8th
6th Sept. 2013

6th Sept. 2013

8th Annual conference GERMAN SOCIETY FOR STEM CELL RESEARCH

Prof. Dr. Gustav Steinhoff, Prof. Dr. Robert David
Department of Cardiac Surgery, University of Rostock

TERUMO

GSZ
DEUTSCHE GESELLSCHAFT FÜR STAMMZELLFORSCHUNG

Universität Rostock
Traditio et Innovatio

STEMCELL™ TECHNOLOGIES

CONTENT

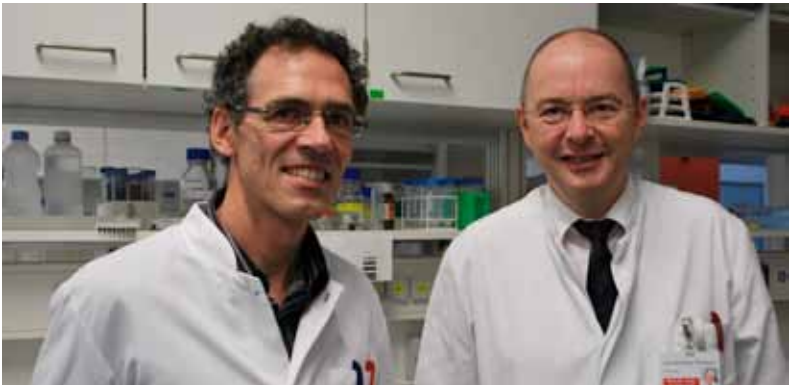
GSZ 2013

Supported by

BALTIC SUMMERACADEMY 2013

Supported by

DEAR COLLEAGUES



we would like to announce the final program of the 8th annual conference of the GSZ to be held in Rostock, September 6, 2013. The meeting will take place at the centre of Rostock in the congress venue of the Radisson Blue Hotel. We are looking forward to presentations of latest development in stem cell research. The meeting is combined with the 12th Baltic Summeracademy for Cardiac Surgery on September 7, 2013, which is focusing on „Regenerative Medicine in Cardiac Surgery". The two-day program is highly interesting and focuses on presentations by young researchers. The congress fee is valid for both days in the historic hanseatic scenery of Rostock.

Looking forward to see you in Rostock in September,

Prof. Dr. med. Gustav Steinhoff
University Medicine Rostock
Congress President

Prof. Dr. rer. nat. Robert David
University Medicine Rostock
Vice-Congress President

CONFERENCE SITE

GSZ 2013 // BALTICSUMMERACADEMY 2013

RADISSON BLU HOTEL
Lange Straße 40
18055 Rostock | Germany

The Radisson BLU Hotel, Rostock is conveniently located in the heart of the city and offers fabulous views of the city's harbour. The hotel is just 1 kilometre from the train station, while trams and buses stop right outside the hotel. Rostock-Laage Airport is just 35 kilometres away while the Hamburg International Airport is 190 kilometres from the hotel. The city of Rostock is just a short journey to the unspoilt coastline of Warnemünde with its open sandy beach. Boats from Scandinavia and the Baltic States arrive at the Rostock international harbour every day, and Rostock-Warnemünde is a popular stop for cruise ships sailing along Germany's Baltic Coast.

Note: Advance booking is highly recommended.

RADISSON BLU HOTEL ROSTOCK
Lange Straße 40 | 18055 Rostock
phone: +49 (0)381 375 00

e-mail: axel.behrendt@radissonblu.com
web: www.radissonblu.com

FACTS FOR THE TRAVELLER

air travel

Some regional airlines serve the local airport at Rostock-Laage, but most large airlines serve the airport in Hamburg and Berlin. From the airport Rostock-Laage shuttle busses go to the central bus station next to the railway station. At the Rostock main railway station there will be the tramway 5 or 6 to Rostock „Kröpeliner Tor" running nearly every 15 min at day time with a break between 1:00 am and 4:00 am.

ferry travel

Ferries go from Helsinki (Finland), Trelleborg (Sweden) and Gedser (Denmark). For further information visit the homepage of Scandlines Deutschland GmbH.

car travel

Comming from the south of Germany traveling in direction Berlin (beltway west). Change from beltway to A24 direction Hamburg/Rostock for 60km. At the junction Wittstock/Dosse change from A24 onto the A19 in direction Wittstock, Rostock via A19. Follow the A19 to exit Rostock-Süd. After leaving the A19, follow the signs to Rostock-City.

train

From Hamburg: Most trains from Hamburg go directly to Rostock. Travelling time is about 2h 15min and the costs are about 31.- EUR.
From Berlin: Leaving from Berlin-Lichtenberg or Berlin-Zoologischer Garten trains go directly to Rostock. Traveling time is about 2h 45min and tickets cost 22-45.- EUR.

SOCIAL PROGRAM

GSZ 2013 - FRIDAY, SEPTEMBER 6TH

Käpt'n Piet

adress
Stadthafen 71
18057 Rostock

time
start 19:00 - open

The particpiants of the 8th annual conference of the German Society For Stem Cell Research can have a relaxed evening at a special location for their own. "Kapt n Piet" is a boat at the harbor of Rostock. You find a lounge atmosphere at a unique place and can talk with colleagues and friends.

BALTIC SUMMERACADEMY 2013 - SATURDAY, SEPTEMBER 7TH

Ursprung

adress
Alter Markt 16
18055 Rostock

time
start 20:00

Subsequent to the baltic summer academy the congress presidents invite all participants to come to a charming restaurant and club called "Ursprung" in the historic city centre of Rostock. Enjoy a charity concert of the band "Five Men On The Rocks" who are known for their excellent stage performance including cover songs from bands like AC/DC, Deep Purple or Led Zeppelin. Professor David, part of the rostock medical team, is going to join the band on the guitar as a special guest.

The proceeds are going to support a stem-cell-laboratory for students.

PROGRAM SEPTEMBER FRIDAY 6TH

GSZ 2013

12:00	**Welcome**	Prof. Hescheler, Prof. Steinhoff
12:05 – 13:00	**Podiumsdiskussion** „Stammzellforschung: Sensationelle Ergebnisse im Tiermodell versus Realität am Krankenbett" Moderation: **R. Heusch-Lahl, G. Steinhoff**	
12:00 – 12:15	**Impulsreferat:** „Was haben die Forscher adulter Stammzellen erreicht? Warum ist die klinische Umsetzung von Laborergebnissen so schwierig, was hindert uns daran?" Prof. Dr. Kl. Cichutek (Präsident Paul-Ehrlich Institut, Langen) Teilnehmer: **Prof. Dr. K. Cichutek,** Langen; **Prof. Dr. A. Ho,** Heidelberg; **Prof. Dr. Hescheler,** Köln; **Prof. Dr. David,** Rostock; **Prof. Dr. U. Martin,** Hannover; **Prof. Dr. M. Freund,** Rostock	

SESSION I: Stem Cell Therapy

Moderation: G. Steinhoff (Rostock) and W. Franz (Innsbruck)

13:00 – 13:15	**L. Schneider,** Milan	DNA damage in mammalian neural stem cells leads to senescence-associated secretory phenotype and BMP2/JAK-STAT mediated astrocytic differentiation
13:15 – 13:30	**A. Schade,** Rostock	Efficient modifications of human mesenchymal stem cells using novel non-viral gene carriers for regenerative medicine
13:30 – 13:45	**T. Faltus,** Leipzig	Current trends in European and German stem cell law – criminal prosecution in basic research and unpatentability of inventions
13:45 – 14:00	**S. Saito,** Hannover	In vivo integration of pluripotent stem cell-derived bioartificial cardiac tissue
14:00 – 14:15	**R. Zweigerdt,** Hannover	Suspension culture of human pluripotent stem cells in stirred bioreactors
14:15 – 14:45	Coffee Break and Poster Viewing (Moderated Poster Session 1)	

SESSION II: (Re-)programming and Tissue Engineering

Moderation: I. Gruh (Hannover) and R. David (Rostock)

14:45 – 15:00	**G. Schumann,** Langen	Reprogramming triggers mutagenic endogenous L1 and ALU transposition and the accumulation of intact L1 elements in hiPSC genomes
15:00 – 15:15	**R. Schwanbeck,** Kiel	Chromatin protein high mobility group A2 (HMGA2) – a promoter of neural stem cell self-renewal – is regulated by notch1 cell-context dependently
15:15 – 15:30	**M. Lehmann,** Köln	Evidence for a critical role of catecholamines for cardiomyocyte lineage commitment in murine embryonic stem cells
15:30 – 15:45	**A. Rada-Iglesias,** Köln	Characterization of human developmental enhancers. from whole genomes to single snaps
15:45 – 16:00	**PM. Dohmen,** Berlin	Six years of clinical follow up with endothelial cell seeded small diameter vascular grafts during coronary bypass surgery
16:00 – 16:30	Coffee Break and Poster Viewing (Moderated Poster Session 2)	

SESSION III: Animal and Disease Models / Imaging Technologies

Moderation: U. Martin (Hannover) and I. Kutschka (Magdeburg)

16:30 – 16:45	**C. Klopsch,** Rostock	Spray-and laser-assisted cell seeding for autologous stem cell-plus-fibrin heart valve tissue engineering
16:45 – 17:00	**C. Zechel,** Lübeck	Hierarchical organization of brain tumor stem cells
17:00 – 17:15	**K. Schuetze,** Bernried	Early detection of stem cell differentiation with Raman spectroscopy
17:15 – 17:30	**KAT. Carvalho,** Curitiba, PR, Brazil	The nanostructured membrane could be used for stem cell therapy to tissue repair.
17:30 – 17:45	**C. Klopsch,** Rostock	Cardiac erythropoietin patch restores cardiac functions and induces stem cell mediated regeneration after myocardial infarction
17:45 – 18:00	**H. Murua Escobar,** Hannover/Rostock	MRI contrast enhancing labelling of mammalian cells via manganese and superparamagnetic nanoparticles as tools for in vivo animal models
18:15 – 19:00	General Meeting of GSZ-Members	
19:00 – open	Get together	

POSTERSESSIONS

SESSION I

1)	**A. Limpert,** Lübeck	Approach to sensitize glioma stem cells to standard chemotherapy
2)	**D. Reichert,** Dresden	Are murine mesenchymal stromal cells a trap for human hematopoietic stem and progenitor cells?
3)	**EC. Buss,** Heidelberg/ Rehovot, Israel	Mechanisms of mobilization of human primary precursor-B-ALL cells in an in vivo model system by the CXCR4-antagonist AMD3100 and by catecholamines
4)	**V. Lepperhof,** Köln	Bioluminescence in vivo imaging of genetically selected iPS cell-derived cardiomyocytes after transplantation into infarcted heart
5)	**S. Ulrich,** Hannover	Differentiation of human pluripotent stem cells into lung epithelial cells
6)	F. **Schlegel,** Leipzig	A new possibility to reprogram bone marrow stem cells into endothelial like cells
7)	**M. Schweinlin,** Würzburg	In vitro differentiation of murine embryonic stem cells into intestinal cells

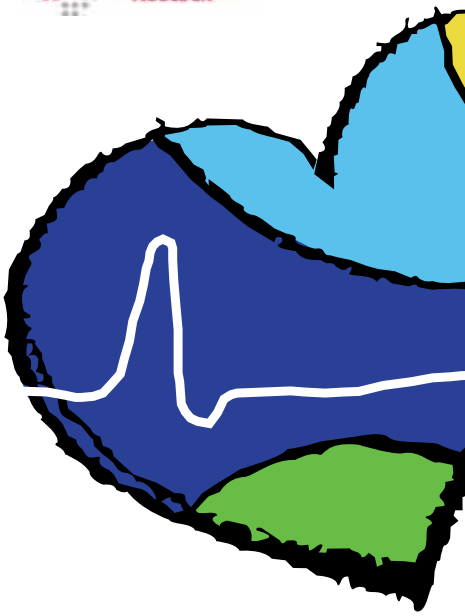
SESSION II

8)	**CO. Heras,** Köln	A bioengineered matrix with physiological elasticity facilitates long-term culture of pluripotent stem cell-derived cardiomyocytes
9)	**N.-Z. Mehrjardi,** Köln/Tehran, Iran	Genetic manipulation of neural progenitor cells derived from human induced pluripotent stem cells by using zinc finger nucleases
10)	**R. Zweigert,** Hannover	Generation of endothelial cells from scalable cultures of undifferentiated human pluripotent stem cells
11)	**A. Witthuhn,** Langen	Line1-mediated retrotransposition in human pluripotent stem cells: consequences for genomic stability of HES and HIPS cells and their derivatives
12)	**K. Osetek,** Hannover	Young versus aged cell sources - genetic aberrations in iPS cells and the effect of primary cell proliferation on reprogramming efficiency
13)	**L. Gan and B. Denecke,** Aachen	Transcription factors, microRNAs and their interactions during cardiomyocyte-specific differentiation of murine embrionic stem cells
14)	**I. Gruh,** Hannover	Optogenetic control of stem cell-derived bioartificial cardiac tissue
15)	**K. Burkert,** Köln	Monitoring differentiation of pluripotent stem cells to cardiomyocytes by measuring cardiac Troponin T release into the cell culture medium.

