



Stem Cell and Tissue Engineering Conference

Hotel Munkebjerg
June 4-5

2015

INTRODUCTION

Stem Cell and Tissue Engineering Conference 2015

Stem cells hold the potential to generate virtually every cell type of our body. Within the last decade, the regenerative capacity of adult stem cells have been widely exploited to generate tissues including blood vessels, bone, cartilage and more complex organs such as liver, intestine and heart. Particularly in the field of tissue engineering, research into developing the correct biomaterials and tissue-specific scaffolds have been given a tremendous push forward and with our increased understanding of fundamental stem cell biology, stem cell-based treatments and drug screening of patients is within reach. These translational stem cell activities are essential for realizing the promise of stem cells for future clinical treatments. Research groups at universities and in industry around the world are already pursuing clinical trials based on stem cells. In the present conference, we will explore and discuss the most recent developments and highlight important clinical potentials as presented by some of the best researchers in this field.

The Stem Cell and Tissue Engineering conference 2015 will include sessions and presentations on

- Cloning and reprogramming
- Stem cells in the treatment of diabetes
- Production, banking and upscaling of stem cells for disease modelling
- Ethics in stem cell research
- Bioengineering and transplantation
- Tissue engineering and organ design
- Regenerative medicine

In addition, the conference contains a poster session covering a wide range of stem cell research as well as a commercial exhibition of equipment, consumables and services to Danish biotechnology.

On behalf of the organizers, Danish Stem Cell Society (DASCS) and Danish Biotechnological Society (DBS), we wish you a fruitful and stimulating conference.



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PROGRAM DAY 1

9.00 - 9.30	Registration, coffee and tea
9.30 - 10.00	Welcome
Lars Haastrup Pedersen	Danish Biotechnological Society (DBS).
Christian Clausen	Danish Stem Cell Society (DASCS).
10.00 - 11.00	Session 1 > Cloning and reprogramming
L1 Jerome Jullien	Gurdon Institute, University of Cambridge, UK. "Programming and reprogramming cell fate in xenopus"
L2 Xianmin Zeng	Buck Institute, USA "Human pluripotent stem cell-derived dopaminergic neurons: Therapy and screening for Parkinson's disease."
11.00 - 13.00	Posters, exhibitions and lunch
13.00 - 14.00	Session 2 > Production and banking of stem cells for disease modelling
L3 Timothy Allsopp	Neusentis, Pfizer, UK. "European Bank for Pluripotent Stem Cells (EBISC) - introduction and perspective"
L4 Paul De Sousa	University of Edinburgh, UK. "Defining novel epigenetically regulated determinants of a human pluripotent state"
14.00 - 15.00	Posters, exhibitions and hotel check-in
15.00 - 16.00	Session 3 > Tissue Engineering and organ design
L5 Kim Jensen	BRIC, University of Copenhagen. "A developmental perspective on tissue maturation and stem cell function in the intestinal epithelium"
L6 Petter Björngquist	NovaHep AB, Sweden. "Stem cell bio-engineering and individualisation of human organs - applications in regenerative medicine and toxicity testing"
16.00 - 17.00	Posters, exhibitions and meet the speakers
17.00 - 17.30	Session 4 > Regulatory and ethics in stem cell research
L7 Peter Sandøe	University of Copenhagen. "Use of human SC's - what are the ethical issues?"
17.30 - 19.00	Pre-dinner beer, posters and exhibitions
19.00 - ...	Dinner at Panorama Restaurant followed by social interaction and drinks

PROGRAM DAY 2

8.30 - 9.30	Session 5 > Bioengineering
L8 Jens Vinge	Aarhus University. "Scaffold Mechano Transduction for Stem Cell Guidance"
L9 Casper Foldager	Aarhus University. "Cartilage regeneration and targets for prevention of early osteoarthritis"
9.30 - 10.30	Posters and exhibitions, meet the speakers, hotel checkout
10.30 - 11.30	Session 6 > Stem cells and diabetes
L10 Anne Grapin-Botton	DanStem, Copenhagen University. "Self-organizing 3D models of the pancreas - from stem cells towards diabetes therapies"
L11 Christian Honoré	Novo Nordisk, Department for Stem Cell Biology. "Pluripotent stem cell in diabetes disease modeling and drug discovery"
11.30 - 13.00	Lunch, posters, exhibitions and meet
13.00 - 13.45	Session 7 > Poster and sponsor presentations
	Poster presentation I Poster presentation II Poster presentation III
13.45 - 14.00	Selected technology by exhibitor
14.00 - 14.30	Posters, exhibitions and meet the speakers
14.30 - 15.30	Session 8 > Regenerative Therapies
L12 Amanda Carr	University College London, Institute of Ophthalmology. "A vision for the future of blindness: Stem Cell Therapies for Age-related Macular Degeneration"
L13 Ulrich Martin	Center for Regenerative Medicine, Hannover Medical School, Germany. "Development of iPSC-based Cardiac Therapies"
15.30 - 15.45	Closing remarks

SPEAKER ABSTRACTS

L1: Jerome Jullien

TITLE

Programming and reprogramming cell fate in xenopus

AUTHOR

Jerome Jullien

AFFILIATION

University of Cambridge, The Gurdon Institute

ABSTRACT

For a long time it has been assumed that the only role of sperm at fertilization is to introduce the male genome into the egg. Recently, ideas have emerged that the epigenetic state of the sperm nucleus could influence transcription in the embryo^{1,2}. However conflicting reports have challenged the existence of epigenetic marking of sperm genes^{3,4}, and there are no functional test supporting the role of sperm epigenetic marking on embryonic gene expression. Here, we show that sperm is epigenetically programmed to regulate embryonic gene expression. By comparing the development of sperm- and spermatid-derived frog embryos we show that the programming of sperm for successful development relates to its ability to regulate transcription of a set of developmentally important genes. During spermatid maturation into sperm, these genes lose H3K4me2/3 and retain H3K27me3 marking. Experimental removal of these epigenetic marks, at fertilization, deregulate gene expression in the resulting embryos in a paternal chromatin dependent manner. This demonstrates that epigenetic instructions delivered by the sperm at fertilization are required for correct regulation of gene expression in the future embryos. The epigenetic mechanism of developmental programming revealed here are likely to relate to the mechanisms involved in transgenerational transmission of acquired traits. Understanding how parental experience can influence development of the progeny has broad potential for improving human health.



L2: XIANMIN ZENG

TITLE

Human pluripotent stem cell-derived dopaminergic neurons: therapy and screening for Parkinson's disease

AUTHOR

Xianmin Zeng^{(1),(2)}

AFFILIATION

(1) Buck Institute, 8001 Redwood Blvd, Novato, California

(2) XCell Science Inc, 200 Professional Center Dr. Suite 211, Novato, California

ABSTRACT

For cell-based therapy to treat Parkinson's disease (PD), we have developed a GMP-compatible process for generating authentic midbrain dopaminergic neurons using defined media from human pluripotent stem cells (PSC). We have also performed IND-enabling preclinical efficacy and safety studies using cells manufactured by this process. To optimize the transplantation process we have helped develop and test a surgical delivery catheter that can be integrated with an FDA-approved iMRI skull-mounted aiming device and targeting software. Finally we have coordinated with clinicians on developing a delivery procedure and a surgical trial design. I will outline our effort/path to an IND/Phase I trial.

