed in mature MSNs. After 70 days of differentiation, a large population of GABAergic neurons (78% GABA+ cells from neurons expressing TUJ1) and DARPP32-positive striatal MSNs (about 35% DARPP32+ cells expressing GABA) was generated. Electrophysiological analysis using calcium imaging and whole-cell patch-clamp recordings are used to analyze neuronal function as well as synaptic activity to identify disease-relevant phenotypes linked to myoclonus dystonia compared to MSNs derived from 3 healthy controls. Furthermore, the expression of GABA receptor subunits in MSNs is evaluated by quantitative real-time PCR. The *in vitro* modelling of myoclonus dystonia using iPSC-derived medium spiny neurons can contribute to identify potential drug targets for novel therapeutic strategies in dystonia with *SGCE* mutations.

33. The impact of non-myocytes on the electromechanical properties of hPSC-derived bioartificial cardiac tissue

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Currently, one the most critical aspects of cardiac tissue engineering is the maturity status of human pluripotent stem cell (hPSC)-derived cardiomyocytes (CM). In the heart, CMs are coupled to capillary endothelial cells (EC), mural cells (e.g. pericytes) and fibroblasts (Fb) promoting structural and electrophysiological tissue maturation as well as vascular network formation. Here, an in vitro model is shown for the investigation of the role of ECs, pericytes (PC) and different Fb sources in hPSC-derived bioartificial cardiac tissue (BCT) formation and function.

The hPSC-based CMs (αMHC+) [1], ECs (CD31+) [2], and PCs (PDGFRβ+) were differentiated, purified via genetic or surface-marker based selection, and characterized for cell-type specific marker expression and function. The obtained hPSC-PCs showed a similar gene expression profile to primary PCs and Fbs, and their angiogenic potential was demonstrated in a co-culture assay with hPSC-ECs.

Differentiated hPSC-PCs were used with or without hPSC-ECs to generate BCTs and to address their effect on tissue morphology and electromechanical parameters compared to control tissues containing primary dermal or cardiac Fbs. Addition of hPSC-ECs resulted in spontaneously formed and maintained endothelial network structures, which were distributed throughout the BCTs. Interestingly, tissues with hPSC-PCs exhibited similar tissue remodeling compared to both controls, but more organized sarcomere structures as well as improved longitudinal cell- and extracellular matrix orientation. Moreover, electrophysiological properties of the PC and cardiac Fb-containing BCTs were alike.

Due to their dual ability in supporting EC function and matrix remodeling, hPSC-PCs together with hPSC-derived CMs and ECs, represent a promising cell combination for future myocardial tissue replacement therapy. Additionally, tri-culture BCTs could also be used as a screening platform to address complex effects on multiple cell types.

1. Halloin, C., et al., *Continuous WNT Control Enables Advanced hPSC Cardiac Processing and Prognostic Surface Marker Identification in Chemically Defined Suspension Culture*. Stem Cell Reports, 2019. **13**(2): p. 366-379.
2. Olmer, R., et al., *Differentiation of Human Pluripotent Stem Cells into Functional Endothelial Cells in Scalable Suspension Culture.* Stem Cell Reports, 2018. **10**(5): p. 1657-1672.

34. Engineered heart tissues: Systematic assessment of batch- and clone-related variability

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The potential of human induced pluripotent stem cells (hiPSC) in disease modeling approaches might be limited by technical variability occurring on the way from reprogramming to phenotypic readouts. To address this, we performed a prospective, standard operating procedure-controlled assessment of the degree and sources of variability of hiPSC-cardiomyocytes (CM). We generated >500 3D engineered heart tissues (EHT) with 1 Million hiPSC-CM each, derived from 27 differentiation batches representing 9 clones of 3 healthy donors. Low quality of hiPSC influenced all further processes including cardiac differentiation. Functional characterization of EHT with high-quality hiPSC showed low variability with a reliability of baseline contractility measurements (coefficient of variation, CV: 0.29) that compares well with established cardiovascular disease models (CV: 0.23 – 0.82). Analysis of the proportion of variance showed a dominance of variability between batches (30%) over donor (9%) and clone (11%) related variance. The data argues for technical repetition on batch rather than clone level and underscores the potential of hiPSC and EHT as a reliable model in cardiovascular research.

35. Generation of disease-specific BMPR2mutated iPSCs and development of transgenic reporter cell lines as tools for pulmonary hypertension disease modelling and drug discovery

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Introduction: Bone morphogenetic protein receptor 2 (BMPR2) mutations resulting in reduced transcription of genes linked to proliferation and apoptosis in endothelial cells (ECs), such as ID-1 (inhibitor of differentiation), occur frequently in pulmonary hypertension (PH). Despite molecular insights from studies mainly using primary cells or immortalized cell lines, underlying mechanisms of PH remain unclear. Induced pluripotent stem cells (iPSCs) can proliferate and differentiate into cell types of all three germ layers, offering valuable source of disease-relevant cells. This project aims at generating ECs from PH patient-specific iPSCs harbouring BMPR2 mutations as an in vitro disease model to elucidate the role of EC malfunction in PH and for drug screening.