Cardiac Surgery // Faculty of Medicine // University of Rostock

Universität Rostock Traditio et Innovatio

Universitäres Herzzentrum Rostock



Regenerative Medicine in Cardiac Surgery

Prof. Dr. Gustav Steinhoff, PD Dr. Alexander Kaminski, Prof. Dr. Robert David
Department of Cardiac Surgery, University of Rostock

TERUMO MAQUET Edwards Lifesciences MACS Miltenyi Biotec

GORE ETHICON Biosurgery SORIN GROUP Medtronic

BALTIC SUMMER ACADEMY 2013
07th September 2013 // Radisson Hotel // Rostock // Germany
www.baltic-summeracademy.com

REGENERATIVE MEDICINE IN CARDIAC SURGERY

DEAR COLLEAGUES

Cardiac surgery is a fastly changing field of specialized medicine exposing high challenges in specialist training and innovation activity. The 12th conference of the BSA 2013 will address this field focussing Regenerative Medicine prone to make changes in treatment strategies and technologies. The field is emerging and gets major attention from young researchers and clinicians to lead the development to improved patient outcomes in cardiac surgery and intervention. The meeting between experts in the field and young researchers is held this year again in the Radisson Blue Hotel located in the center of the hanseatic Rostock city. The symposium is intended as a small but high-end meeting to discuss new and interdisciplinary developments in cardiac surgery. We cordially invite you to Rostock and hope you will enjoy an extraordinary scientific program.

Yours,
Prof. Dr. med. Gustav Steinhoff
PD Dr. med. Alexander Kaminski
Prof. Dr. rer. nat. Robert David

PROGRAMM BALTIC SUMMERACADEMY 2013

SATURDAY, SEPTEMBER 7TH 2013

SESSION I – **STEM CELLS**
Chair: G. Faggian, A. Patel

08:00 – 08:30	**G. Steinhoff,** Rostock	Application of stem cells in cardiac surgery
08:30 – 09:00	**A. Patel,** Utah (USA)	Regenerative technologies in cardiac surgery
09:00 – 09:30	**A. Nemkov,** St. Peterburg (RUS)	Bone marrow cell therapy of the heart in patients with coronary artery disease, SPbSMU experience
09:30 – 10:00	**R. David,** Rostock	Regulation of cardiac cell differentiation
10:00 – 10:15	**J. Jung,** Rostock	Directed forward programming of pluripotent stem cells towards pacemaker cells using the transcription factor Tbx3
10:15 – 10:30	**C. Lang,** Munich/Rostock	Positron Emission Tomography Based In-vivo Imaging of Early Phase Stem Cell Retention after Intramyocardial Delivery in the Mouse Model
10:30 – 11:00	Coffee break	

SESSION II – **EVOLVING STRATEGIES IN CARDIAC SURGERY**
Chair: G. Steinhoff, A. Nemkov

11:00 – 11:30	**G. Faggian,** Verona (IT)	Aortic surgery: Reconstructive and regenerative concepts
11:30 – 12:00	**H. Ince,** Rostock	Interventional Cardiac Surgery: An interventionalist's view
12:00 – 12:30	**A. Kaminski,** Rostock	Interventional Cardiology: A surgeon's view
12:30 – 13:00	Lunch break	

SESSION III – **REGENERATIVE STRATEGIES**
Chair: R. David, G. Pompilio

13:00 – 13:30	**G. Pompilio,** Milano (IT)	Mechanisms of cardiac regeneration
13:30 – 14:00	**L. M. Popescu,** Bucharest (ROU)	Stem Cells Alone: The dogma of regenerative medicine challenged by telocytes
14:00 – 14:30	**B. Vollmar,** Rostock	Erythropoetin and dermal wound healing
14:30 – 15:00	**A. Patel,** Utah (USA)	SDF-1 and sternal wound healing
15:00 – 15:15	**C. Klopsch,** Rostock	Erythropoetin and cardiac repair
15:15 – 15:30	**P. Donndorf,** Rostock	Quantification of the in vivo migratory capacity of human CD133+ bone marrow stem cells after intraoperative harvesting from the sternal bone marrow
15:30 – 16:00	Coffee break	

SESSION IV – **HEART VALVE TISSUE ENGINEERING: EMERGING TECHNOLOGIES**
Chair: A. Kaminski, B. Vollmar

16:00 – 16:15	**C. Klopsch,** Rostock	Cell seeding technologies in heart valve tissue engineering
16:15 – 16:30	**I. Sabel,** Munich	Tissue engineered transcatheter heart valves after crimping and perfusion
16:30 – 16:45	**T. Mayer,** Munich	Influence of different fixatives on the mechanical properties of cell-seeded scaffolds
16:45 – 17:00	**N. Haller,** Munich	Conditioning of colonized homografts for a time period of 12 days under sterile and controlled conditions
17:00 – 17:15	**S. Eilenberger,** Munich	Mechanical integrity of decellularised, stented and re-seeded human aortic valves after crimping and perfusion
17:15 – 17:30	**A. Erle,** Ulm	Co-cultures of cardiomyocytes and bone marrow mesenchymal stromal cells: modelling myocardial MSC integration

Closing comments: G. Steinhoff, A. Kaminski, R. David

FACULTY BALTIC SUMMERACADEMY 2013

SATURDAY, SEPTEMBER 7TH 2013

Amit N. Patel, M.D., B.S., M.S.
Director of Clinical Regenerative
Medicine and Tissue Engineering
Ass.Professor of Cardiothoracic Surgery
University Hospital
Salt Lake City, USA

Giuseppe Faggian, M.D.
Professor of Cardiac Surgery
Department of Cardiac Surgery,
University of Verona
Verona, Italy

Gustav Steinhoff, M.D.
Professor of Cardiac Surgery
Department for Cardiac Surgery
University Medicine Rostock
Rostock, Germany

Brigitte Vollmar, M.D.
Professor of Experimental Surgery
Institute of Experimental Surgery
Rostock, Germany

Alexander Kaminski, M.D.
PD of Cardiac Surgery
Department of Cardiac Surgery
University Medicine Rostock
Rostock, Germany

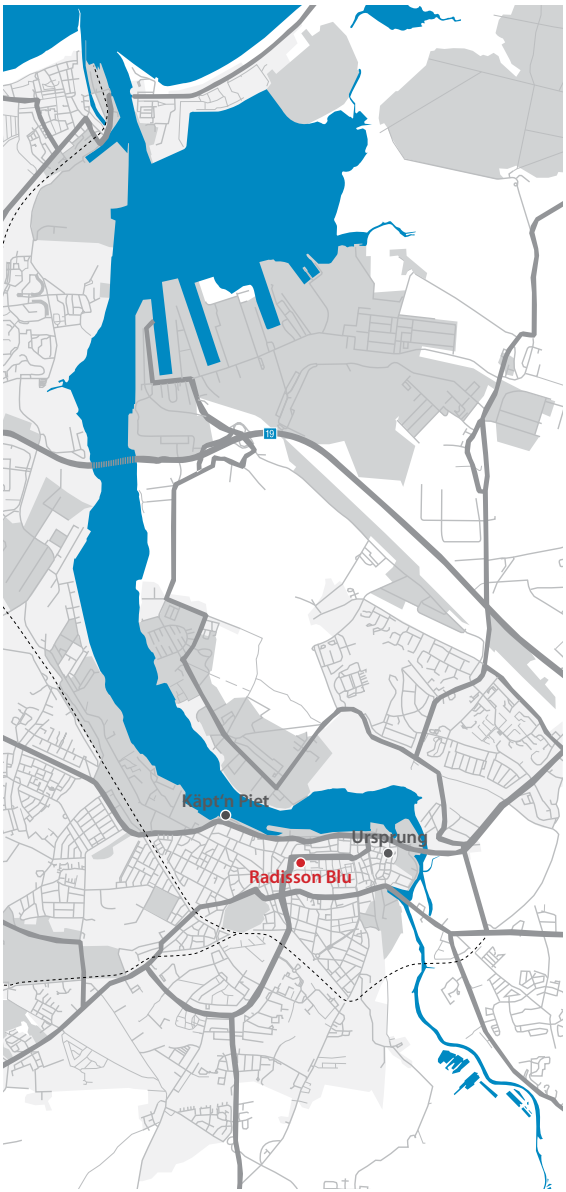
Hueseyin Ince, M.D.
Ass. Professor of Cardiology
Department of Internal Medicine
University Medicine Rostock
Rostock, Germany

Alexander Nemkov, MD, PhD
Professor of Cardiac Surgery
Director Cardio surgery department
St. Petersburg Pavlov State
Medical University
St. Petersburg, Russia

Robert David, Ph.D.
Professor of Regenerative Medicine
in Cardiac Surgery
Department of Cardiac Surgery
University Medicine Rostock
Rostock, Germany

L.M. Popescu, MD, PhD, dr.h.c.mult.
Professor of Cellular and Molecular
Medicine Head of the National
Institute of Pathology,
Bucharest, Romania

Giulio Pompilio, M.D., Ph.D..
Assistant Professor of Cardiac Surgery
Centro Cardiologico Monzino IRCCS
Milano, Italy



ABSTRACTS

GSZ 2013

September 6th 2013 | Rostock | Germany

BALTICSUMMERACADEMY

September 7th 2013 | Rostock | Germany

01 Abstract no.

DNA DAMAGE IN MAMMALIAN NEURAL STEM CELLS LEADS TO SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE AND BMP2/JAK-STAT MEDIATED ASTROCYTIC DIFFERENTIATION

title

L. Schneider, F. d'Adda di Fagagna

Authors

OBJECTIVES: Recent research demonstrates the necessity to understand the impact of DNA damage on somatic stem cells. Especially for neural stem cells (NSC), this impact is poorly elucidated, despite the obvious implications for developmental and adult neurogenesis and age- and stress-related neurodegeneration.
MATERIALS & METHODS: We exposed to X-ray induced DNA damage wildtype and gene-deficient embryonic stem cell-derived NSC, but also adult NSC in vitro and in vivo, using a Sox2—Cre reporter mouse model for NSC fate tracing.
RESULTS: Irradiated NSC rapidly enter cellular senescence, despite a widespread transcriptional downregulation of DNA damage response (DDR) signaling machinery. Concurrently, the cells lose their self-renewal potential, downregulate the expression of typical stem cell markers and undergo astrocytic differentiation. Importantly, this occurs cell-intrinsically and niche-independently, i.e under self-renewal-promoting culture conditions. Indeed, we showed the phenomenon of the senescence-associated cytokine secretion to be the key mechanism. Of these cytokines, BMP2, but not IL-6 or LIF, was responsible for expression of astroglial marker GFAP in irradiated NSC via its novel non-canonical signalling through JAK-STAT. Moreover, the DDR gene ATM plays a supportive role in cytokine-induced differentiation, while this process can be efficiently potentiated through p53 deficiency. In a murine model, cranial irradiation results in astrocytic differentiation of SOX2-expressing NSC residing in the adult subventricular zone.
CONCLUSIONS: DNA damage studies on NSC reveal a novel kind of irreversible cellular senescence, which does not rely on ongoing DDR signalling. Moreover, astroglial differentiation was shown to be mediated through a novel non-canonical signaling pathway, adding surprising new aspects to the unique physiology of somatic stem cells.

abstract

02 Abstract no.

EFFICIENT MODIFICATIONS OF HUMAN MESENCHYMAL STEM CELLS USING NOVEL NON-VIRAL GENE CARRIERS FOR REGENERATIVE MEDICINE

title

A. Schade, E. Delyagina, A. Skorska, C.A. Lux, R. David, G. Steinhoff

Authors

OBJECTIVES: Genetic modifications of human mesenchymal stem cells (hMSCs) before transplantation can expand their regenerative capacity. However, safe and efficient gene delivery methods, suitable for clinical application, have not been discovered, yet. Therefore, our aim is to develop efficient magnetic non-viral carriers for DNA or microRNA (miR) delivery in hMSCs using cationic polymer polyethylenimine (PEI) bound to iron oxide magnetic nanoparticles (MNP) and to study the transfection process on the intracellular level.
MATERIALS & METHODS: Transfection efficiency of magnetic complexes, containing DNA or miR was investigated in hMSCs using luciferase assay or flow cytometry. We performed intracellular visualization and colocalization studies of fluorescently labeled complexes using laser scanning microscopy (LSM). Additionally, release, processing and functionality of precursor miR delivered with magnetic complexes were studied by LSM, real-time PCR and live cell imaging.
RESULTS: Transfection efficiency of DNA/PEI complexes bound to MNP was 10-fold higher compared to commercially available PEI complexes already 12 hours post-transfection. Colocalization studies showed that magnetic complexes released DNA faster into the cytosol than DNA/PEI complexes. Furthermore, this approach was suitable for efficient miR delivery with an uptake efficiency of ~75% and moderate cytotoxicity in hMSCs. We determined an improved transfection performance of MNP – containing complexes compared to PEI complexes 72 hours post-transfection indicating a prolonged effect of MNP complexes.
CONCLUSIONS: We concluded that our magnetic construct is advantageous for delivery of both, DNA and miR. Hence, our findings may become of great importance for in vivo targeting and tracking of magnetically modified cells after transplantation.

abstract

Abstract no. 03

title **CURRENT TRENDS IN EUROPEAN AND GERMAN STEM CELL LAW – CRIMINAL PROSECUTION IN BASIC RESEARCH AND UNPATENTABILITY OF INVENTIONS**

Authors T. Faltus

abstract The presentation will first focus on the rules governing international and transnational stem cell research cooperation. Against public and professional opinion, in the field of human embryonic stem cells there is still the risk of criminal prosecution for German researchers.
The presentation will also illustrate the European and German legal system for market approval of stem cell based products. One of the major statutes in that field is the so called European ATMP-Regulation, which covers stem cell based therapies. In principle, any product which falls within the scope of the ATMP-regulation must undergo a centralized European market approval regime by the European Medicines Agency in London. However, this practice of the regular centralized market approval can be very costly and may be oversized for small and medium sized companies which are the major developer of stem cell based products. Therefore, there are a number of financial amenities for small and medium sized companies as well as exemptions and shortcuts from the requirement for a centralized market authorization. The presentation therefore will explain these amenities as well as the requirements to enter the European market without a centralized market approval. The presentation also illustrates the differences between the legal requirements for market approval and health care reimbursement.
The presentation will further focus on the impact and trends of the European Court of Justice's recent decision on the unpatentability of human embryonic stem cell based inventions within the European Union. The meaning of this decision will also be compared with the decision of the European Patent Offices' decision on the so called WARF stem cell patent.