For iPSC-based screening, we have developed a large panel of genetically engineered human iPSC lines including 1) control lines, 2) lines from patients with mono allelic disease, 3) knock-in reporter lines, 4) isogenic controls of single and double knock-outs. Equally importantly, we are able to produce large numbers of differentiated cells including neural stem cells, neurons and astrocytes in an assay ready format from these engineered iPSC lines, and run demonstration screens for neuroprotective and neurotoxic effects. I will illustrate this approach by a PD panel we have developed, and discuss our recent results and the utilities of the rich resources/tools we have generated for iPSC-based screening for neurodegenerative diseases.



SPEAKER ABSTRACTS

L3: TIMOTHY ALLSOPP

TITLE

European bank for pluripotent stem cells (EBiSC)
– introduction and perspectives.

AUTHOR

Timothy Allsopp

AFFILIATION

Neusentis, Pfizer Research Unit



ABSTRACT

Induced pluripotent stem (iPS) cells have the potential to significantly advance drug development and health research, yet collections of stem cells are scattered across the world, their quality cannot always be guaranteed, and accessing them is often difficult. The goal of EBiSC is to establish a European iPS cell bank that will be the 'go-to' resource for the characterization, storage and distribution of high quality iPS cells. Ultimately, EBiSC will become an independent organization, distributing high quality iPS cells on a not-for-profit basis to scientists worldwide. EBiSC is designed to address the increasing demand by iPSC researchers for quality-controlled, disease-relevant research grade iPSC lines, data and cell services. The EBiSC Consortium including leading pharmaceutical companies boasts the leadership, scientific expertise, facilities, networks and experience to achieve these goals and, being representative of all stakeholders from tissue donors to clinical and academic iPSC researchers and industrial users, to respond appropriately to advances in science and society. The initiative is driven by joint collaborations between leading iPSC research groups across Europe and major European pharmaceutical companies.



SPEAKER ABSTRACTS

L4: PAUL DE SOUSA

TITLE

Defining novel epigenetically regulated determinants of a human pluripotent state

AUTHORS

Paul A. De Sousa

AFFILIATION

Centres for Clinical Brain Sciences and Regenerative Medicine, University of Edinburgh

ABSTRACT

Human embryonic stem cells (hESCs) undergo epigenetic changes in vitro which may compromise function, so an epigenetic pluripotency “signature” would be invaluable for line validation. To identify such a signature we have assessed Cytosine-phosphate-Guanine Island (CGI) methylation in hESCs by genomic DNA hybridisation to a CGI array, and detected substantial variation in CGI methylation between lines. By comparison of hESC CGI methylation profiles to corresponding somatic tissue data and hESC mRNA expression profiles we have identified a conserved hESC-specific methylation pattern associated with expressed genes. Transcriptional repressors and activators were over-represented amongst genes whose associated CGIs were methylated or unmethylated specifically in hESCs, respectively. Knockdown of candidate transcriptional regulators induced differentiation in hESCs, whereas ectopic expression in fibroblasts modulated iPSC colony formation. Chromatin immunoprecipitation confirmed interaction between the candidates and the core pluripotency transcription factor network. Our research identifies prospectively novel pluripotency associated genes on the basis of a conserved and distinct epigenetic configuration in human stem cells.



L5: KIM JENSEN

TITLE

A developmental perspective on tissue maturation and stem cell function in the intestinal epithelium

AUTHOR

Kim Jensen

AFFILIATION

University of Copenhagen, BRIC

ABSTRACT

The adult intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. Deregulation of the normal regulatory pathways that control stem cell turnover and differentiation are associated with the development of diseases such as cancer. Interestingly, epithelial progenitors present in the intestine during foetal stages share many of these features with cells isolated from tumour lesions. This includes their growth factor dependency, morphology and expression patterns associated with differentiation. Importantly, these features are conserved between human and mouse. We propose that characterising intestinal foetal progenitors along side epithelial cells isolated from both normal and malignant intestinal epithelium will provide key insights into pathways important for the development of malignant cancer. Here I will present recent data related to the control of cells behaviour and tissue maturation in late stage human and mouse foetal development, and the emergence of cells with adult stem cell properties.



SPEAKER ABSTRACTS

L6: PETTER BJORQUIST

TITLE

Stem cell bio-engineering and individualisation of human organs
– applications in regenerative medicine and toxicity testing

AUTHOR

Petter Björquist

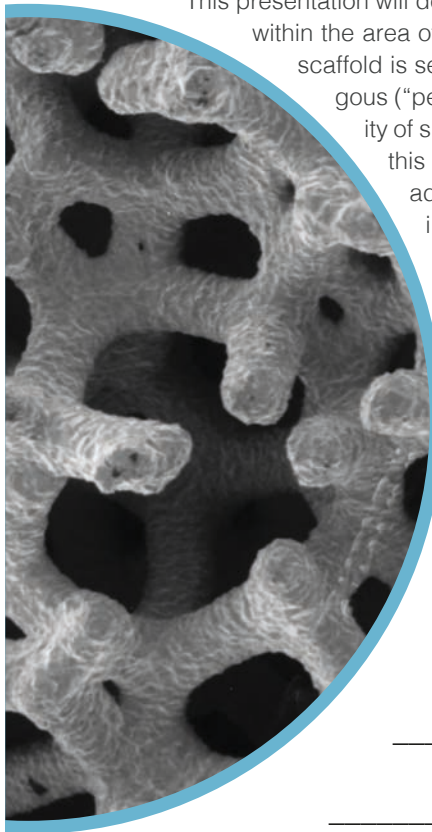
AFFILIATION

NovoHep AB

ABSTRACT

This presentation will describe a novel stem cell and tissue engineering technology, currently being explored for applications within the area of regenerative medicine. In short, a donated tissue is decellularised followed by a process when this scaffold is seeded with the patient's own stem cells, resulting in an allogeneic ("foreign") tissue becoming autologous ("personalised"). This addresses the two major shortcomings in transplantation surgery of today; availability of suitable donor tissue and the severe side-effects following lifelong immunosuppressive treatment. Using this technology, the next generation of tissue-engineered products for replacement therapy is developed, addressing a huge unmet medical need as well as a growing commercial market. The technology will be illustrated by successful engineering of multiple organs, including liver, kidney and pancreas.

Vascular disease of the lower extremities is very common, affecting 25% of adults in westernized societies. Such pathological conditions represent an enormous burden, both to patients and to the healthcare systems. With clinical proof of concept of the above mentioned technology achieved in 2011 (results published in the Lancet), we are currently preparing for GMP-adaptation and a pivotal clinical trial using personalised tissue-engineered veins (P-TEV) for surgical treatment of chronic venous insufficiency (CVI). The process how P-TEV are produced will be described in detail.



SPEAKER ABSTRACTS

L7: PETER SANDOE

TITLE

Use of human SC's – what are the ethical issues?

AUTHOR

Peter Sandøe & T.J. Kasperbauer

AFFILIATION

Department of Food and Resource Economics, University of Copenhagen.

ABSTRACT

This talk reviews four main ethical issues raised by research on stem cells. First, we discuss early controversies over the use of human embryos in stem cell research. We trace these early concerns to more recent debates over the use of adult stem cells. Second, we discuss issues raised about the privacy of donors and patients in stem cell research. These concerns are discussed in the context of informed consent. Third, we identify potential risks in using stem cells for therapy. And fourth, we discuss the ethical importance of distinguishing between using stem cells for therapy and using stem cells for enhancement.