Methods: PH patient-specific iPSCs were generated from CD34pos cells using sendai virus reprogramming vectors. BMPR2mutated iPSCs were differentiated into ECs using growth factors (BMP4/VEGF). Viability, function and expression of ID-1 after BMP4 stimulation were assessed.

Results: PH patient-specific iPSCs expressed pluripotency markers and randomly differentiated in vitro into cell types of all three germ layers. Differentiated ECs expressed CD31, VE-Cadherin, and vonWillebrand factor. Compared to controls, BMPR2mutated ECs showed impaired network formation on Matrigel and delayed migration into scratch wounds, suggesting impaired angiogenic ability. Moreover, the cells were prone to apoptosis with higher Caspase3/7 activity. Furthermore, cellular dehydrogenase activity was lower in BMPR2mutated ECs implying slower proliferation or lower metabolic activity. Immunofluorescence staining revealed BMPR2 localised to the cell membrane in primary ECs but mainly nuclear in iPS-derived ECs. Interestingly, however, BMPR2 was located in the Golgi complex in one patient with deletion in BMPR2 gene exon1 (extracellular domain), indicating abnormal cellular trafficking of the mutated protein. QRT-PCR showed expression of ID-1 was lower in ECs from the same patient, suggesting reduced BMP/Smad signalling. In conclusion, BMPR2mutated ECs from PH patient-specific iPSCs could be a valuable tool for PH disease modelling.

36. Patient-specific stem cell-derived cardiomyocytes with β-myosin mutation Arg723Gly show altered function and morphology, typical for hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is the most frequent inherited cardiac disease. Even so, the pathomechanism leading to HCM is still not sufficiently explored. Heart muscle tissue from HCM patients is hard to obtain. Therefore, cardiomyocytes differentiated from HCMpatient-derived induced pluripotent stem cells (hiPSC-CMs) appear attractive as a model to investigate pathomechanisms and direct effects of HCM mutations. More than one third of mutation-positive HCM-patients have a mutation located in the β-cardiac myosin heavy chain protein (β-MyHC).

Here we asked if patient-derived hiPSC-CMs carrying the HCM-relevant β-MyHC mutation Arg723Gly reflect functional and morphological alterations typical for HCM. To approach this question, we characterized the impact of mutation Arg723Gly on electrically evoked twitch contractions, intracellular calcium transients and morphology by comparing hiPSC-CMs carrying the mutation (MT) with wildtype hiPSC-CMs without mutation (WT). To exclude unspecific effects, only hiPSC-CMs expressing exclusively the β-isoform of MyHC were investigated.

Analysis of twitch contractions showed larger contraction amplitude and significantly longer time to peak and half relaxation time of twitch for MT in comparison to WT. Also, intracellular calcium transients had longer time courses for MT than for WT. Notably, for some MT-CM batches arrhythmic calcium transients in up to ~ 20 % of the CMs were found, which may be related to the often observed ventricular tachycardias in the patient's family. Morphologically, MT-CMs exhibited a significant larger cell area under basal conditions and even further growth under stimulation with hypertrophic agonist isoprenaline than WT. Hence, HCM-patient-derived hiPSC-CMs show many of the phenotype traits typical for HCM heart tissue and might serve as a promising model for further exploration of HCM mechanisms.

37. Chemical defined, biomimetic matrices for serum-free culture of MSC and beyond

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The natural cellular environment is a complex interplay of extracellular matrix (ECM) components such as glycosaminoglycans (GAGs) and proteoglycans, soluble factors and cell-cell interactions. Current matrices facilitating *in vitro* culture of stem cells are either poorly defined ECM extracts or single component polymers that lack the natural complexity. We have developed a library of selfassembling matrices that are suitable for expansion of stem cells for therapeutic applications. By combining GAGs with biofunctional peptides, these matrices present multiple essential cues of the natural ECM in a chemically defined manner. Our technology enables a screening approach to identify the relevant composition for the cell type of choice. Screening the material library led to a candidate coating supporting growth of mesenchymal stromal cells (MSC) in the absence of serum. Long-term culture studies showed enhanced cell proliferation in animal-free media for up to 40 population doublings. We further identified another matrix that facilitates the maintenance and proliferation of induced pluripotent stem cells (iPSC). iPSC growth was studied and maintained for 3 passages while preserving their differentiation capacity. After neuronal induction of iPSC-derived neural precursor cells, the maturating neurons switched their biomatrix preferences. Analysis of the differentiation efficiency provided evidence that the incorporated GAGs supported neuronal development. Stability tests on FGF2 demonstrated that GAG-containing matrices maintain FGF2 activity for up to 3 days. Thus, the presented matrices provide optimal conditions for the expansion and differentiation of adult and induced stem cells. The developed matrices are biologically relevant, modular, chemically defined and scalable and thus enable defined cell culture protocols for stem cell research, drug-development and cell therapy applications.