Abstract no. 04

title **IN VIVO INTEGRATION OF PLURIPOTENT STEM CELL-DERIVED BIOARTIFICIAL CARDIAC TISSUE**

Authors S. Saito, H. Baraki, G. Kensah, M. Baker, A. Haverich, U. Martin, I. Gruh, I. Kutschka

abstract OBJECTIVE: We evaluated the histological, functional and electromechanical characteristics of bioartificial cardiac tissue (BCT) made from induced pluripotent/embryonic stem-cell derived cardiomyocytes (iPSC-CM/ESC-CM) transplanted into infarcted rat hearts.
METHODS: BCTs (6x0.5mm) made from murine iPSC-CM were conditioned in a bioreactor for 21 days, and implanted into the myocardium of nude rats with chronic myocardial infarction (n=15). Heart function was compared to control animals without BCT (sham, n=15) four weeks postoperatively using echocardiography and magnetic resonance imaging. The histological characteristics of the BCTs were evaluated by α-Actinin, CD31 and connexin 40/43/45 staining. To evaluate the electrical coupling with the host myocardium, BCTs made from channelrhodopsin-2 transgenic mouse ESC-CM were implanted into the myocardium of nude-rats (n = 6). Stimulation of the whole hearts with blue light (475 nm) was performed 4 weeks after the implantation and compared to control hearts (n=3). Electrocardiograms were recorded during stimulation.
RESULTS: At 4 weeks, heart function was significantly better preserved in the BCT-group compared to the sham-group (LVEF; 54±11 vs. 40±18, p= 0.004; LV end-systolic volume; 0.23±0.01mL vs. 0.45±0.01mL, p=0.001). Histological examination revealed abundant survival of cardiomyocytes within the BCTs (α-Actinin), gap-junction protein expression (connexin 40/43/45), and neo-vascularization (CD31). Blue light stimulation induced a stimulation rate dependent ventricular arrhythmia of the host heart, suggesting relevant electromechanical coupling.
CONCLUSIONS: Bioartificial cardiac tissue made from murine iPSC-CM/ESC-CM engrafts with electromechanical connection into ischemic nude rat myocardium. Therefore, an actual functional contribution to left heart performance is conceivable.

05 Abstract no.

SUSPENSION CULTURE OF HUMAN PLURIPOTENT STEM CELLS IN STIRRED BIOREACTORS title

R. Olmer, U. Martin, R. Zweigerdt Authors

OJECTIVE: Envisioned therapeutic and industrial applications of human pluripotent stem cells (hPSCs) and their derivatives will require large cell quantities generated in defined conditions. To overcome limitations of conventional, adhesion-dependent, 2 dimensional (2D) cell culture we have established single cell-inoculated suspension cultures of hPSCs as matrix free cell-only aggregates in stirred tank bioreactors.
METHODS: These systems are widely used in biopharmaceutical industry, allow straight forward scale up and detailed online monitoring and control of key process parameters. To ensure minimum medium consumption but in parallel functional integration of all probes mandatory for process monitoring (including pO2, pH, and biomass sensors) experiments were performed in 100 ml culture volume in a "mini bioreactor platform" consisting of 4 independently controlled vessels.
RESULTS: By establishing defined parameters for tightly controlled cell inoculation and aggregate formation, up to 2 x 108 hiPSCs / 100 ml were readily generated in a single process run in 7 days. Expression of pluripotency markers and cells ability to differentiate into derivates of all three germ layers in vitro was maintained, underlining practical utility of this new process.
CONCLUSION: Despite substantial progress, yet only linear rather than exponential growth rates were achieved in suspension culture and a relative low cell density of up to ~2 x 106 hiPSCs / ml was obtained in our system, suggesting a great potential for further process development. Towards this end we will present new strategies and data on process optimization leaping forward towards the controlled mass expansion of hPSCs and their progenies. abstract

06 Abstract no.

REPROGRAMMING TRIGGERS MUTAGENIC ENDOGENOUS L1 AND ALU TRANSPOSITION AND THE ACCUMULATION OF INTACT L1 ELEMENTS IN HIPSC GENOMES title

S. Klawitter, N.V. Fuchs, K.R. Upton, Munoz-Lopez, J.L.Garcia-Perez, Z. Izsvak, G.J. Faulkner, G.G. Schumann Authors

OBJECTIVES:The use of human induced pluripotent stem cells (hiPSCs) holds great therapeutic promise for regenerative therapies and disease modeling. However, reprogramming and cultivation of hiPSCs can induce genetic and epigenetic abnormalities that can result in tumorigenic hiPSCs and undermine the use of iPSCs or their derivatives in regenerative medicine. Activation of endogenous mobile retrotransposon families LINE-1 (L1), Alu and SVA can cause such mutations, that were shown to be the cause of genetic disorders and tumor diseases. We investigated if endogenous L1, Alu and SVA elements mobilize in hiPSCs and contribute to their genomic destabilization.
METHODS:To explore if L1 elements are activated in hiPSC lines, we analyzed CpG methylation of L1 promoters by bisulfite sequencing, and L1 expression by qRT-PCR and immunoblot analysis in 17 iPSC lines and their somatic parental cells. To map individual mobilization events that occurred during or after reprogramming, we used a novel high-throughput protocol named retrotransposon capture sequencing (RC-seq). New transposition events that were only present in the iPSC lines, were validated as de novo by insertion site PCR and capillary sequencing.
RESULTS:Reprogramming reduced methylation of the L1 promoter significantly, induced full-length L1 mRNA expression by 4-25,000-fold relative to parental cells. 50% of all de novo insertions were found in protein-coding genes, including genes impairing oncogenic growth and invasion of breast cancer cells.
CONCLUSIONS: L1-mediated mobilization occurs during or after reprogramming into iPSCs and during cultivation, amplifying the number of functional, mutagenic L1 elements. These mobilization events can perturb key protein-coding genes with unknown consequences in differentiated cells, questioning biosafety of hiPSCs and their derivatives. abstract

Abstract no. 07

title **CHROMATIN PROTEIN HIGH MOBILITY GROUP A2 (HMGA2) – A PROMOTER OF NEURAL STEM CELL SELF-RENEWAL – IS REGULATED BY NOTCH1 CELL-CONTEXT DEPENDENTLY**

Authors R. Schwanbeck, S. Martini, U. Just

abstract OBJECTIVES: Hmga proteins are non-histone chromatin factors involved in several gene regulatory processes, regulation of cell cycle, proliferation and differentiation. Recently, Hmga2 was shown to promote fetal and adult neural stem cell self-renewal and regulate the neurogenic potential of neural precursor cells (NPCs) via modulating the global chromatin state. Notch1 plays a pivotal role in cell lineage decisions and is critical for neurogenesis. Here, we investigate how Notch signaling and Hmga2 are connected in the regulatory network during neurogenesis.
MATERIALS & METHODS: ESCs carrying an inducible form of Notch1 were differentiated along the neuroectodermal lineage or as embryoid bodies preferring mesodermal differentiation. Using qPCR analysis the expression of Hmga2 and Hmga1 was monitored during neuroectodermal and mesodermal differentiation upon Notch1 induction. Protein expression of Hmga2 was analyzed by Western blot. Sox9, a mediator of Notch1 induced changes during neuroectodermal differentiation, was knocked down in parallel to Notch1 induction to investigate the role of Sox9 for Hmga regulation.
RESULTS: During mesodermal differentiation Hmga2 expression strongly peaked at day 7. When Notch1 was induced this Hmga2 peak was fully quenched. However, if the ES cells were differentiated along the neuroectodermal lineage we observed a small rise of Hmga2 peaking at day 6. This peak was strongly increased by induction of Notch1. Knock down of Sox9 during Notch1 induction had only a marginal effect on this increase.
CONCLUSIONS: Here we show for the first time that Hmga2, a promoter of neural stem cell self-renewal, is a target gene of Notch1 during neuroectodermal differentiation, possibly mediating important functions of Notch1. The induction of Hmga2 occurs in a highly cell-context specific way, since in mesodermal cells Hmga2 is suppressed by induction of Notch1. This regulation is may be a critical step in the regulatory network of Notch signaling during neurogenesis.

Abstract no. 08

title **EVIDENCE FOR A CRITICAL ROLE OF CATECHOLAMINES FOR CARDIOMYOCYTE LINEAGE COMMITMENT IN MURINE EMBRYONIC STEM CELLS**

Authors M. Lehmann, F. Nguemo, V. Wagh, K. Pfannkuche, J. Hescheler and M. Reppel

abstract OBJECTIVES: Catecholamine function and regulation in adults is well described, yet, little is known about the role of catecholamines during heart development. In this study we sought to evaluate the effects of catecholamines on early heart development in vitro using embryonic stem (ES) cell-derived cardiomyocytes.
METHODS: Effects of catecholamine depletion induced by reserpine were examined in murine ES cells (line D3, PIG44) during differentiation. Cardiac differentiation was assessed by immunocytochemistry, qRT-PCR, quantification of beating clusters, flow cytometry and pharmacological approaches. Proliferation was analyzed by EB cross-section measurements, while functionality of cardiomyocytes was studied by extracellular field potential (FP) measurements using microelectrode arrays (MEAs). To further differentiate between substance-specific effects of reserpine and catecholamine action via - and -receptors we proved the involvement of adrenergic receptors by application of unspecific receptor antagonists.
RESULTS: Reserpine led to remarkable down-regulation of cardiac-specific genes, proteins and mesodermal marker genes. In more detail, the average ratio of ~40 % spontaneously beating control clusters was significantly reduced by 100 %, 91.1 % and 20.0 % on days 10, 12, and 14, respectively. Flow cytometry revealed a significant reduction (by 71.6 %, n=11) of eGFP positive CMs after reserpine treatment. MEA measurements underlined those findings. By contrast, reserpine did not reduce EB growth while number of neuronal cells in reserpine-treated EBs was significantly increased. Interestingly we found that developmental inhibition after - and -adrenergic blocker application mimicked developmental changes with reserpine.
CONCLUSIONS: Thus, our data suggest that reserpine inhibits cardiac differentiation, and that catecholamines play a critical role during development.

09 Abstract no.

CHARACTERIZATION OF HUMAN DEVELOPMENTAL ENHANCERS: FROM WHOLE GENOMES TO SINGLE SN title

A. Rada-Iglesias Authors

OBJECTIVES: Distal regulatory elements, such as enhancers, play a preponderant role in the establishment of cell-type and developmental-stage specific gene expression profiles. However, these elements are difficult to identify, since they lack strong defining features and show limited sequence conservation. Our goal was to develop strategies to identify and characterize key human developmental enhancers.
MATERIALS & METHODS: Using a combination of epigenomic approaches (ChIP-seq, RNA-seq) and ESC-based differentiation protocols, we have characterized the full enhancer repertoire of pluripotent (i.e. human embryonic stem cells (hESC)) and multipotent (i.e. human neural crest cells (hNCC)) human embryonic cell populations.
RESULTS: In hESC, we uncovered a unique chromatin signature that identifies a novel class of enhancers, which are inactive but poised in hESC and that become active upon differentiation in a lineage-specific manner. Similarly, our epigenomic approach allowed us to characterize enhancers in hNCC, a hitherto largely inaccessible and biochemically intractable vertebrate-specific embryonic cell population that contributes to the formation of multiple tissues and organs, such as the peripheral nervous system and most of the facial bones and cartilages. Using the sequence information contained within hNCC enhancers, we uncovered NR2F1 and NR2F2, two orphan nuclear receptors, as novel neural crest and craniofacial regulators.
CONCLUSIONS: The genomic characterization of human enhancers in relevant cell types might streamline the identification of functional non-coding genetic variants, which can have far-reaching implications in our understanding of the genetic basis of human complex diseases and human morphological evolution. abstract

References:
Rada-Iglesias, A., et al.; A unique chromatin signature uncovers early developmental enhancers in humans. Nature. (2011);470, 279–283. Rada-Iglesias, A., et al.; Epigenomic annotation of enhancers predicts transcriptional regulators of human neural crest. Cell Stem Cell . (2012);11, 633–648.