L8: JENS VINGE

TITLE

Scaffold Mechano Transduction for Stem Cell Guidance

AUTHOR

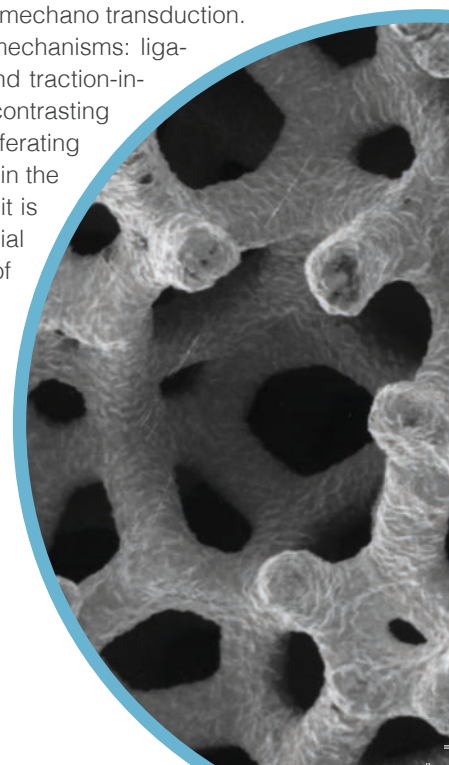
Jens Vinge Nygaard

AFFILIATION

Mechanical and Materials Engineering
Department of Engineering (ENG), Aarhus University, Aarhus, Denmark.

ABSTRACT

The emergence of tissue phenotypes through stem cell cultivation in scaffolds can be guided by incorporating functionalisation into the material design. Successful tissue emergence arises from proper temporal biochemical and mechano transduction. Mechano transduction through cell interaction produces two different but concurrent signaling mechanisms: ligation-induced signaling, which depends on biological stimuli from the surrounding environment, and traction-induced signaling, which depends on mechanical stimuli, [1]. Different substrate stiffnesses have contrasting effects on migration and proliferation, where cells migrate faster on softer substrates while proliferating preferentially on the stiffer ones. This implicates that substrate rigidity is a critical design parameter in the development of scaffolds aimed at eliciting maximal cell and tissue function, [2]. From mechanics it is known that the stiffness of 3D porous structures scales with the relative density of the porous material and thus the porosity, [3]. Stem cell lineage specifications can be controlled by optimal selection of scaffold porosity.



L9: CASPER FOLDAGER

TITLE:

Cartilage regeneration and targets for prevention of early osteoarthritis

AUTHOR

Casper Foldager

AFFILIATION

Aarhus University

ABSTRACT

Articular cartilage is a specialized avascular, aneural, and alymphatic tissue with limited ability for self-renewal following injury. In this talk I will address traumatic injuries and early osteoarthritis with special attention to the pathological development and disease progression and the potential intrinsic regenerative abilities.



L10: ANNE GRAPIN-BOTTON

TITLE

Self-organizing 3D models of the pancreas - from stem cells towards diabetes therapies

AUTHORS

Manuel Figueiredo-Larsen(1), Chiara Greggio(2), Filippo De Franceschi(2), Henrik Semb(1), Samy Gobaa(3), Adrian Ranga(3), Matthias Lutolf(3), Florian Buettner(4), Fabian Theis(4) and Anne Grapin-Botton(1),(2)

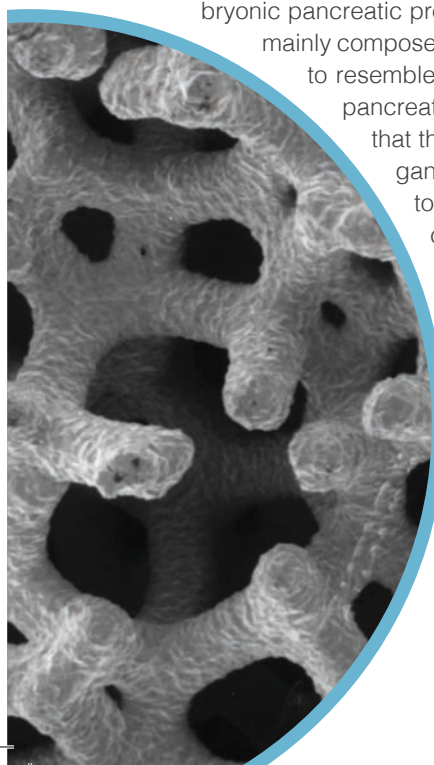
AFFILIATION

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(2) Ecole Polytechnique Fédérale de Lausanne, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland
(3) Ecole Polytechnique Fédérale de Lausanne, Institute of Bioengineering, Lausanne, Switzerland
(4) Institute of Bioinformatics and Systems Biology, Helmholtz-Zentrum München, Neuherberg, Germany

ABSTRACT

We recently established three-dimensional (3D) culture conditions that enable the efficient expansion of dissociated mouse embryonic pancreatic progenitors. By manipulating the composition of the culture medium we generate either hollow spheres, mainly composed of pancreatic progenitors, or complex organoids which differentiate and spontaneously self-organize to resemble the embryonic pancreas. We have conducted multiplexed single-cell PCR analysis to compare the pancreatic cells seeded, the cells grown in organoids and in spheres. Principal component analysis revealed that the sphere cells are molecularly homogenous and similar to the seeded progenitors. In contrast, all organoid cells diverge from the original cells after 7 days of culture and become heterogenous with respect to the expression of a subset of genes. More recently, we showed that organoids start from several seed cells, which aggregate and cross-talk. Heterogeneity in this seed is needed to initiate organogenesis. To understand how this heterogeneity later translates into self-organization, we combine live imaging, expression profiling and mathematical modelling. We will discuss our recent experiments attempting to recapitulate the process uncovered in mice to human embryonic stem cells that we drive to pancreatic differentiation and expand into organoids in Matrigel. Our aim is to develop a human model of pancreas development, of potential use for diabetes modelling, toxicity assay and drug development.

We will discuss our recent experiments attempting to recapitulate the process uncovered in mice to human embryonic stem cells that we drive to pancreatic differentiation and expand into organoids in Matrigel. Our aim is to develop a human model of pancreas development, of potential use for diabetes modelling, toxicity assay and drug development.



SPEAKER ABSTRACTS

L11: CHRISTIAN HONORÉ

TITLE

Induced pluripotent stem cells in diabetes disease modelling and drug discovery

AUTHOR

Christian Honoré.

AFFILIATION

Department of Islet & Stem Cell Biology. Novo Nordisk A/S.

ABSTRACT

Induced pluripotent stem cells (iPSC) can be derived from somatic cells through forced overexpression of key pluripotent transcription factors. This ground-breaking technique allows for the establishment of pluripotent stem cells from both healthy individuals as well as from patients suffering from numerous disorders including various forms of diabetes. The genetic composition of patient-specific iPSC should thus be identical to the individual from which it was derived including the mutation(s) contributing to the specific disease. Combined with the ability of these cells to differentiate into potentially all cell types of the human body, iPSC holds a great potential as a novel tool for disease modelling. We have recently embarked on evaluating the potential use of iPSC cells for disease modelling of various diabetic disorders as well as drug discovery efforts. In this talk, I will present initial results from these efforts as well as dis-



L12: AMANDA CARR

TITLE

A vision for the future of blindness.
Stem Cell Therapies for Age-related Macular Degeneration.