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38. hiPSC-derived POis: generating Pancreatic islet Organoids with in vivo-like characteristics

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Diabetes mellitus is a chronic metabolic disease characterized by increased blood glucose levels resulting from insufficient insulin responses due to the destruction or dysfunction of pancreatic β-cells residing within the pancreatic islet. The transplantation of islets represents a promising therapy but major drawbacks comprising donor shortage or loss of the natural islet niche account for limited graft survival rates.

Islet survival and function intensely relies on the interplay of all cell types including not only hormone-releasing cells but also other components such as the vascular and/or the nervous system of the islet. As the pancreatic islet is at the center of islet transplantation success, we hypothesize that bioartificial hiPSC-based substitutes have to closely resemble native islet characteristics. We therefore aim to develop pancreatic islet organoids from hiPSCs with in vivo-like characteristics regarding islet size, structure and cellular composition combining endocrine islet cells with e.g. a vascular and neuronal system. For the derivation of islet-specific cell types, we apply state-of-the-art differentiation protocols that allow islet organoid formation. Generated islet organoids are characterized by gene and protein expression profiling but also functionally by studying their hormone-releasing capacities. To enable standardized up scaling of hiPSC maintenance culture and differentiation, we recently developed a hiPSC-specific suspension bioreactor platform with an on board microscopy unit that allows live monitoring of hiPSC and organoid culture.

Future work aims to establish the long-term culture of islet organoids in suspension bioreactors to improve the maturity of cells, one drawback of current 2D-differentiated hiPSC-derived pancreatic cells. Furthermore, the development of GMP-graded islet organoids based on GMP-qualified hiPSC cell lines and differentiation protocols will serve as platform for standardized ATMP development for clinical translation in future.

39. Vascular constructs using human pluripotent stem cells in the therapy of peripheral arterial disease

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Introduction: Tissue engineering has emerged as a promising alternative for vascular grafts. The aim of this study was to determine the feasibility and safety of tissue-engineered vascular grafts, obtained using canine decellularized aortae reseeded with human induced pluripotent stem cell-derived endothelial cells (hiPSC-EC) in a large animal model.
Methods: In the present work, we compared the expression of endothelial markers in 2D hiPSC-EC cultures maintained under either static or continuos dynamic cell culture conditions, using the Alvetex multi-welled system. Human iPSC-EC were used to reseed decellularised canine aortic tissues. Generated tissue-engineered grafts were implanted in a clinically relevant large animal model of abdominal aortic replacement for 1 week (n = 4). To demonstrate cell retention in vivo, we performed endoscopic analysis, and evaluated size and patency of the constructs by colour Doppler ultrasound and CT angiography. Finally, vascular grafts were collected for gene expression analyses and histological / immunohistochemical examinations ex vivo.
Results: Our results demonstrated that hiPSC-EC cultures responded to dynamic circulation and continuos perfusion of medium for 1 week in 2D, by increasing the expression of endothelial markers, such as CD31 and VE-cadherin (6 and 5 times upregulated, respectively; $p \leq 0.05$), and YAP (downstream effector of the Hippo pathway, 9 times upregulated; $p \leq 0.001$), as compared to cells maintained in static conditions. Human iPSC-EC was able to recellularise 3D vascular decellularised biomatrices and thereby it developed a mature, functional phenotype (e.g. antiplatelet and vasoactive effects) in vitro. hiPSC-EC cultured on 3D decellularised vascular biomatrices showed increased expression of arterial, venous, common endothelial marker genes and angiogenesis-related proteins (e.g. angiopoietins, endoglin, FGFs), compared to 2D hiPSC-EC cultures grown on collagen-coated dishes. Cell-matrix adhesion proteins (e.g. collagen XVIII, MMP8 and 9, TIMP1) were also upregulated, suggesting the increased adhesive capacity of cells upon reseeding. In vivo, ultrasonographic and CT angiography confirmed that implanted vascular constructs displayed good patency with no obvious thrombi. No adverse immunoreactions were reported in any of the treated animals. Post-implantation analysis of the grafts showed that hiPSC-EC responded to shear stress and dynamic flow in vivo, by upregulating Notch signalling (Notch 1 and Notch 2; 3

and 5 times upregulated, respectively) and mechanosensitive YAP (2 times upregulated) / TAZ (5 times upregulated) compared to 3D static cultures.

Conclusions: Our results showed that hiPSC-EC can be used to repopulate decellularised aortic walls. Optimising dynamic cell culture conditions is necessary to obtain functional vascular tissue-engineered grafts for therapeutic applications.

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