10 Abstract no.

SIX YEARS OF CLINICAL FOLLOW UP WITH ENDOTHELIAL CELL SEEDED SMALL DIAMETER VASCULAR GRAFTS DURING CORONARY BYPASS SURGERY title

P.M. Dohmen, A. Pruß, C. Koch, A.C. Borges, W. Konertz Authors

OBJECTIVE: This clinical study was performed to investigate the patency rate of endothelial cell seeded small-diameter expanded polytetrafluorethylene grafts during coronary artery bypass surgery.
MATERIAL AND METHODS: Between September 1995 and December 1998 fourteen patients (median age 71 years, range 61-79 years) received 21 endothelial cell seeded small-diameter grafts. Forty-three percent of the performed implantations were re-operations. Endothelial cells were harvested from a forearm vein, expanded and characterized in the laboratory until a sufficient number was available. After in vitro seeding, the grafts were allowed to mature for another 10 days, prior to implantation. Graft patency was investigated with angiography, angioscopy and intravascular ultrasonography during follow up.
RESULTS: Cumulative data represented 58 patient's years and was 100 % completed. The seeded autologous vascular endothelial cell density was 105000 +/- 12000 cells/cm^2 with a cell viability of 95.5 +/- 1.5 %. Operative mortality was 7.1 % (one patient). Patency rate at discharge was 95.2 % and at a mean follow up of 27 months 90.5 %. The proven patency rate at up to 72 months was at least 50.0 %, as 5 patients refused angiographic evaluation. None of these 5 patients suffered from angina pectoris and so the best scenario would have shown a patency rate of 85.7 %. Angioscopy and intravascular ultrasonography showed absence of atheromas or stenosis in the investigated patent grafts.
CONCLUSIONS: Autologous vascular endothelial cell seeding improves patency rate of small caliber expanded polytetrafluorethylene grafts in patients without suitable autologous graft material. abstract

Abstract no. 11

title **SPRAY- AND LASER-ASSISTED CELL SEEDING FOR AUTOLOGOUS STEM CELL-PLUS-FIBRIN HEART VALVE TISSUE ENGINEERING**

Authors C. Klopsch, R. Gaebel, A. Kaminski, B. Chichkov, S. Jockenhoevel, G. Steinhoff

abstract OBJECTIVE: At present intensive investigation aims at the creation of optimal valvular prostheses. We introduced and tested two advanced cell-plus-matrix seeding technologies, the spray-assisted-bioprocessing (SaBP) and the laser-assisted bioprocessing (LaBP), for intraoperative table-side autologous tissue engineering (TE) of bioresorbable artificial grafts.
METHODS: Three-leaflet valves were manufactured following TE of electrospun poly ε-caprolactone (PCL) tissue equivalents. For SaBP, human mesenchymal stem cells (HMSC), umbilical cord vein endothelial cells (HUVEC) and fibrin were simultaneously spray-administered on PCL substrates. For LaBP, HUVEC and HMSC were separately laser-printed in stripes followed by fibrin sealing. Tissue equivalents were monitored in-vitro under static and dynamic conditions in bioreactors.
RESULTS: SaBP and LaBP resulted in TE of grafts with homogenous cell distribution and accurate cell pattern, respectively. Engineered valves demonstrated immediate sufficient performance, complete cell coating, proliferation, engraftment, HUVEC-mediated invasion, HMSC differentiation and extracellular matrix deposition. SaBP revealed higher efficiency with at least 12-fold shorter processing time than the applied LaBP setup. LaBP realized coating with higher cell density and minimal cell-to-scaffold distance. Fibrin and PCL stability remain issues for improvement.
CONCLUSIONS: The introduced TE technologies resulted in complete valvular cell-plus-matrix coating, excellent engraftment and HMSC differentiation. SaBP might have potential for intraoperative table-side TE considering the procedural duration and ease of implementation. LaBP might accelerate engraftment with precise patterns.

Abstract no. 12

title **HIERARCHICAL ORGANIZATION OF BRAIN TUMOR STEM CELLS**

Authors C. Zechel, E. Hirseland, S. Roth, I. Choschzick, J. Leppert, Y. Alkanan, S. Lichthardt, V. Tronnier

abstract INTRODUCTION AND OBJECTIVE: Gliomas originate from glial cells, their precursors (NPC) or neural stem cells (NSC), and harbor distinct populations of cells, so-called BTSC (brain tumor stem cells) and non-BTSC (bulk tumor cells). BTSCs exhibit similarities to NSC/NPC, self-renew and produce orthotopic tumors in mouse models. Recent data proposed that BTSCs constitute a lineage of self-renewing cell types expressing a range of markers of forebrain lineages. The hierarchical organization of BTSC lineages and their significance is poorly understood.
MATERIALS AND METHODS: We isolated BTSC from different glioma entities, such as glioblastoma multiforme (GBM), gliosarcomas (GSarc), oligoastrocytomas (OAC) and oligodendromas (OD). BTSC lines were clonally expanded in serum-free medium by limited dilution assays and transplanted into SCID mice. Expression of stemness-associated factors was investigated.
RESULTS AND CONCLUSIONS: We identified BTSC in biopsies from all GBMs, GSarcs, OACs and ODs. BTSCs were transplanted into the brain of SCID mice to prove their tumorigenic potential. All BTSC lines expressed (i) the intermediate filaments Nestin and GFAP, (ii) the regulators of neural differentiation Dlx-2 and REST/NRSF, (iii) the transcription factors Sox2, c-Myc, Oct4 and Nanog, and (iv) low levels of neuronal proteins. Clonally expanded BTSCs exhibited differences in the expression of stemness factors, as well as neural and BTSC markers. In particular, the expression of GFAP, CD133 and Sox2 differed. Spontaneous differentiation of a subfraction of cells was observed during progressive passaging, generating BTSC-derived cell lineages. The extent of differentiation of the distinct BTSCs and their progeny differed and the lineages displayed substantial heterogeneity. Our data suggest that GFAP is similarly associated with a high degree of stemness in BTSC and NSC/NPC lineages. Moreover, the regulation of the expression of Sox2, Oct4 and Nanog, but not the BTSC marker c-Myc, appeared linked.

13 Abstract no.

EARLY DETECTION OF STEM CELL DIFFERENTIATION WITH RAMAN SPECTROSCOPY title

K. Schuetze, S. Koch, E. Lorbach Authors

abstract

OBJECTIVES: An important issue in stem cell research is monitoring cell differentiation. Working with tedious stem cells requires a non-contact and stress free method. Raman spectroscopy (RS) recognizes and discriminates cells through their interaction with focused laser light i.e. without the need of biochemical stains or antibodies.
In this work we demonstrate the power of RS for the early detection of osteogenic differentiation in mesenchymal stem cells (MSC). We also tested RS to identify subpopulations of labeled murine MSC in comparison with gene expression assays.
MATERIALS & METHODS: Poietics® Human MSC isolated from bone marrow were cultured according to manufacturers' instructions. After 14 days expansion 3000 cells/ well were plated in a 96 well MatriPlate with or without collagen coating. For osteogenic differentiation cells were cultured in osteogenic differentiation BulletKit™. After 5 and 7 days cells were fixed with 4% PFA. Spectra were taken and analyzed using principal component analysis (PCA).
For the detection of subtypes of mouse MSC (Gibco) cells were labeled with CD 44, CD 90 and Sca-1 surface markers. Sample preparation for RS was 4% PFA to fix the cells for 15 min at RT and then washed in PBS. The MACS-sorted cell populations were analyzed by RS and compared with results of gene expression assays.
RESULTS: Osteogenic differentiation: On day 5 after plating Raman spectra of MSC cells revealed a tendency of difference between control cells and BulletKit™- incubated cells. On day 7 osteogenic differentiation was visible in cells plated on collagen. Until now common methods for analyzing osteogenic differentiation require long-term cultivation of 21-28 days. Raman spectra of labeled MSC subtypes yield a strong overlap in the PCA-plots but there were cells that clearly differ. Gene expression analysis confirmed these findings.
CONCLUSIONS: RS is an exciting new tool for non-destructive cell analysis and drug reaction survey. RS enables discrimination of cell types, differentiation states and fate of cells and tissue in a much earlier and less expensive manner.

14 Abstract no.

THE NANOSTRUCTURED MEMBRANE COULD BE USED FOR STEM CELL THERAPY TO TISSUE REPAIR. title

Carvalho K.A.T., Souza CMCO, Souza CF, Woehl MA, Irioda AC, Francisco JC, Guarita-Souza LC, Sierakowski M-R Authors

abstract

OBJECTIVES: to use hydrocolloids as tamarind xyloglucan-XG, gellan gum-GL or collagen-C, to be incorporated on regenerated bacterial cellulose membranes and prospect the improvement as cell therapy.
MATERIALS & METHODS: the total mass based on dried films was performed according to the commercial samples of bacterial cellulose (BC). In addition, the inclusions of drugs (lysozyme-LZ and / or fluconazole-FZL), with antibacterial and antifungal properties, are also evaluated in these systems. The bionanocomposites were obtained after defibrilation using a never-dried BC to form a pulp and the films of BC regenerated (RBC), in the presence or not of hidrocolloids (10% w/w) and/or drugs (10% w/w), were developed by dry casting. Cell adhesion and proliferation tests were done on different membranes, allowing the evaluation of their performance. Human adipose-derived mesenchymal stem cells were seeded on the membranes and after a period of 2 hours (adhesion test), and 7 days (proliferation test). Optical and Electron Microscopies were done in membrane. The evaluation was performed in burned skin and in infarcted myocardium as curative and patch in vivo. Histopathological analysis were done by H&E, immune assay with antibody anti-Brdu and anti-VIII factor in skin and myocardial.
RESULTS: the MTT cell counting technique, among the 5 tested nanostructured membranes and the commercial BC, the RBC-tamarind xyloglucan nanostructured membrane incorporated with lysozyme presented the best performance in adhesion and proliferation tests, that represents 35.3% more efficiency than the control. The angiogenesis were demonstrated in myocardium as well regeneration in both tissues.
CONCLUSIONS: the results allow the utilization of these membranes in tissue repair in skin and heart.

Abstract no. 15

title **CARDIAC ERYTHROPOIETIN PATCH RESTORES CARDIAC FUNCTIONS AND INDUCES STEM CELL MEDIATED REGENERATION AFTER MYOCARDIAL INFARCTION**

Authors C. Klopsch, G. Kleiner, M. Ludwig, A. Skorska, R. Gäbel, P. Mela, S. Jockenhoevel, G. Steinhoff

abstract OBJECTIVE: We investigated the therapeutic efficacy of Erythropoietin (EPO) at a moderate dose level and compared local and systemic delivery routes in a rat myocardial infarction (MI) model.
METHODS: Following MI, EPO (300 U/kg) was delivered immediately by intraperitoneal (EPO-S, n=25) injection, intramyocardial (EPO-L, n=23) injection or epicardially as an EPO-plus-fibrin patch (EPO-X, n=26). Groups were compared to each other and MI control groups with saline injections (MIC, n=25) or saline-plus-fibrin patch application (MIC-X, n=29). Heart functions were examined under baseline and Dobutamine-induced stress conditions using conductance catheter method 6 weeks after MI. Moreover, blood and myocardium were analysed including candidate genes and proteins at early (24 hours) and late (6 weeks) stages after MI.
RESULTS: Results illustrated superior cardiac functions and healing in EPO-X compared with all groups at 6 weeks after MI. Pressure-volume loops in EPO-X demonstrated 38% improved contractility (dpdtmax), 47% better elasticity (dpdtmin) and 15% enhanced ejection fraction compared with MIC-X. Furthermore, dpdtmax was 33% and 22% higher and dpdtmin was 32% and 23% greater in EPO-X compared with EPO-S and EPO-L, respectively. Infarction size, wall thinning, fibrosis and cardiomyocyte hypertrophy in EPO-X were reduced by 34%, 53%, 48% and 22% compared mit MIC-X, respectively. Early real-time PCR analyses in EPO-X revealed exclusively elevated gene levels of intracardiac stem cell homing factors (SDF-1, CXCR-4, CD34), tissue-transformation factors (TGF-beta, MMP-2), anti-apoptotic Bcl-2 and cell cycle progression factor Cdc2.
CONCLUSIONS: Immediate epicardial EPO-plus-fibrin patch better than systemic or local protein delivery restored cardiac performance after MI and early induced key molecules in myocardial regeneration. Therapeutic efficacy might be local-dose dependent.

Abstract no. 16

title **MRI CONTRAST ENHANCING LABELLING OF MAMMALIAN CELLS VIA MANGANESE AND SUPERPARAMAGNETIC NANOPARTICLES AS TOOLS FOR IN VIVO ANIMAL MODELS**

Authors H. Murua Escobar, K. Sterenczak, S. Willenbrock, M. Meier, S. Petri, C. Junghanß, H. Hedrich, I. Nolte

abstract OBJECTIVE: In vivo animal models play a key role for the evaluation of cell therapeutic approaches in cancer as well as stem cell research. Thereby the possibility to monitor the biologic behaviour of the administered cells in vivo is of major interest. Magnetic resonance imaging (MRI) based visualisation offers a non-toxic, non-invasive and non-lethal option. Superparamagnetic nanoparticles (SPIOs) and manganese allow enhancing MRI contrast providing the possibility to observe specifically labelled cells in vivo.
METHODS: Different mammalian cell types (human CD34+ cells, canine CD34+, various cell lines, and canine dendritic cells,) and numbers were labelled by manganese or SPIOs and their respective MRI detection limit was characterised at 1.0 T or 7.0 T. The in vivo detection was performed using either 10e4–10e5 manganese or 10e5 SPIO-labelled cells injected s.c ot intra spinal into NOD-SCID mice and scanned with 7.0 T MRI, respectively.
RESULTS: In vitro detection limit was determined as 5x10e4 to 10e5 manganese or SPIO labelled cells in 1.0 T MRI and 2.5x10e4 cells in 7.0 T MRI for SIPO labelled cells. In vivo detection showed clear signals of 10e4-10e5 s.c. injected manganese labelled tumor cells as well as 10e5 implanted SPIO-labelled CD34+cells. The manganese labelled tumor cells could be observed up to 15 days. The implanted CD34+ SPIO labelled cells were observed for 96h showing clearly detectable signals.
CONCLUSION: MRI in combination with manganese or SPIOs represents a valuable tool for non-invasive, non-toxic in vivo cell-tracking helping to elucidate the migration characteristics of implanted cells in cell therapeutic approaches or tumor models.