AUTHOR

Amanda Carr

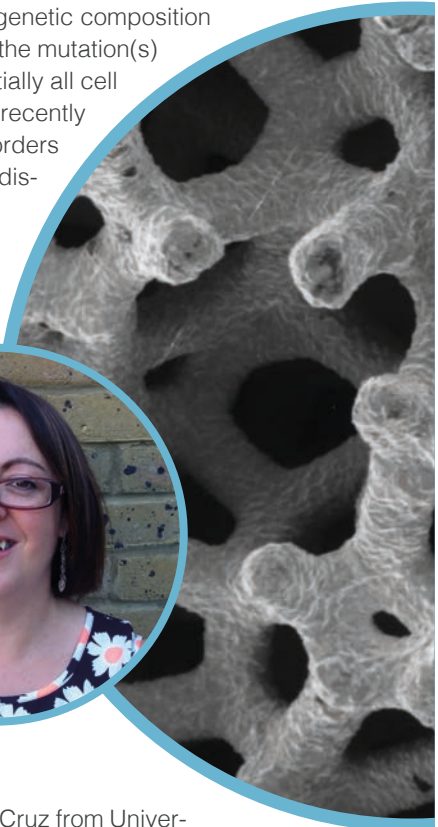
AFFILIATION

University College London (UCL)
Institute of Ophthalmology

ABSTRACT

The London Project to Cure Blindness is collaboration between Professor Pete Coffey and Lyndon da Cruz from University College London and Moorfields Eye Hospital. The project aims to develop novel therapies for the treatment of Age-related Macular Degeneration (AMD), the largest cause of blindness worldwide. Initial aims are to use human embryonic stem cells to produce a monolayer of cells affected in AMD, known as the retinal pigment epithelium (RPE). These cells will be transplanted on an engineered patch under the retina in imminent clinical trials. The group is also creating an induced pluripotent stem cell bank that will enable us to produce autologous and haplotyped RPE, and is investigating the production of therapeutic photoreceptor cells from pluripotent stem cells.

AMD is the leading cause of blindness in the western world, resulting in loss of high acuity central vision required for fine details tasks. Defects within a layer of cells in the macular region of the eye, known as the retinal pigment epithelium (RPE), result in the loss of overlying photoreceptor cells and vision loss. As a single layer of cells, the RPE is an ideal target for stem cell therapy. This talk will provide an overview of the current status and the future perspectives for AMD therapies using pluripotent stem cells.



L13: MARTIN ULRICH

TITLE

Development of iPSC-based Cardiac Therapies

AUTHOR

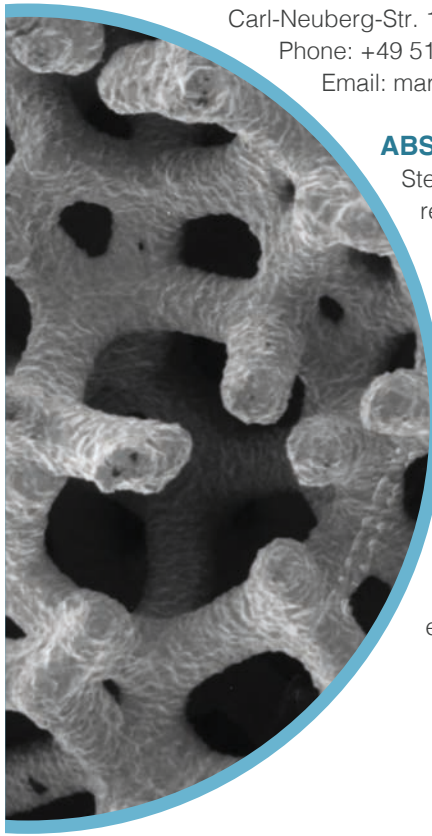
Ulrich Martin

DEPARTMENT

Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO),
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ABSTRACT

Stem cell based therapies are considered as promising and innovative therapeutic strategies for heart repair. Patient-derived induced pluripotent stem cells (iPSCs) are now available, which combine the advantages of autologous adult stem cells with the unlimited potential of embryonic stem cells for proliferation and differentiation. Intense research has driven forward recent dramatic progress in various areas of iPSC technology relevant for clinically applicable iPSC-based cellular therapies. At this point, transgene-free autologous iPSCs can be easily obtained e.g. from small blood samples, scale up of the expansion and differentiation of iPSCs to clinically required dimensions is possible, and highly enriched cardiomyocyte preparations can be produced using small molecule-based defined protocols. On the other hand, potential risks including genetic abnormalities in iPSCs and critical hurdles such as the targeted specification of distinct cardiomyocyte subpopulations, survival and proper functional integration of cellular transplants after myocardial infarction, and in vitro engineering of prevascularized muscle patches have yet to be overcome. Nevertheless, concepts of cellular cardiomyoplasty seem to have come of age. Based on our recent developments we are currently preparing for preclinical animal studies, and we expect first clinical applications of iPSC-based heart repair to become realized within the coming years.



POSTER ABSTRACTS

P1 TITLE

Increased mucin expression in primary cultures of oral mucosal epithelial cells by mycophenolate mofetil finds application in limbal stem cell deficiency

AUTHORS

Sandeep Kumar Agrawal(1)*, Aditi Bhattacharya(1), Janvie Manhas(1), Krushna Bhatt(2), Yatin Kholakiya(2), Nupur Khera(1), Ajoy Roychoudhury(2), Sudip Sen(1)

AFFILIATIONS

(1) Department of Biochemistry, (2) Department of Oral & Maxillofacial Surgery, Centre for Dental Education & Research, All India Institute of Medical Sciences, New Delhi, India. *drsandeepagrawal25@gmail.com, +919953014166

ABSTRACT TEXT

Background: Autologous cultured explants of human oral mucosal epithelial cells (OMEC) are a potential therapeutic modality for limbal stem cell deficiency (LSCD). Most of these patients suffer from incapacitating dry eye with significantly reduced mucin production. Mycophenolate mofetil (MMF) has been shown to upregulate the mucin expression in conjunctival goblet cells in vitro. The objective of this study was to evaluate the effects of MMF on mucin expression in primary cultures of oral mucosal epithelial cells.

Methods: With informed consent, thirty oral mucosal tissue samples were obtained from patients undergoing oral surgery for non-malignant conditions. OMEC were grown on human amniotic membrane scaffold for 2 weeks in growth media containing DMEM & Ham's F12 with FBS and growth factors. Mucin gene expression was quantified using RT-PCR and q-PCR before and after treating cultured OMEC with MMF for 24 hours. Protein expression was validated using immunocytochemistry.

Results: Morphological studies revealed a confluent sheet of proliferating, stratified oral mucosal epithelial cells. The expression of MUC1, MUC15 and MUC16 mucin mRNA was found to be upregulated in MMF treated primary cultures of OMEC, compared to untreated controls as quantified by q-PCR with β -actin as internal control. Increased MUC1 protein expression was validated using immunocytochemistry.

Conclusions: Our findings demonstrate that MMF can be a novel enhancer of mucin production in OMEC in vitro. It has the potential to improve dry eye in patients undergoing OMEC transplantation for LSCD. Further clinical trials are required to establish the role of MMF in patients undergoing OMEC transplantation.

P2 TITLE

Translating Regenerative Science into Clinical Applications – The Catapult Approach

AUTHORS

Dr Nina G Bauer(1)

AFFILIATIONS

(1) Business Development Executive EMEA, The Cell Therapy Catapult Ltd., Guy's Hospital, London, United Kingdom

ABSTRACT TEXT

Cell and gene therapies represent the most recent phase of the biotechnology revolution in medicine. As with most remedies, these therapies are based on ground-breaking scientific discoveries. Importantly, progression of cell and gene therapies from an academic setting into the clinic brings with it a whole raft of scientific, manufacturing, regulatory, health economics and commercialisation issues to be solved. Very few organisations that work in the space currently have all these capabilities in-house. Thus, it is clear that collaborations between academic institutions, pharmaceutical enterprises, biotechnology companies and other stakeholders are necessary to accelerate therapeutic developments.

In the UK, the major barriers for the growth of a cell and gene therapy industry have been identified as being regulatory, business, manufacturing and supply chain related. The Cell Therapy Catapult was established by Innovate UK in 2012 as an independent organisation to advance the growth of the UK cell and gene therapy industry, by bridging the gap between scientific research and commercialisation to address the issues outlined above. New ways of effective collaboration for innovation and investment in innovation will be described, as will strategies to overcome the manufacturing and regulatory challenges that exist today.



POSTER ABSTRACTS

P3 TITLE

Effects of Beetroot Extract on Cell Proliferation and Overdose H₂O₂-Induced Apoptosis in Human Gingival Fibroblasts and Human Mesenchymal Stem Cells

AUTHORS

E. Sacide Çağlayan(1), Z. Dilşat Çoban(2), Şefik Güran(2)

AFFILIATIONS

(1) Department of Nutrition & Dietetics, Yıldırım Beyazıt University, Ankara/TURKEY
(2) Department of Medical Biology, Gülhane Military Medicine Academy, Ankara/TURKEY

ABSTRACT TEXT

Complex effects of natural polyphenols are well-studied in cancer cells. Recently, researches have been focused on polyphenol-induced cell cycle regulations in immunity, wound-healing and stem cell fields. In addition, striking results have been obtained from resveratrol-related mechanisms in stem cell researches. Polyphenol-rich content and thus anti-apoptotic functions of beetroot (Br) is well-known in cancer studies. However, effects of Br on stem cell cycle are needed to be clarified. In the current study, we evaluated low-dose (Ld=0.5-1 mg/ml) and high-dose (Hd=5-10mg/ml) effects of Br extract on human gingival fibroblasts (hGF) and human bone marrow mesenchymal stem cells (hBM-MSC) by using XTT-cell proliferation assay. We also assayed protective effect of Ld and Hd Br concentrations on overdose H₂O₂ (2mM in 2h) induced apoptosis by Ethidium bromide-Acrydine orange (EB-AO) staining. Statistics of cell proliferation rate were calculated by one-way Anova tests. According to results, statistic differences were calculated as significant between Hd-Br (10mg/ml) and controls (p<0.001) both within 24h and 48h of hGF. However, a significant reduction in 0.5mg/ml (p=0.001) and an escalation in 10 mg/ml (p<0.001) were calculated in 72h of hGF. Dramatic reduction of 0.5-5mg/ml Br-treated hBM-MSC have been calculated within 24h and 48h according to controls (p<0.001). Early apoptosis (EA)-rate was counted as 70% in H₂O₂-treated controls of hGF and %22 in hBM-MSC. In Br-treatment, EA-rate was counted in %16 of 5mg/ml and %6 of 10mg/ml-treated hGF and %6 of both 5mg/ml and 10mg/ml-treated hBM-MSC. According to striking results, advanced molecular researches will be beneficial for enlightening of Br effects in stem cell field.

P4 TITLE

Stem cell-based therapies in horses and dogs and their potential as pre-clinical models

AUTHORS

Carmon Co1 and Thomas G Koch(1),(2)

AFFILIATIONS

(1) Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada.
(2) Department of Clinical Studies, Orthopaedic Research Lab, Aarhus University, Denmark.

ABSTRACT TEXT

Horses and dogs suffer from a number of conditions with close resemblance to human conditions. Contemporary veterinary medicine rivals human medicine in many aspects including advanced clinical specialty training and state-of-the art diagnostic and treatment modalities. Research in the Koch lab is focused on development of stem cell-based therapies for horses and dogs to enhance animal welfare as well as provide pre-clinical data for strategies to treat human diseases. In the past 10 years we have developed a robust isolation method for the derivation of cryotolerant equine umbilical cord blood-derived mesenchymal stromal cells (eCB-MSC). eCB-MSC are lymphocyte suppressive, devoid of MHC I and II expression and possess high chondrogenic potential in vitro. Based on these findings, our rationale is that these cells are candidates for allogeneic cytotherapeutic strategies and we have ongoing evaluation of these cells for various conditions in live horses. To date, we have administered allogeneic eCB-MSC to horses with natural tendon and ligament injuries as well as intra-articular injuries in research horses with LPS-induced synovitis. Responses have been favorable. For cartilage repair we are pursuing osteochondral implants with an eCB-MSC-derived chondral phase. We have extended our research scope to include the canine model in the Koch lab, utilizing somatic MSC and induced pluripotent stem cells. Past collaborations have lead to the exchange of Danish and Canadian graduate students. Investigators and students are very much encouraged to contact us to explore the possibility of collaboration in this space of human and veterinary stem cell medicine.

POSTER ABSTRACTS

P5 TITLE

Defining the earliest step of cardiovascular development using CRISPR genome technology

AUTHORS

Tilde V. Eskildsen(1),(2), Edward Shin(2), Mohammad A. Mangedar(2), Ditte C. Andersen(1),(3), Bruce R. Conklin(2), Søren P. Sheikh(1),(3)

AFFILIATIONS

(1) Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Denmark.

(2) Institute of Molecular Medicine, University of Southern Denmark, Denmark.

(3) Department of Cardiovascular Diseases, Gladstone Institutes, University of California San Francisco, USA.

ABSTRACT TEXT

We hypothesize that modified versions of the imprinted gene Dlk1 affect the stem cell potential of iPS cells and thus the differentiation into cardiovascular cell types.

Understanding how stem cells determine their cell fate and differentiate into the 3 interdependent cardiovascular cell types; cardiomyocytes, endothelial cells and smooth muscle is crucial in order to facilitate tissue regeneration e.g. in a disease state of heart failure by promoting new vessels in the damaged area of the heart.

Novel powerful CRISPR techniques have emerged that allow genome engineering in a variety of cell types including human iPS cells. Dlk1 is a maternal imprinted gene, believed to be necessary for full pluripotent potential of iPS cells and to play a crucial role in the development of blood vessels. To investigate the impact of Dlk1 during cardiovascular differentiation, we use the CRISPRi technology. This technique facilitates inducible and sequence-specific repression and re-expression of the genes of interest.

The CRISPRi modified Dlk1 cell lines will be characterized during differentiation and analyzed for preferences of cell fate. The analysis will be performed using flow cytometry and immunocytochemistry to quantify the number of pluripotent cells, mesodermal cells or any of the terminal differentiated cardiovascular cell types. Results obtained in the present project are expected to add significant knowledge to the regulation of stem cell fate in general, and this may on a long term be used in advancing novel stem cell therapeutics.