17 Abstract no.

APPROACH TO SENSITIZE GLIOMA STEM CELLS TO STANDARD CHEMOTHERAPY title

A. Limpert, J. Leppert, A. Kerstein*, Y. Alkanan, L. Bähr, V. Tronnier, C. Zechel Authors

INTRODUCTION & OBJECTIVE: Glioblastoma multiforme (GBM) grow invasively, develop resistance to radiation and chemotherapy, and frequently recur. Experimental data proposed that a subpopulation of GBM cells (so-called BTSC) with similarities to neural stem cells survive standard radio-/chemotherapy and become responsible for tumor regrowth. Recently, a genetically engineered mouse model proved that a restricted cell population of slowly dividing GBM cells propagated tumor growth after standard chemotherapy with temozolomide (TMZ).
MATERIALS & METHODS: Self-renewing BTSC from GBMs were transplanted into SCID mice to prove their tumorigenicity. Expression of stemness factors and markers was investigated by immunological techniques and qRT-PCR. BTSC in culture and xenoplants were treated with TMZ and the malaria drug chloroquine (CQ). Effect of CQ/TMZ on BTSC proliferation and survival was determined by BrdU-ELISA, MTT and LDH assays and by tumor growth in SCID mice.
RESULTS & CONCLUSIONS: BTSCs giving rise to proliferating cultures were transplanted into the brain of SCID mice to prove their tumorigenic potential. The BTSC lines which induced tumors in the mouse model with the highest efficacy were selected for further analyses. Firstly, we performed dose curves with TMZ and CQ to determine the effects on proliferation and cell vitality in vitro. Then TMZ was analyzed under suboptimal concentrations in the presence of CQ. Together our results provide evidence that CQ sensitized BTSC for TMZ treatment in a subset of BTSC lines, whereas the synergistic effect of both compounds was absent in others. In general, the effects on proliferation were stronger than those on cell vitality, and CQ promoted autophagy rather than apoptosis. Shrinkage of orthotopic tumors was observed in TMZ-treated SCID mice; in vivo analysis of the co-application of TMZ and CQ is under investigation. Finally, preliminary data suggest an influence of the degree of stemness on the responsiveness of BTSC to TMZ and TMZ/CQ. abstract

18 Abstract no.

ARE MURINE MESENCHYMAL STROMAL CELLS A TRAP FOR HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS? title

D. Reichert, J. Friedrichs, S. Ritter, T. Merkel, C. Werner, M. Bornhäuser and Corbeil Authors

OBJECTIVES: Humanized mice give the opportunity to study human biology with less limitation, and many models were developed for the exploration of the human hematopoietic system. Understanding all molecular details underlying differences between human and mouse system is urged to establish an ideal model.
MATERIALS & METHODS: To dissect these issues, we analyzed the behavior of human CD34+ hematopoietic stem/progenitor cells (HSPCs) growing on either human or mouse mesenchymal stromal cells (MSCs) as feeder cell layer.
RESULTS: We observed after 7 days of culture that human HSPC pools are expanding on MSCs, irrespective of their species origin, by comparison to fibronectin used as supporting matrix. Although the number of CD34+CD133– HSPCs increased in similar proportion, those harboring the more primitive CD133+ phenotypes were significantly reduced on murine MSCs indicating a substantial difference between species. Using scanning electron and time-lapse video microscopies, we observed that the number of human HSPCs exhibiting migrating morphologies was significantly decreased upon culture with murine MSCs by comparison to human ones suggesting that adhesive properties that influence the HSPC polarization may differ between species. By applying atomic force microscopy-based single-cell force spectroscopy, we found that detachment forces of freshly isolated and 7-days cultured CD34+ HSPCs are threefold higher on murine MSCs by comparison to human cells.
CONCLUSIONS: Our data demonstrate that the adhesion properties of human CD34+ HSPCs differ on human by comparison to murine MSCs. Such information raises some caution in data interpretation obtained when murine models are used to study the primitive properties of human HSPCs as well as their migration behaviour for instance in the homing process. abstract

Abstract no. 19

title **MECHANISMS OF MOBILIZATION OF HUMAN PRIMARY PRECURSOR-B-ALL CELLS IN AN IN VIVO MODEL SYSTEM BY THE CXCR4-ANTAGONIST AMD3100 AND BY CATECHOLAMINES**

Authors E.C. Buss, S. Kalinkovich, B. Saeed, A. Schajnovitz, O. Kollet, A. Dar, M. Tesio, S. Fruehauf, M. Hotfilder, L.D. Shultz, A.D. Ho, T. Lapidot

abstract INTRODUCTION: Leukemia stem cells (LSC) are well protected by adhesion to their niche in the bone marrow. Mobilization of LSC to the circulation might render them vulnerable to anti-leukemia therapy. The aim of this study was to explore mechanisms of leukemia mobilization from the BM with AMD3100 (AMD) in a pre-clinical immune deficient mouse model.
METHODS: Immunodeficient mice were engrafted with the childhood pre-B-ALL leukemic cell line G2 and with primary childhood precursor-B-ALL cells from 4 patients with up to 100% of transplanted mice being engrafted.
RESULTS: Treatment with AMD lead to a significant mobilization of all transplanted leukemias with a mobilization level of between 3-8 times above baseline. We also showed that the G2 cell line and all 4 examined precursor-B-ALL samples expressed the catecholamine receptors D3, D5 and beta-2. Treatment with high doses of epinephrine alone lead to leukemia mobilization in vivo similar to AMD treatment. Lower doses of norepinephrine in combination with AMD increased significantly mobilization of leukemia blasts to a maximum of 20 times above baseline.
CONCLUSIONS: We have demonstrated an in vivo xenotranplantation system of primary human precursor-B-ALL cells for research into mobilization of leukemia cells. These leukemic cells can be mobilized efficiently by the CXCR4 antagonist AMD3100 and synergistically by catecholamines. This model for mobilisation of human ALL cells could be potentially used for researching the eradication of minimal residual disease.

Abstract no. 20

title **BIOLUMINESCENCE IN VIVO IMAGING OF GENETICALLY SELECTED IPS CELL-DERIVED CARDIOMYOCYTES AFTER TRANSPLANTATION INTO INFARCTED HEART**

Authors V. Lepperhof, O. Polchynski, K. Kruttwig, C. Brüggemann, F. Drey, K. Neef, Y. Zheng, M. Höhn, Y.-H. Choi, J. Hescheler, T. Šarić

abstract OBJECTIVES: Cell loss after transplantation is a major limitation for their therapeutic applications. To efficiently assess cell retention and facilitate the optimization of procedures for enhancing the cell survival, tools for longitudinal in vivo tracking of viable transplanted cells are needed.
MATERIALS & METHODS: Transgenic murine induced pluripotent stem (iPS) cell lines which, in addition to cardiomyocyte (CM)-specific expression of puromycin N-acetyl-transferase and EGFP, also constitutively express firefly luciferase (Fluc) for bioluminescence (BL) in vivo imaging were generated by electroporation. Fluc-iPSM were used for follow-up by BLI after transplantation into syngeneic murine skeletal muscle and murine cryoinjured hearts.
RESULTS: Puromycin selected Fluc-iPS-CM displayed normal structural and functional properties. Their Fluc activity was significantly lower than in iPSC most likely due to transgene silencing but was still high enough to allow their dose dependent detection in the heart and hind limb muscle when injected above the threshold of 2.5-5x104 cells. Drug selected iPS-CM were poorly retained in the infarcted myocardium but a small population (5%) of iPS-CM still survived for 28 days at the site of injection of most animals.
CONCLUSIONS: These data indicate that BL imaging is a suitable method for noninvasive monitoring of iPS-CM survival in vivo and that Fluc expressing iPS-CM generated in this study will enable further optimization of approaches for improving the survival, integration and therapeutic efficacy of transplanted CM.

21 Abstract no.

DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO LUNG EPITHELIAL CELLS

title

A.S. Ulrich, R. Haller, M. Sgodda, C. Mauritz and U. Martin

Authors

OBJECTIVE: Pluripotent stem cells (PSCs), like embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), offer promising new perspectives for the treatment of lung diseases, e.g. cystic fibrosis (CF), by cellular or tissue replacement therapy, disease modelling and drug screening. On that account, we are aiming at an efficient protocol for the differentiation of human (h)PSCs into functional lung epithelial (progenitor) cells.
METHODS: We make use of a hESC (hES3) NKX2.1-eGFP knockin reporter cell line (kind gift of A. G. Elefanty) to monitor and optimize the first respiratory differentiation steps. The percentage of NKX2.1-eGFP+ cells is measured by flow cytometry. In addition, we are currently characterizing the phenotype of FACS-sorted pure populations of NKX2.1-eGFP+ versus NKX2.1-eGFP- cells by analyzing specific markers using qRT-PCR. Cystic fibrosis transmembrane conductance regulator (CFTR) expressing cells are analyzed by qRT-PCR, immunostaining and western blots.
RESULTS: With our current serum-free monolayer-based protocol we receive around 10 % NKX2.1-eGFP+ cells from hESCs. Preliminary data indicate the respiratory origin of these NKX2.1-eGFP+ progenitors since the thyroidal or neuronal phenotype seem not to be favored. Furthermore, we were able to detect hESC-derived CFTR+ cells, a cell type affected in CF patients. Characterization of those CFTR+ cells is ongoing as well as the differentiation of wildtype hiPSC and mutant or gene-corrected CF-iPSCs into CFTR+ cells.
CONCLUSION: The efficient generation of NKX2.1+ lung epithelial progenitors and CFTR+ cells provide the basis for innovative therapeutic options for the treatment of respiratory diseases like CF.

abstract

22 Abstract no.

A NEW POSSIBILITY TO REPROGRAM BONE MARROW STEM CELLS INTO ENDOTHELIAL LIKE CELLS

title

F. Schlegel, S. Dhein, Ö. Akhavuz, F.W. Mohr, P.M. Dohmen

Authors

OBJECTIVE: This study was performed to investigate the reprogrammation potential of bone marrow stem cells (BMSCs) into functional venous endothelial cells (VECs).
MATERIAL AND METHODS: Pelvic bone marrow aspiration was performed in six mini-pigs to harvest BMSC using a filter with a 100µm cell strainer to determinate bone remains and tissue fragments. Bone marrow-derived mononuclear cells were isolated by sucrose gradient centrifugation, adding endothelial growth factor (16µg/ml) into the culture medium to transform BMSCs into VECs. BMSCs transformation and characterization was examined by immunofluorescence staining for CD 31 (PECAM) and von Willebrandt factor (vWF) staining as well as the expression of endothelial nitric oxide synthase (eNOS). Further NO release was examined by spectrophotometric investigation. To investigate whether transform BMSCs could form angiogenetic tubes in a 3D culture, angiogenesis assay with matrigel was performed.
RESULTS: Reprogrammed BMSCs exhibited a typical cobblestone-like endothelial cell phenotype. Immunofluorescence staining showed that differentiated BMSCs were comparable with mature VECs. Comparing eNOS expression by immunfluorescence staining and western blot of BMSCs and VECs showed no differences. Furthermore reprogrammed BMSCs were positive for the typical mature endothelial cell marker called vWF in all cultures. Spectrophotometric examination showed comparable NO release for reprogrammed BMSCs (550.0±115.0µmol/ml) and VEC (507.5±92.5µmol/ml) under stimulation with 1mmol/l ATP and under control conditions (BMSCs: 72.5±47.5µmol/ml vs. VEC: 55.0±3.8µmol/ml). Angiogenesis assay with matrigel indicated the tube formation of reprogrammed BMSC in vitro after 18h as seen in VECs.
CONCLUSION: This study showed that reprogrammation of BMSCs can successfully performed into typical phenotype VECs. Further studies are needed to evaluate if BMSC can also transformation in other typical phenotype endothelial cells.

abstract

Abstract no. 23

title **IN VITRO DIFFERENTIATION OF MURINE EMBRYONIC STEM CELLS INTO INTESTINAL CELLS**

Authors C. Berger, S. Wilhelm, M. Schweinlin, H. Walles, M. Metzger

abstract OBJECTIVE: In our study, we aim to generate functional intestinal epithelial cells in order to use them as in vitro test model for various biomedical applications. These in vitro differentiated cells provide an alternative, more standardized, source for intestinal cells, which can be used for studying aspects of (tumor) stem cell biology or to build up organotypic tissue cultures as preclinical tool e.g. to test absorption or toxicity of drugs.
METHODS: Murine embryonic stem (ES) cells (CGR8) were stimulated with activin, gsk-3 inhibitor and dorsomorphin to form definitive endoderm. To induce formation of hindgut we used FGF and GSK-3 inhibitor and subsequently formed organoids were cultured in matrigel. Final maturation was achieved by addition of EGF, Noggin and R-spondin. Cells were characterized on mRNA and protein level using qPCR, immunocytochemistry and flow cytometry techniques. Markers were sox17 foxa2 (definitve endoderm), cdx2, klf5, cxcr4 (hindgut specification) and villin, cdx1, Bmi1, Mucin2, chromogranin A, IAP, lysozcyme, E-cadherin, ZO-1 (intestinal differentiation).
RESULTS: Treatment with GSK-3 inhibitor, Dorsomorphin and Activin led to a population expressing markers for DE (Sox17 and Foxa2) and simultaneous loss of embryonic markers (Oct4, Sox2, nanog). FACS analysis revealed a Sox17+/Foxa2+ population of about 20%. Further specification to hindgut cells was confirmed by up-regulation of specific genes such as cdx2. Long-time culture of intestinal organoids showed cell types expressing markers for proliferative, secretory and resorptive cell entities, which are organized as polarized structure including crypts, villi and tight junctions. Organoids could be propagated over several passages.
CONCLUSIONS: In order to build up a reliable and easy to reproduce intestinal test system, ES cells could be a novel and elegant cell source. In line with recently published data for human ES cells, our findings indicate that our differentiation protocol is also feasible to generate murine intestinal cells in vitro. Next steps would be a functional characterization of the generated epithelial cells and the attempt to culture these cells on a biological scaffold in order to provide a tight barrier system suitable for biomedical applications mentioned above.

Abstract no. 24

title **A BIOENGINEERED MATRIX WITH PHYSIOLOGICAL ELASTICITY FACILITATES LONG-TERM CULTURE OF PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES**

Authors C.O. Heras, N. Schloerer, S. Diluweit, K. Brockmeier, J. Hescheler, K. Pfannkuche

abstract OBJECTIVES: Induced pluripotent stem cell derived cardiomyocytes (iPSCMs) have the ability to actively sense their mechanical environment and respond to substrate stiffness by altering their adhesion, morphology and function. To elucidate the impact of different stiffnesses ranging from embryonic myocardial tissue (6-10 kPa) to polystyrene (3-3.5 gPa) on iPSCMs, we used the polyacrylamide hydrogel model.
MATERIALS & METHODS: PA gels were used as substrate for iPSCMs attachment. Three values of stiffness were selected. Elastic modulus of each selected stiffness was measured by AFM (Atomic Force Microscopy) using the Hertz model. To facilitate cell adhesion, a protein was cross-linked to the PA gel surface. Three cross-linkers were used, two commercially available and one which was synthetized and characterized using NMR.
RESULTS: The elasticity properties of the gel were obtained by AFM. Different spots of each gel were measured and the force-displacement curves were plotted. Sample A=12 kPa (k=1,1N/m, Cone:20°), Sample B=49 kPa (k=42N/m, Cone:18°) and Sample C=165 kPa (k=42N/m, Cone:18°). Fibronectin was coupled the polyacrylamide matrix by means of a cross-linker. N-Succinimidyl Acrylate and Sulfo-Sanpah were tested and compared to the synthetized cross-linker. NMR spectroscopy showed a purity of 99% for the synthesized Compound I. Our results indicate that the attachment of iPS cell derived cardiomyocytes was improved for the Compound I, with the possibility of long term culture (+6 weeks).
CONCLUSIONS: Our preliminary data suggests that long term culture of cardiomyocytes is supported by the elastic matrix, facilitating detailed characterization of long-term effects of various matrix stiffnesses and surface ligands on iPS cell-derived cardiomyocytes.

25 Abstract no.

GENETIC MANIPULATION OF NEURAL PROGENITOR CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS BY USING ZINC FINGER NUCLEASES

title

N. Zare Mehrjardi, H. Baharvand, K Neef, J. Hescheler, T. Šarić

Authors

OBJECTIVE: Induced pluripotent stem cell derived neural progenitor cells (NPCs) represent a valuable source of neural cell lineages for research, drug testing and potential therapeutic applications. Optimal utilization of NPCs in these areas requires improved protocols for their genetic manipulation. Random insertion of transgenes into the NPC genome is frequently accompanied by transgene silencing and may lead to insertional mutagenesis. Here, we tested the utility of zinc finger nuclease (ZFN) technology for targeted insertion of selectable markers into a genomic safe harbor locus of NPCs to avoid gene silencing and deleterious effects on cell function.
METHODS: Long-term self-renewing NPCs capable of differentiation into neurons, astrocytes and oligodendrocytes were generated from human iPSCs using published protocols. ZFNs were used to target the insertion of a gene cassette in which EF1-α promoter drives the expression of a puromycin resistance gene and a reporter marker GFP into AAVS1 locus in NPCs. Stable transgenic NPCs were selected and characterized.
RESULTS: DNA sequencing confirmed that the transgene cassette was successfully integrated into the targeted AAVS1 locus. Transgenic NPCs were indistinguishable from parental NPCs in regard to proliferation rate, marker expression and potential for in vitro differentiation into neurons, astrocytes and oligodendrocytes. Moreover, ZFN-engineered NPCs integrated into the neonatal mouse brain cortex, differentiated toward neural cell lineages retaining the GFP expression and did not form teratoma two months after transplantation.
CONCLUSION: These data indicate that ZFN can be successfully employed for gene manipulation in human iPSC-derived NPCs without causing gene silencing and perturbation of cell function.

abstract

26 Abstract no.

GENERATION OF ENDOTHELIAL CELLS FROM SCALABLE CULTURES OF UNDIFFERENTIATED HUMAN PLURIPOTENT STEM CELLS

title

R. Olmer, S. Becker, R. Voswinckel, U. Martin

Authors

OBJECTIVE: Full endothelialisation of gas exchange membranes in extracorporal membrane oxygenation devices for improved hematocompatibility, or cell therapy of pulmonary hypertrophy requires large amounts of (patient-specific) ECs. ECs can be isolated from peripheral blood or explanted vessels, however, success rates of EC isolation from blood are low and especially ECs from older individuals show a limited proliferation capacity. Patient specific ECs from hPSCs might be an alternative cell source. The opportunity to generate large amounts of hPSC in defined media under scalable monitored conditions allows for the generation of cell numbers in dimensions which are suitable for cellular therapies. By differentiation of these well monitored cell populations a virtually unlimited number of autologous ECs may become available.
METHODS & RESULTS: Utilization of BMP4 and VEGFA for the differentiation resulted in up to 12% of CD31+ cells. Substitution of BMP4 by a small molecule could increase the amount to 20% CD31+ cells. FACS-sorted CD31+ iPSC derivatives are currently characterized in detail. In addition, the generation of transgenic hPSC reporter lines, which express a fluorescence reporter / antibiotic resistance under the control of EC specific promotors for further improvement of differentiation is in progress.
CONCLUSION: Resulting (patient-specific) iPSC-derived ECs will represent a novel cell source for disease modelling or biofunctionalization of gas exchange membranes. In addition, TALEN-based gene correction in iPSCs might enable novel concepts of ex vivo gene therapy for respiratory diseases.

abstract

Abstract no. 27

title **LINE1-MEDIATED RETROTRANSPOSITION IN HUMAN PLURIPOTENT STEM CELLS: CONSEQUENCES FOR GENOMIC STABILITY OF HES AND HIPS CELLS AND THEIR DERIVATIVES**

Authors A. Witthuhn, U. Martin, A. Haase, G. G. Schumann

abstract OBJECTIVES: Human pluripotent stem cells (hPSCs) are considered as favourite cell source for regenerative medicine. Recent findings indicate that potentially tumorigenic chromosomal abnormalities and mutations arise in hPSCs during their generation, expansion and differentiation. Such mutations could be induced by human non-LTR retrotransposons (LINE1, Alu, SVA). It has been reported that the reprogramming process towards hiPSCs might enhance the activation of LINE1s. Similar levels of active LINE1s were found in hESCs and hiPSCs but not in parental cells. Hence, we aim to ascertain how LINE1 mobilization affects the genomic integrity of hPSCs and their differentiated derivatives.
METHODS: Aim of the study is to investigate retrotransposition rates in hPSCs and their derivatives. LINE1 expression levels will be assessed using immunoblottings, immunofluorescence stainings and RT-PCR. Using a novel retrotransposition reporter assay. we will assess whether LINE1 activity may cause genetic aberrations. Furthermore, we will analyse the LINE1 mediated genomic destabilization and preferential integration sites by Array-CGH and high-throughput sequencing.
RESULTS: We adapted LINE1 reporter vectors to the use in hPSCs and for stable integration into the AAVS1 safe harbour locus. Successful reporter expression and retrotransposition, in hiPSCs were proven by expression of retrotranspositon dependent G418 resistance. Furthermore, we detected increased LINE1 protein levels via immunoblottings and immunofluorescence stainings.
CONCLUSIONS: We successfully proved that retrotransposition of engineered LINE1s is supported in hiPSCs. Experiments are ongoing to assess retrotransposition rates and integration preferences in hPSCs during long-term culture and differentiation. Also ongoing experiments will demonstrate whether LINE1 retrotransposition may induced genetic aberrations and can be considered as one underlying reason for the reported instabilities of hiPSCs and hESCs. would be a functional characterization of the generated epithelial cells and the attempt to culture these cells on a biological scaffold in order to provide a tight barrier system suitable for biomedical applications mentioned above.

Abstract no. 28

title **YOUNG VERSUS AGED CELL SOURCES - GENETIC ABERRATIONS IN iPS CELLS AND THE EFFECT OF PRIMARY CELL PROLIFERATION ON REPROGRAMMING EFFICIENCY**

Authors K. Osetek, A. Haase, G. Göhring, U. Martin

abstract OBJECTIVE: The quality of iPSCs derived from somatic cells of aged individuals may be critical for the production of clinically useful cell products. In fact, the proliferative capacity of primary cells from aged individuals is typically lower than in young cell sources. Also, nuclear and mitochondrial mutations in adult stem cells and differentiated somatic lineages appear to accumulate over a lifetime and have been suggested to contribute to aging and cancer formation.
It is thus aim of our study to comparatively investigate reprogramming efficiencies and rates of genomic abnormalities in iPSCs from old versus young cell sources. To exclude cell-type-specific effects as far as possible, we decided to focus on endothelial cells (ECs) derived from different cell sources including cord blood and umbilical cord, as well as on ECs isolated from peripheral blood and saphena veins of adult / elderly donors.
METHODS: Population doublings (PDs) were determined in early passage ECs. Cells were reprogrammed with lentiviral vectors expressing Thomson or Yamanaka factors and reprogramming efficiencies were correlated to PDs. Karyotype analyses of iPSCs were performed. Exom sequencing has been performed and detected SNPs will be confirmed by PCR.
RESULTS: ECs from blood of adult patients could be isolated in a very small portion of samples, only. Although not significant, a tendency for higher proliferation rates in cells from younger sources was observed. In contrast, reprogramming rates both with Thomson and Yamanaka constructs were 10-100 fold higher in young cell sources. ECs from old sources could be reprogrammed with c-Myc, only. Karyotyping revealed chromosomal aberrations in some of iPSC from elderly patients, whereas all iPSC clones from young sources had normal karyotype. Bioinformatic analysis of exom sequencing data is ongoing.
CONCLUSIONS: Our preliminary data indicate that young cell sources are easier to reprogram and iPSCs derived from young cell source may be of superior quality compared to iPSCs from elderly patients. These features make them promising candidates for future clinical applications.

29 Abstract no.

TRANSCRIPTION FACTORS, MICRORNAS AND THEIR INTERACTIONS DURING CARDIOMYOCYTE-SPECIFIC DIFFERENTIATION OF MURINE EMBRIONIC STEM CELLS

title

L. Gan, and B. Denecke

Authors

OBJECTIVES: In the present study, we profiled the gene- and microRNA (miRNA)-expressions of murine ESCs during their differentiation to cardiomyocytes to improve the knowledge of gene expression regulation during the differentiation process of embryonic stem cells (ESCs)on the transcriptional level.
METHODS: Murine ESCs were differentiated to cardiomyocytes with over 99% purity under selection marker. Gene expressions of undifferentiated ESCs (day0) and cardiomyocytes at different maturation stages (day12, day19, and day26) were profiled using Affymetrix GeneChip® Mouse exon arrays. miRNA expressions were measured for the same samples on both Affymetrix miRNA 1.0 and Febit Biochips miRNA platforms. Differentially expressed genes and microRNAs during differentiation of ESCs were identified with Bioconductor packages under R. An undirected Gaussian graphical Markov model was applied on expression profiles in the purpose of exploring gene expression regulatory networks involved in cardiomyocyte-specific differentiation of murine ESCs.
RESULTS: Under strict conditions 1374 genes (69 of which were transcription factors) were identified as regulated between ESC-derived cardiomyocytes and undifferentiated ESCs. Furthermore, fifty microRNAs were found regulated from the result of both Affymetrix and Febit platforms. Comprehensive regulation networks with transcription factors, microRNAs and regulated expressed genes could be constructed based on the gene and microRNA expression profiles with the help of targeting information of transcription factors and miRNAs.
CONCLUSIONS: The regulatory mechanisms of transcription factors and miRNAs are not isolated but interacted. With our study, both gene and miRNA expressions were profiled for cardiomyocyte-specfic differentiation process of murine ESCs. These results reveal to us the possibility to improve the understanding of the transcription factor/miRNA regulatory networks during cardiomyocyte-specific differentiation in an interactive way.

abstract

30 Abstract no.

OPTOGENETIC CONTROL OF STEM CELL-DERIVED BIOARTIFICIAL CARDIAC TISSUE

title

M. Bakar, G. Kensah, S. Saito, J. Dahlmann, H. Baraki, U. Martin, I. Kutschka, P. Sasse, I. Gruh

Authors

OBJECTIVE - Electrical stimulation is used in cardiac tissue engineering for the enhancement of cell contractility and maturation. However, tissue damage due to faradaic reactions might occur and shows the need for alternative stimulation approaches. Therefore, we aimed to construct a light-sensitive bioartificial cardiac tissue (BCT) from murine embryonic stem cell (mESC)-derived cardiomyocytes expressing channelrhodopsin, a light-activated cation channel.
METHODS - Cardiomyocytes were generated from a transgenic mouse ESC line expressing channelrhodopsin under control of the chicken β-actin promoter. BCTs were prepared from cardiomyocytes and fibroblasts with liquid collagen and Matrigel. Stimulation was performed with a 470 nm high power LED and contraction forces measured in a bioreactor. Threshold light intensity for pacing and effect of long-term (14 d) stimulation of BCT were tested. Micro-electrode-arrays (MEA) were used to investigate the light-induced field potentials in BCTs.
RESULTS - Light-inducible BCTs showed spontaneous beating activity, indicating that channelrhodopsin did not cause a leaky current. The magnitude of light-induced contractions was found to depend on two factors: light intensity and stimulus duration. Beating frequency and field potential analysis showed that the frequency could be controlled with 1:1 capture up to 300 bpm. Furthermore, no side effects were observed in BCTs after long-term light stimulation.
CONCLUSIONS - Light stimulation can be used as an alternative approach to electrical stimulation of cardiac constructs and its potential for improvement of cardiomyocyte maturation and tissue function will be further investigated. We are currently testing BCT excitability and coupling to the host myocardium after transplantation to rat myocardium.

abstract

Abstract no. 31

title **MONITORING DIFFERENTIATION OF PLURIPOTENT STEM CELLS TO CARDIOMYOCYTES BY MEASURING CARDIAC TROPONIN T RELEASE INTO THE CELL CULTURE MEDIUM.**

Authors K. Burkert, D. Ivanyuk, B. Baudis, K. Ko, H. Schöler, J. Hescheler and T. Šarić

abstract OBJECTIVE: In order to achieve a reliable and reproducible production of cardiomyocytes (CM) derived from embryonic stem (ES) and induced pluripotent stem (iPS) cells for regenerative medicine approaches, suitable strategies for monitoring the kinetic and efficiency of stem cell differentiation are mandatory.
METHODS: In this study cardiac Troponin T (cTnT) in medium supernatant was detected by ELISA during and after cardiac differentiation in vitro.
RESULTS: We detected rapid cTnT release into the medium supernatant from purified CM derived from transgenic murine iPS cells. In spontaneously differentiating cultures cTnT was detectable in the medium concomitant with the first appearance of CM in embryoid bodies. Robust cTnT release was also detected in medium of native CM isolated from murine neonatal hearts, as well as in cardially differentiating cultures of unselected murine germline-derived pluripotent stem (gPS) cells and human iPS cells. Moreover, the enhancement of cardiac differentiation efficiency in murine ES cell cultures by ascorbic acid treatment was accompanied by higher cTnT concentrations in the medium as compared to non-treated cultures, indicating good correlation between cTnT medium content and CM yield in embryoid bodies. Measurements of lactate dehydrogenase (LDH) activity and caspase activation revealed that cTnT was not released from apoptotic or necrotic cells. Rather, cTnT appeared to be secreted as a full length protein as revealed by immunoprecipitation with cTnT-specific antibody.
CONCLUSIONS: Collectively, these observations provide a potential means for monitoring CM production that is highly specific, simple and broadly applicable to any cell line without requirements for a specific genetic cell modification. The biology and mechanism behind the cTnT secretion by viable CM remain to be elucidated.