P6 TITLE

Electrospun biomimetic nanofibers for pelvic floor repair – An in vivo study in rats

AUTHORS

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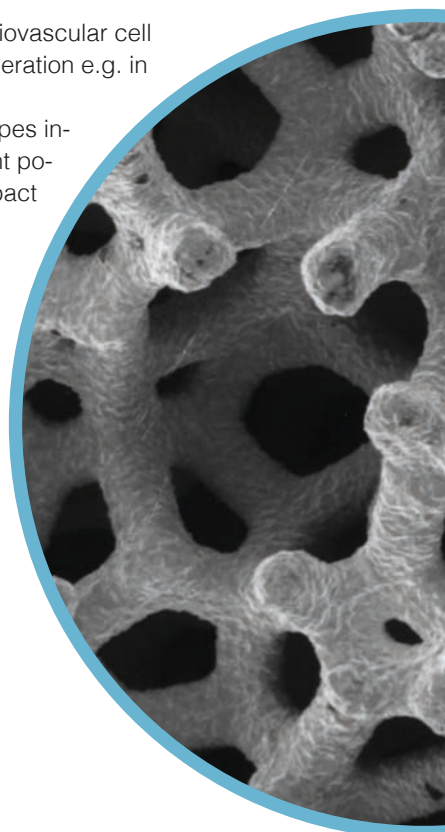
ABSTRACT TEXT

Half of the female population over the age of 50 will experience pelvic organ prolapse (POP) caused by weakening or rupture of fascias in the pelvic floor. We suggest a new treatment approach based on tissue engineering principles to functionally reconstruct the anatomical structures of the female pelvis.

The aim of this study was to investigate the mechanical performance of a degradable mesh of polycaprolactone (PCL) releasing basic fibroblast growth factor (bFGF) to enhance collagen production. We implanted the mesh in the abdominal wall of 40 rats. Samples were explanted after four, eight and 24 weeks, and tested for tensile strength, strain, maximum stiffness, energy absorption, and total amount of collagen. The mechanical integrity of the implant was evaluated by accessing the hernia size.

The study showed an increase in tensile strength, maximum stiffness and energy absorption over time but a difference between bFGF- and no-bFGF-group was only seen after four weeks. Over time, a significant increase of total collagen was seen but there was no difference between bFGF- and no-bFGF-group. Tensile strength and stiffness increased significantly with increasing hernia size.

Conclusively, a tissue-engineered treatment for POP with a degradable PCL mesh seems possible. The results suggest that the mesh promotes tissue formation with increasing collagen amount and biomechanical properties over time but that the addition of bFGF does not add any positive long-term effect. The occurrence of hernia indicates a mismatch between the degradation time of the mesh and the formation of the new tissue.



P7 TITLE

Degradable mesh for pelvic floor repair – An in vivo study in rats

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ABSTRACT TEXT

Half of the female population over the age of 50 will experience pelvic organ prolapse (POP) caused by weakening or rupture of fascias in the pelvic floor. We suggest a new treatment approach based on tissue engineering principles to functionally reconstruct the anatomical structures of the female pelvis.

The aim of this study was to investigate the mechanical performance of a degradable mesh of polycaprolactone (PCL) releasing basic fibroblast growth factor (bFGF) to enhance collagen production. We implanted the mesh in the abdominal wall of 40 rats. Samples were explanted after four, eight and 24 weeks, and tested for total amount of collagen, mRNA expression and protein amount of collagen-I, collagen-III, and fibronectin.

Outcome was related to the size of herniation in the abdominal wall.

After four weeks implantation, mRNA expression of collagen-I and collagen-III increased significantly in the mesh with bFGF. The difference equalized after eight and 24 weeks. No difference between the bFGF-group and no-bFGF-group at any time was seen on either protein level or hernia size. Histologically, a visible difference was seen after four weeks with a decreased cell popularization in the group with bFGF. No difference between the bFGF-group and no-bFGF-group was seen at eight and 24 weeks.

Conclusively, the implantation of mesh promotes tissue formation but addition of bFGF in the dosage used in this study is not efficient to enhance production of collagen. Furthermore, the evolution of hernia indicates a mismatch between the degradation time of the mesh and the formation of new tissue.

P8 TITLE

The landscape of alternative splicing in hematopoietic stem cells

AUTHORS

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ABSTRACT TEXT

Elucidation of the transcriptome of immune cells and their progenitors has enabled many discoveries and systematic understanding of these cells. Alternative splicing (AS) is a feature of eukaryotic cells, enhancing transcriptome complexity beyond the known number of genes, allowing for each gene to encode for more than one protein. Hematopoietic stem cells (HSCs) represent a form of prototypic adult stem cells that were intensively studied. Despite the high interest in them, data critical for research were not available until technical advancements were made. The emergence of RNA-sequencing has allowed a closer inspection of the HSCs' transcriptome. Analysis of AS from high throughput sequencing is not yet trivial; therefore, we have chosen an approach to focus on the numbers of exon junctions rather than the individual quantification of exons. Indeed, our validation of predicted AS in HSCs found RNA-seq junctions as a valid tool to make AS predictions. Moreover, given the correct analysis, new exon-exon junctions (which could occur in some AS events) can also be identified. AS detection via a bioinformatic pipeline followed by real time PCR verification has proved to be a useful and reliable tool in our studies, revealing multiple AS events of genes that are known to play major roles in HSCs (such as Pbx1, Meis1 and more). While some of the identified AS events are known and documented, such as with HoxA9, some are novel isoforms. These data provide, for the first time, a comprehensive whole transcriptome view of AS in HSCs. Our findings shed a new light on the way we view the transcriptome's complexity of adult stem cells.

POSTER ABSTRACTS

P9 TITLE

3D printed hydrogel scaffolds to define cell cluster morphology

AUTHORS

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ABSTRACT TEXT

Abstract text (max. 250 Words): Availability of increasingly complex 3D microenvironments for in vitro cell culture allows researchers to study stem cells organized with a spatial distribution that more closely mimics their in vivo organization. Recent studies have shown that mesenchymal stem cells cultured as 3D clusters or spheroids can support tissue regeneration through secretion of anti-inflammatory compounds that are not secreted when cultured as 2D cell layers. Defining proper and well-organized stem cell cluster formation could be essential to study this feature in vitro. However, the state-of-the-art hanging drop technique requires a skilled experimentalist to achieve proper 3D aggregation and provides little freedom in controlling cluster morphology. As a further complication, the resulting 3D cell spheroids are not easily handled during further analysis or application process steps. Here we present a novel 3D printed soft scaffold manufactured by (micro)stereolithography that allows detailed control of the stem cell cluster morphology. Soft hydrogel scaffolds with defined microscopic structure and low cell adhesion properties were manufactured by light-guided crosslinking of poly(ethylene glycol) diacrylate. Mesenchymal stem cells were seeded on the manufactured scaffolds at different cell densities, and stem cell cluster morphologies were recorded at different time points by time lapse microscopy. Furthermore, live/dead staining assays were performed to assess the 3D stem cell clusters viability. Results indicate that the use of these soft scaffolds stabilizes the 3D cluster formation compared to the hanging drop procedure, thus enabling the design of more complex structures that could be used in the future as stem cell based biomedical devices.