1 Abstract no.

DIRECTED FORWARD PROGRAMMING OF PLURIPOTENT STEM CELLS TOWARDS PACEMAKER CELLS USING THE TRANSCRIPTION FACTOR TBX3 title

Julia Jung1, Juliane Stieber2, Britta Husse3, Christian Rimmbach1, Christoph Brenner3, Evelyn Fischer3, Andreas Dendorfer4, Wolfgang-Michael Franz3 and Robert David Authors

OBJECTIVE: The "sick sinus syndrome" comprises pathological, symptomatic sinus bradycardia, sinoatrial block, sinus arrest as well as the tachycardia-bradycardia syndrome. It is often accompanied by general cardiac diseases such as an ischemic heart disease, cardiomyopathies or myocarditis. As an alternative to the present therapeutical approach of artificial pacemakers, the objective was to generate "biological pacemakers" out of pluripotent stem cells. abstract

METHODS: Our group has previously shown that "forward programming" of pluripotent stem cells towards specific cardiomyocyte subtypes is feasible via overexpression of distinct early cardiovascular transcription factors such as MesP1 (early/intermediate type cardiomyocytes) and Nkx2.5 (ventricular cells). In order to generate pacemaker cells we used the T-box transcription factor Tbx3, as it is required for normal size and function of the sinoatrial node (SAN). Tbx3 is a transcriptional repressor acting to suppress the atrial myocyte phenotype and therefore specifying SAN versus atrial cells.

RESULTS: Here we first demonstrate that forced expression of the T-box transcription factor Tbx3 similar to MesP1 and Nkx2.5 is sufficient to enhance cardiogenesis in murine embryonic stem cells (mES). The number of spontaneously contracting foci increases dramatically. Likewise, protein expression levels corresponding to cardiac and in particular SA/AV markers are enriched. Detailed patch clamping analyses and funnel channel density measuring revealed electrophysiological characteristics corresponding to all subtypes of cardiac cell differentiation, yet with a highly distinct percental distribution as compared to controls. In particular, Tbx3 enhanced the appearance of pacemaker cells by 2fold, similar to the effect of Nkx2.5 and MesP1 on ventricular and early/intermediate cell types, respectively.
To further increase the effect, we next developed optimized culture conditions of the programmed cells. This led to cellular aggregates termed "programmed nodal bodies ("pNBs") with highly increased beating frequencies of 300-400bpm, containing 70% of SA/AV cells as evident from single cell patch clamping and FACS analyses.
Additionally performed Ca2+ measurements showed that the Calcium gradients within pNBs are sensitive to inhibition of the HCN4 channel ("funny channel"), a characteristics of pacemaker cells. Furthermore, they undergo a typical decrease in their beating frequencies after inhibition of voltage-sensitive Calcium channels (L- and T- type Ca2+-channels). Likewise, administration of caffeine induced a Ca2+ peak with an amplitude equivalent to the spontaneous Ca2+-transients and blockage of the Na-Ca-exchanger suppressed normal Ca-transients. Additional inhibition of SERCA led to intracellular Ca2+ accumulation and a Ca2+depleted sarcoplasmic reticulum (SR) as evident from a reduced response to caffeine. Therefore various physiological parameters confirm the pacemaker phenotype of our cells.
To further address the functionality of the pNBs we took advantage of the ex vivo system of cultivated ventricular slices. Remarkably, the pNBs are capable of integrating into the slice tissue, will remain spontaneously active and are able to elicit robust contractions of the adult mouse heart slice in their vicinity.

CONCLUSION: These data show that our pNBs achieved via Tbx3 based programming in addition to optimized differentiation exhibit electrophysiological characteristics of cardiac pacemaker aggregates and are able to stimulate contractions within adult mouse heart tissue ex vivo. Therefore, to the best of our knowledge we provide the first example of pluripotent stem cell derived functional nodal tissue.of the transcription factor/miRNA regulatory networks during cardiomyocyte-specific differentiation in an interactive way.

ABSTRACTS

Abstract no. 2

title **POSITRON EMISSION TOMOGRAPHY BASED IN-VIVO IMAGING OF EARLY PHASE STEM CELL RETENTION AFTER INTRAMYOCARDIAL DELIVERY IN THE MOUSE MODEL**

Authors Cajetan Lang3 ,Sebastian Lehner, MD2 , Andrei Todica, MD2 , Guido Boening, PhD2, Wolfgang-Michael Franz, MD4, Peter Bartenstein, MD2, Marcus Hacker, MD2, Robert David, Ph

abstract PURPOSE: To establish Positron Emission Tomography (PET) as a tool for in-vivo quantification and monitoring of intramyocardially transplanted stem cells after labeling with [18F]-fluorodeoxyglucose (FDG) in mice with induced myocardial infarction.
METHODS: After inducing myocardial infarction in C57BL/6 mice, murine embryonic stem cells were labeled with FDG and transplanted into the border zone of the infarction. Dynamic PET-scans were acquired from 25 to 120 min after transplantation, followed by a scan with 20 MBq FDG i.v. for anatomical landmarking. All images were reconstructed using the OSEM3D and MAP reconstruction algorithms. FDG data was corrected for cellular tracer efflux and used as marker for cellular retention. FACS analyses of EGFP expressing transplanted cells were performed to validate the PET data.
RESULTS: We observed a rapid loss of cells from the site of transplantation, followed by stable retention over 120 minutes. Amounts of retention were 5.3±1.1 % at 25 min, 5.0±0,9 % at 60 min and 5.7±1.2 % at 120 min. Results from FACS analyses showed a high correlation without significant differences between the groups (P>0.05). FDG-labelling did not have any adverse effects on cell proliferation and differentiation.
CONCLUSIONS: Relying on up-to-date imaging we cloud equip the mouse model with a powerful method for tracking and quantifying intramyocardially transplanted stem cells in-vivo. This revealed a massive cell loss within minutes whereas thereafter a relatively stable amount of ~ 5% remaining cells was observed. Our method may become crucial for further optimization of cardiac cell therapy in the widely used mouse model of infarction.

Abstract no. 3

title **ERYTHROPOETIN AND CARDIAC REPAIR**

Authors C Klopsch, G Kleiner, R Gäbel, A Skorska, M Ludwig, P Mela, S Jockenhoevel, G Steinhoff.

abstract OBJECTIVE: High-dose intramyocardial Erythropoietin (EPO) therapy protects the myocardium from ischemic injury and promotes beneficial remodelling. We investigated the therapeutic efficacy of EPO at a moderate dose level and compared local and systemic delivery routes in a rat myocardial infarction (MI) model.
METHODS: Following MI, EPO (300 U/kg) was delivered immediately by intraperitoneal (EPO-S, n=25) injection, intramyocardial (EPO-L, n=23) injection or epicardially as an EPO-plus-fibrin patch (EPO-X, n=26). Groups were compared to each other and MI control groups with saline injections (MIC, n=25) or saline-plus-fibrin patch application (MIC-X, n=29). Heart functions were examined under baseline and Dobutamine-induced stress conditions using conductance catheter method 6 weeks after MI. Moreover, blood and myocardium were analysed including candidate genes and proteins at early (24 hours) and late (6 weeks) stages after MI.
RESULTS: Results illustrated superior cardiac functions and healing in EPO-X compared with all groups at 6 weeks after MI. Pressure-volume loops in EPO-X demonstrated 38% improved contractility (dpdtmax), 47% better elasticity (dpdtmin) and 15% enhanced ejection fraction compared with MIC-X. Furthermore, dpdtmax was 33% and 22% higher and dpdtmin was 32% and 23% greater in EPO-X compared with EPO-S and EPO-L, respectively. Infarction size, wall thinning, fibrosis and cardiomyocyte hypertrophy in EPO-X were reduced by 34%, 53%, 48% and 22% compared mit MIC-X, respectively. Histological improvements in EPO-L and EPO-S were only moderate. Early real-time PCR analyses in EPO-X revealed exclusively elevated gene levels of intracardiac stem cell homing factors (SDF-1, CXCR-4, CD34), tissue-transformation factors (TGF-beta, MMP-2), anti-apoptotic Bcl-2 and cell cycle progression factor Cdc2. Blood analyses suggested, healing effects in EPO-X might have been hematopoiesis independent.
CONCLUSIONS: Immediate epicardial EPO-plus-fibrin patch better than systemic or local protein delivery restored cardiac performance after MI and early induced key molecules in myocardial regeneration. Therapeutic efficacy might be local-dose dependent.

This work was supported by the German Ministry of Education (BMBF; Germany, Berlin; funding indicator 0312138 A), the Ministry of Economy (Mecklenburg-West Pommerania, Schwerin; funding indicator RTC V220-630-08-TFMV-F/S-035), and the German Research Foundation (DFG; Germany, Berlin; funding indicator SFB TR37, TPA4).

4 Abstract no.

QUANTIFCATION OF THE IN VIVO MIGRATORY CAPACITY OF HUMAN CD 133+ BONE MARROW STEM CELLS AFTER INTRAOPERATIVE HARVESTING FROM THE STERNAL BONE MARROW title

P Donndorf, D Useini, CA Lux, B Vollmar, E Delyagina, M Laupheimer, A Kaminski and G Steinhoff Authors

OBJECTIVES: Human bone marrow stem cell populations have been applied for cardiac regeneration purposes within different clinical settings in the recent past. The migratory capacity of applied stem cell populations towards injured tissue, after undergoing specific peri-interventional harvesting and isolation procedures, represents a key factor limiting therapeutic efficacy. We therefore aimed at quantifying the migratory capacity of human cluster of differentiation (CD) 133+ bone marrow stem cells in vivo after intraoperative harvesting from the sternal bone marrow.
METHODS: Human CD133+ bone marrow stem cells were isolated from the sternal bone marrow of patients undergoing cardiac surgery at our institution. Migratory capacity towards stromal cell-derived factor 1 alpha (SDF-1α) gradients was tested in vivo by intravital fluoresecence microscopy, utilizing the cremaster muscle modell in severe combined immunodeficiency mice and analysing CD133+ cell interaction with the local endothelium. Furthermore, the role of a local inflammatory stimulus for CD133+ cell interaction with the endothelium was studied. Endothelial response upon chemokine stimulation was described using laser scanning microscopy of histological cremaster muscle samples.
RESULTS: SDF-1α alone was capable to induce relevant early CD133+ cell interaction with the endothelium, indicated by the percentage of rolling CD133+ cells (45.9 ± 1.8 % in 'SDF-1' vs. 17.7 ± 2.7% in 'control', $p < 0.001$) and the significantly reduced rolling velocity after SDF-1α treatment. Furthermore, SDF-1α induced firm endothelial adhesion of CD133+ in vivo. Firm endothelial adhesion, however, was significantly enhanced by additional inflammatory stimulation with tumor necrosis factor-alpha (TNF-α) (27.9 ± 4.3 cells/mm2 in ' SDF-1+TNF' vs. 2.2 ± 1.1 cells/mm2 in 'control', $p < 0.001$).
CONCLUSIONS: CD133+ bone marrow stem cells exhibit sufficient in vivo homing towards SDF-1α gradients in an inflammatory microenvironment after undergoing standardized intraoperative harvesting from the sternal bone marrow. abstract

5 Abstract no.

CELL SEEDING TECHNOLOGIES IN HEART VALVE TISSUE ENGINEERING title

C Klopsch, R Gaebel, A Kaminski, B Chichkov, S Jockenhoevel, G Steinhoff Authors

OBJECTIVE: At present intensive investigation aims at the creation of optimal valvular prostheses. We introduced and tested two advanced cell-plus-matrix seeding technologies, the spray-assisted-bioprocessing (SaBP) and the laser-assisted bioprocessing (LaBP), for intraoperative table-side autologous tissue engineering (TE) of bioresorbable artificial grafts.
METHODS: Three-leaflet valves were manufactured following TE of electrospun poly ε-caprolactone (PCL) tissue equivalents. For SaBP, human mesenchymal stem cells (HMSC), umbilical cord vein endothelial cells (HUVEC) and fibrin were simultaneously spray-administered on PCL substrates. For LaBP, HUVEC and HMSC were separately laser-printed in stripes followed by fibrin sealing. Tissue equivalents were monitored in-vitro under static and dynamic conditions in bioreactors.
RESULTS: SaBP and LaBP resulted in TE of grafts with homogenous cell distribution and accurate cell pattern, respectively. Engineered valves demonstrated immediate sufficient performance, complete cell coating, proliferation, engraftment, HUVEC-mediated invasion, HMSC differentiation and extracellular matrix deposition. SaBP revealed higher efficiency with at least 12-fold shorter processing time than the applied LaBP setup. LaBP realized coating with higher cell density and minimal cell-to-scaffold distance. Fibrin and PCL stability remain issues for improvement.
CONCLUSIONS: The introduced TE technologies resulted in complete valvular cell-plus-matrix coating, excellent engraftment and HMSC differentiation. SaBP might have potential for intraoperative table-side TE considering the procedural duration and ease of implementation. LaBP might accelerate engraftment with precise patterns. abstract

ABSTRACTS

Abstract no. 6

title **TISSUE ENGINEERED TRANSCATHETER HEART VALVES AFTER CRIMPING AND PERFUSION**

Authors I. Sabel1, T. Hollweck1, C. Fano2, M. Dauner2, E. Wintermantel3, C. Hagl1, B. Akra

abstract OBJECTIVE: Tissue engineered stented heart valves are promising as prospective transcatheter heart valve prostheses. The aim of this study was to analyse the mechanical integrity of polymer-based percutaneous heart valve prostheses after crimping and perfusion.
Methods: Stented polyurethane heart valves were consecutively seeded with human saphenous vein fibroblasts and endothelial cells by using a special 3D-rotating bioreactor. Seeded valves were crimped and exposed to a flow of 1.5 l/min for three days at physiologic conditions. The valve was analysed by micro-computerized tomography and endoscopy. Perfused specimens were analysed by scanning electron microscopy and immunohistochemistry.
Results: Micro-computerized tomography showed no damage of the prostheses after crimping. Endoscopic analysis showed a regular, partial opening of the cusps. A cellular coating of the heart valve with partial damage of the endothelial and fibroblast layer was demonstrated by immunohistochemistry. Scanning electron microscopy revealed a cellular layer with scattered ruptures.
Conclusion: Non-invasive micro-computerized tomography can be used for the detection of structural damage on biological tissue. Crimping process affect strongly the performance of the seeded product.

Abstract no. 7

title **INFLUENCE OF DIFFERENT FIXATIVES ON THE MECHANICAL PROPERTIES OF CELL-SEEDED SCAFFOLDS**

Authors T. Mayer1, T. Hollweck1, D. Fehrenbach1, C. Fano2, M. Dauner2, E. Wintermantel3, C. Hagl1, B. Akra1

abstract OBJECTIVE: Various standardized test methods are not suitable for testing vital tissue engineered heart valves. The aim of this study was to determine the mechanical properties and topographies of cell-seeded patches after different conservation procedures.
METHODS: Non-degradable polyurethane specimens were statically seeded with human saphenous vein derived fibroblasts (group A), endothelial cells (group B) as well as fibroblast and endothelial cells (group C). Specimens from Group A and B were either fixed in glutaraldehyde or in formalin solution for 5 d at 4 °C. In group C, storage was performed using glutaraldehyde solution, formalin solution, medium M199 or Chillprotec® for 5 d at 4 °C as well as in medium M199 supplemented with 10 % dimethylsulfoxide for 5 d at -20 °C. Analogously seeded samples without conservation treatment were used as references. Mechanical properties were evaluated by determining the Young's modulus according to DIN EN ISO 527-1. Confluence and topography of seeded constructs were observed using scanning electron microscopy.
RESULTS: Conservation procedures affected Young's Modulus of seeded samples. After conservation, Young's Modulus increased in group A and B compared to untreated samples. In group C, Young's Modulus decreased after conservation with glutaraldehyde solution, formalin solution, medium M199 and medium M199 supplemented with 10 % dimethylsulfoxide. Chillprotec®-conservation showed a comparable Young's Modulus to untreated samples. SEM analysis of all treated groups displayed a similar topography compared to samples without conservation treatment
CONCLUSIONS: Conservation solutions affect the mechanical properties of tissue engineered constructs dramatically, however, the usage of one of these solutions allow testing of seeded scaffolds.

8 Abstract no.

CONDITIONING OF COLONIZED HOMOGRAFTS FOR A TIME PERIOD OF 12 DAYS UNDER STERILE AND CONTROLLED CONDITIONS

title

N. Haller1,2, T. Hollweck1, F. König2, N. Thierfelder1, E. Wintermantel2, C. Hagl1, B. Akra1

Authors

abstract

Objective: Cardiovascular tissue engineering has emerged as a promising approach to overcome the limitations of conventional heart valve substitutes by mimicking a native heart valve. The present study demonstrates that long-term conditioning of decellularized and re-seeded aortic homografts in a low-flow pulsatile bioreactor results in an improved quality of tissue engineering constructs.
Methods: Cryopreserved and thawed homografts were decellularized by a detergent mixture. Decellularized homografts were primarily seeded with fibroblasts followed by colonization with endothelial cells, both isolated from human saphenous vein segments. Re-seeded homografts were exposed to low-flow conditions (750 – 1100 mL/min) for a time period of 12 d. Topographical examination was performed by scanning electron microscopy. Cell layer thickness, composition of extracellular matrix and inflammatory response was investigated by immunohistochemistry.
Results: Scanning electron microscopy of re-seeded homografts showed a confluent and intact cellular coverage before and after conditioning. Immunohistochemical analysis demonstrated a distinct thickening of cellular layer. Cell specific staining demonstrated a confluent endothelial lining (CD31, VE-cadherin) with a multilayer of fibroblast (TE-7) underneath. The expression of extracellular matrix components (collagen IV, fibronectin), cytoskeletal (α-actin) and gap junctional proteins (connexin 43) increased by conditioning. Inflammatory proteins (ICAM-1, VCAM-1) were expressed in a low level.
Conclusions: Conditioning of tissue engineered constructs results in an increased quality of extracellular matrix in regard to connectivity, stability and cell communication, creating native-like prostheses.

9 Abstract no.

MECHANICAL INTEGRITY OF DECELLULARISED, STENTED AND RE-SEEDED HUMAN AORTIC VALVES AFTER CRIMPING AND PERFUSION

title

S. Eilenberger, T. Hollweck, U. Haas, A. Uhlig, C. Hagl, B. Akra

Authors

abstract

Objective: About 3 % of the population in Germany older than 75 years suffers from aortic valve diseases. In high-risk patients, transcatheter valve replacement is becoming the gold standard for the treatment of end stage aortic valve stenosis. The aim of this study was to develop a transcatheter homograft and to determine the mechanical integrity of decellularised and re-seeded valves after crimping and perfusion procedures.
Methods: Fibroblasts and endothelial cells were isolated from human saphenous vein segments and expanded in culture. Homografts were thawed according to a standard procedure and decellularized by detergents. After decellularisation, valves were stented and consecutively seeded with fibroblasts and endothelial cells using a special dynamic seeding device. To evaluate the mechanical integrity of the new created valves, crimping and perfusion steps were applied. Stented homografts were exposed to physiologic flow conditions (t1 = 24 h at 1 l/min, t2 = 24 h at 1.5 l/min, t3 = 24 h at 2 l/min) using a high flow bioreactor with a computer-guided actuation unit. Nativ, decellularized, re-seeded and perfused samples were analyzed by scanning electron microscopy and immunohistochemistry.
Results: Scanning electron microscopy demonstrated a confluent cellular coating of re-seeded homografts. Perfused samples revealed an inhomogenous cellular coating. A partial damaged endothelial lining with an intact fibroblast layer underneath was also detected by immunohistochemistry.
Conclusions: The perfusion of tissue engineered transcatheter homografts affects the integrity of the endothelial coating. This is probably due to the crimping process that induces invisible damages in the endothelial cell layer.

Abstract no. 10

title **CO-CULTURES OF CARDIOMYOCYTES AND BONE MARROW MESENCHYMAL STROMAL CELLS: MODELLING MYOCARDIAL MSC INTEGRATION**

Authors A. Erle(1), R. Lotfi(1), A. Liebold(2), M. Hoenicka(2), D. C. Tradowsky(1), H. Schrezenmeier(1)

abstract OBJECTIVE: Stem cell therapy of myocardial infarction may have a twofold effect: paracrine interactions between stem cells and cardiomyocytes as well as stem cell transdifferentiation/cell fusions. This study investigated direct cell-cell interactions in a co-culture model.
METHODS: Murine or human bone marrow mesenchymal stromal cells (BM-MSCs) were co-cultured with murine or human cardiomyocytes at different seeding ratios ranging from 1:5 to 1:100. For cell tracking purposes human cardiomyocytes and murine BM-MSCs constitutively expressed RFP and GFP, respectively. Cultures were analyzed by a qPCR panel consisting of 14 cardiomyocyte markers, and co-cultures were assessed by video fluorescence microscopy.
RESULTS: Native BM-MSCs were found to already express three of fourteen tested cardiomyocyte markers: FGF2, GJA1 and MEF2C. Co-cultures remained viable for at least four weeks. Cardiomyocytes showed spontaneous beating which was most prominent at seeding ratios of 1:10 (BM-MSC : cardiomyocyte). Video microscopy revealed a few cells of BM-MSC origin which appeared to be beating, and several fused cells from both cell sources.
CONCLUSIONS: Co-cultures demonstrated that cardiomyocytes and BM-MSCs may coexist for extended periods of time, thus allowing mutual interactions. Depending on relative cell numbers, beating of cardiomyocytes was not hindered by BM-MSCs. Both apparently beating cells of BM-MSC origin as well as fused cells may indicate regenerative effects, albeit at low frequencies. Methods currently under development will allow isolating the cell types after co-culture and analyzing them separately to gain further insight into transdifferentiation.

Miltenyi Biotec

NOW AVAILABLE AS CE-MARKED MEDICAL DEVICE IN EUROPE

The CliniMACS Prodigy®

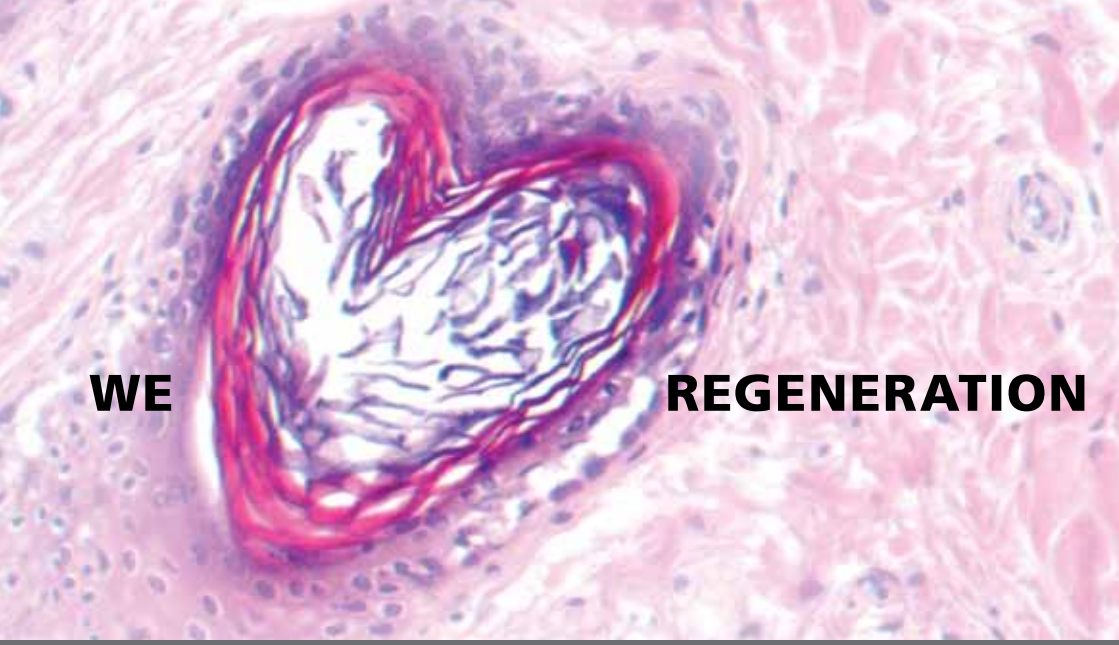
Moving cell manufacturing towards the next level

Automated cell processing from cell sample to final product

- Flexible programming options
- MACS® Cell Separation
- Cell washing procedures
- Fully automated, closed system
- Density gradient fractionation
- Optimized cell culture

Miltenyi Biotec provides products and services worldwide.
Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

▶ **clinimacs-prodigy.com**



world conference on **regenerative medicine**

[Germany | Leipzig | October 23–25, 2013]

ADVANCES IN:

Cell and Immunotherapy * Stem Cells * Cell and Tissue Engineering * Biomaterials and Tissue Interaction * Models of Regeneration * Molecular Mechanisms of Regeneration * Diagnostic and Imaging of Regeneration * Regulatory Affairs

REGENERATION OF:

Neurological Disorders * Autoimmune Disorders * Cardiac and Vascular Disorders * Cancer (Oncology) * Bone and Cartilage * Skin and Soft Tissue * Liver, Lung and Kidney

SPECIALS:

EMA/CAT-TRM Leipzig Collaborative Workshop * Opening Keynotes Hans Schöler and Mick Bhatia * Social Evening: Tropical Experience World Gondwanaland

MORE INFORMATION AND REGISTRATION:
WWW.WCRM-LEIPZIG.COM