P10 TITLE

Clinically-feasible large-scale manufacturing of adipose tissue-derived stromal cells using Quantum cell expansion system and human platelet lysate

AUTHORS

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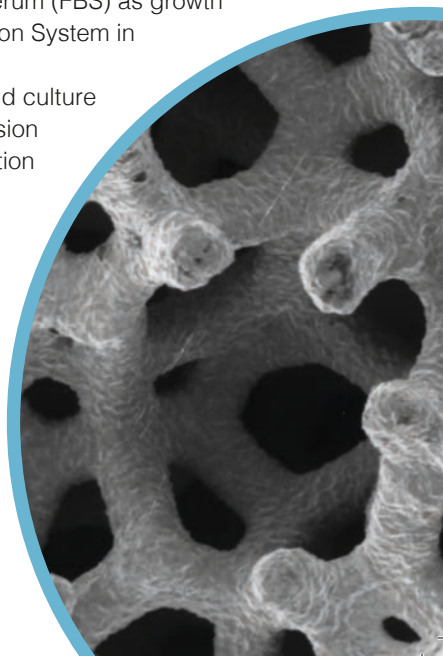
ABSTRACT TEXT

In vitro expanded adipose tissue-derived stromal cells (ASCs) are a useful resource for tissue regeneration. Translation of small-scale autologous cell production into large-scale, allogeneic clinical applications necessitates wise selection of media and cell culture platform. We compare the use of clinical-grade human platelet lysate (hPL) and Fetal Bovine Serum (FBS) as growth supplements in the automated functionally closed hollow fiber bioreactor-based Quantum Cell Expansion System in pursuit of safe and feasible large-scale production of ASCs.

Stromal vascular fraction (SVF) was isolated from abdominal fat, loaded into a bioreactor in and culture expanded α -MEM with 10% FBS or 5% hPL. ASC P0 was harvested and reloaded for continued expansion (P1). Metabolic monitoring guided feeding rate and time of harvest. Viability, sterility, purity, differentiation capacity and genomic stability of ASCs were determined.

Each passage of this two-passages process demonstrates that hPL supports proliferation of ASCs to a greater extent than FBS. ASCs fulfil ISCT criteria in both media types (immunophenotype and triple differentiation). Comparative genomic hybridization demonstrates genomic stability. Sterility, mycoplasma and endotoxin tests were all negative.

The closed automated hollow fiber Quantum Cell Expansion System provides a reliable and efficient mean of manufacturing ASCs while minimising manipulation and risk of contamination. HPL serves as an effective growth supplement for expansion of clinical-grade ASCs. These yields exhibit no chromosomal aberrations and meet all release criteria, indicating a clinically-feasible option for manufacture of large-scale allogeneic ASC products.



P11 TITLE

Exploring the immunosuppressive potential of adipose tissue-derived stromal cells.

AUTHORS

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ABSTRACT TEXT

Mesenchymal stromal cells have been an expanding field of research for more than a decade. At Cardiology Stem Cell Centre, attention has been raised around cells residing in adipose tissue, the adipose tissue-derived stromal cell (ASC). Alongside promising regenerative capabilities, recent findings have indicated a new intriguing aspect: Immunomodulation and –suppression.

As the most potent professional antigen-presenting cell in humans, the dendritic cell plays a crucial role in adaptive immunity. Dendritic cells can be generated from circulating naïve monocytes in peripheral blood by differentiation in culture, and, upon receiving activating stimuli, the formed cells express markers matching their in vivo counterparts. Here, we demonstrate that ASCs do not contribute to maturation of allogeneic DCs in vitro; rather, the DC response to maturation stimuli is suppressed in the presence of ASCs.

Another main feature of immune responses is how circulating lymphocytes react upon encountering foreign antigens. One might expect that the allogeneic origin of ASCs could result in increased proliferation of T cells, yet simple co-cultures of peripheral blood monocytes (PBMCs) and ASCs do not prompt such an increase. Interestingly, the reactions between two PBMC donors, which would normally result in an aggressive increase in proliferation, are greatly lessened by ASCs.

These findings are part of more elaborate ongoing studies of the immunosuppressive capabilities of ASCs, which aim to contribute to documenting the safety of allogeneic ASC therapy while identifying potential new treatments.

P12 TITLE

Modeling human neural tube anatomy through culturing of stem cells under microfluidic gradients

AUTHORS

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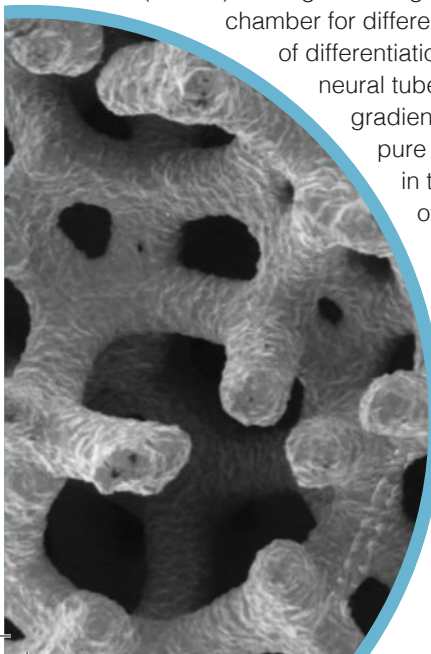
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ABSTRACT TEXT

Knowledge about structural brain development is almost purely derived from rodents or smaller model organisms, since dynamic and mechanistic studies cannot be performed in human embryos. This results in a significant lack of knowledge about human-specific neural development.

Here, we build a simplified 3D model of the developing human neural tube in vitro using human embryonic stem cells (hESCs). Taking advantage of established knowledge on neural tube patterning, we have designed a closed microfluidic culturing chamber for differentiation of hESCs. In this chamber, the cells are exposed to a gradient of chemicals during the first days of differentiation to mimic the anatomical gradients of growth factors present in the embryo around the developing neural tube. We show that hESCs survive well and differentiate into neural precursor cells when cultured in the gradient chamber. In addition, we were able to induce progressive caudalization of neural identity, obtaining pure forebrain cells in the left side of the culture chamber to midbrain cells in the middle and hindbrain cells in the right side of the chamber, indicating an anatomical resemblance to the rostro-caudal organization of the neural tube. Remarkably, we found the gene WNT1 to be very highly expressed in the area of the midbrain-hindbrain boundary in the culture chamber, indicating the formation of an in vitro equivalent to the anatomical structure of the Isthmic Organizer. Our model will be a unique tool for investigating how human brain development differs from that of other species in order to achieve an extraordinary degree of complexity.



POSTER ABSTRACTS

P13 TITLE

Generation of isogenic induced pluripotent stem cells (iPSCs) from Maturity Onset Diabetes of the Young (MODY) patients to study monogenic forms of diabetes

AUTHORS

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ABSTRACT TEXT

Perturbation of pancreatic beta-cells is generally regarded as the cause of type 2 diabetes (T2D). In vitro systems based on human iPS-derived beta cells carrying disease-causing mutations are needed to provide fundamental understanding of the underlying mechanisms and their effects on insulin secretion and beta-cell action. In this project, we aim to establish in vitro models of human monogenic hereditary forms of beta-cell dysfunction using iPSC lines from donors with Maturity Onset Diabetes of the Young 3 (MODY3) - a monogenic form of diabetes. In addition, isogenic control iPSC lines will be generated using state of the art techniques to correct the inherited mutations in the iPSC lines. To this end, iPSC clones from two different MODY3 patients have been generated and characterized. Furthermore, the point mutation in one iPS cell line from patient 2 (iPSC-HNF1aR272C/WT) has been corrected using the CRISPR/Cas9 system. iPSC-HNF1aR272C/WT as well as the corrected isogenic control give rise to insulin+/MafA+/Nkx6.1+ cells, and we are now in the process of testing their glucose-stimulated insulin secretion potential. MODY iPSC-derived beta cells will represent a novel proof-of-principle tool ideal for studying new mutations underlying T2D.

P14 TITLE

Digital drug dosing for cell screening assays

AUTHORS

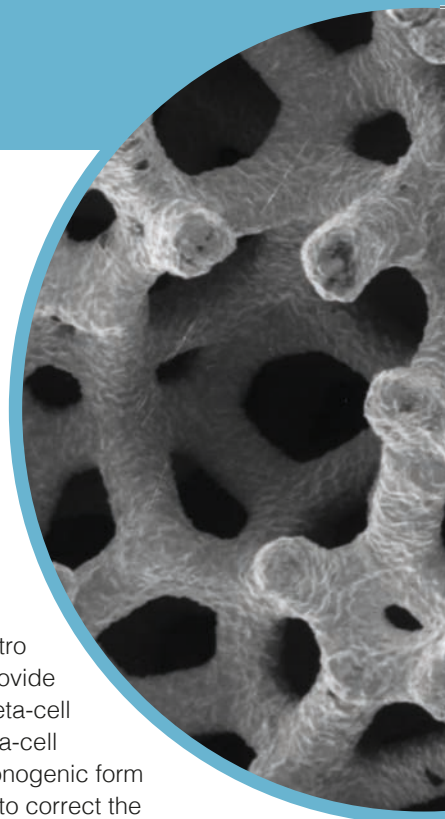
Adele Faralli(1), Fredrik Melander(1), Esben Kjær Unmack Larsen(1), Sergey Chernyy(1), Thomas Lars Andresen(1), Niels B. Larsen(1)

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ABSTRACT TEXT

Screening assays used in biological research and increasingly in clinical patient stratification expose cultured cells to compound combinations at defined concentrations. Dispensing robots offer high flexibility in assay preparation but are costly, require trained personnel, have limited volumetric accuracy, and need dedicated facilities for handling harmful compounds. Multi-well assays with preloaded compound combinations ready for cell culture may overcome these problems given the development of fast and robust fabrication processes. We introduce a new method for defining the final compound concentrations by controlling the volumes of multiple solid polymer gels each loaded with a different compound at constant concentration. The gel volume in each well of a well plate may be easily defined by local light-induced solidification of a solution of pre-polymer and target compound, with the process being repeated for different compounds to introduce freely definable combinations. The light exposure patterns are produced by a standard computer controlled digital projector, thus enabling "Digital drug dosing" in the resulting cell assays. Specifically, we employ light-induced crosslinking of poly(ethylene glycol) (PEG) pre-polymers that have been extensively explored for drug release systems. PEG hydrogel volumes defined by the digital projector were loaded with combinations of cytostatic compounds used in the clinical multi-drug treatment of colorectal cancer to produce a pre-loaded multiwell assay. The resulting hydrogel assay was used for testing the efficacy of the compound combinations on a colorectal cancer cell line as proof of principle, and we observed very good correlation to control wells with direct dispensation of the drug combinations.



POSTER ABSTRACTS

P15 TITLE

Modeling tau pathology using neurons from human induced pluripotent stem cells

AUTHORS

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ABSTRACT TEXT

Tauopathies are neurodegenerative diseases associated with dysfunctions in the microtubule-associated protein tau. This study used induced pluripotent stem cell (iPSC)-derived neurons to investigate neuronal uptake and release of tau, in order to model the cell-to-cell spread that has been implicated in tau pathogenesis. The iPSCs were differentiated to cells with distinct neuronal morphology expressing neuronal markers such as tau, microtubule-associated protein 2 and β -tubulin III. However, they only expressed the fetal 3R τ tau isoform after differentiation and to some extent continued to proliferate, implying an embryonic state of maturation. Neuronal functionality was demonstrated by application of fluorescent probes and image analysis showing increased intracellular calcium levels in response to the neurotransmitters glutamate/glycine, GABA and acetylcholine as well as membrane depolarization by application of extracellular potassium. Moreover, spontaneous calcium oscillations were demonstrated in a subpopulation of cells. Cellular uptake of exogenous recombinant tau was detected by immunostaining and quantified by a Cellomics Array Scan algorithm. The data shows that recombinant tau with disease-causing mutations were taken up to a higher extent than wild type tau. Furthermore, the neurons released endogenous tau into the cell culture media (ELISA measurements) and the levels increased over time. In contrast, extracellular LDH levels did not increase significantly in the same time interval (membrane integrity assay), suggesting the presence of active tau secretion. Together our results imply the applicability of iPSC-derived neurons in modelling the pathogenic cell-to-cell spread of tau.

P16 TITLE

Yap1 is an upstream activator of Notch signalling which controls pancreas morphogenesis and cell specification

AUTHORS

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ABSTRACT TEXT

The mechanisms that regulate organ size during development have remained elusive, despite decades of research addressing this topic. The Hippo signaling pathway is known to play an important role in limiting the organ size by modulating the activity of the transcriptional factors Yap/Taz which control stem/progenitor cell proliferation and apoptosis. Whether Yap/Taz regulate pancreatic progenitor cell expansion and differentiation during early pancreas development is not known. Our data shows that, in mice, the loss of Hippo signaling caused by constitutively active Yap, results in an unexpected phenotype of severe pancreatic hypoplasia and the death of the transgenic pups just after birth. The Yap1 transgenic pancreas displays defects in both exocrine and endocrine cell differentiation; all the terminally differentiated cell types are absent and cells are 'trapped' at a ductal progenitor state. Several signaling pathways are up-regulated in the Yap1 transgenic pancreas, including Notch. Supporting our findings, the Notch1 gain of function pancreas phenocopies Yap1 gain of function phenotype, indicating a link between these two pathways. We further demonstrate that Notch signaling acts downstream of Yap1, as inhibition of Notch signaling in Yap transgenic pancreata rescues the differentiation phenotype of both exocrine and endocrine lineages. Our work shows that the Yap1 mediated regulation of Notch is essential for the maintenance of pancreas organ size by promoting the proliferation of progenitor cells and their differentiation, including insulin producing Beta cells. This novel discovery highlights the importance of a new regulator Yap1 during pancreas morphogenesis and cell specification.

POSTER ABSTRACTS

P17 TITLE

Hepatic Leukemia Factor: an oncogene or a regulator of normal Stem-Cells?

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ABSTRACT TEXT

Hematopoietic stem cells (HSCs) are the only blood cells that are multipotent and self-renewing. Because of their enormous potential in regenerative medicine as units of engraftment, many efforts have been made in order to understand their unique characteristics. Analysis of extensive microarray data enabled the delineation of genes and regulators in HSCs. One prominent gene was found to be the hepatic leukemia factor (Hlf), a transcription factor studied in the context of acute B-cell leukemia as part of the E2A-HLF oncogenic fusion protein. As being rarely investigated in its normal context, a recent study observed that enforced expression of Hlf induced HSC-like immunophenotype, sustained colony-formation activity, and enhanced self-renewal in primary hematopoietic stem- and progenitors ex vivo. Moreover, Hlf was found as one of the core-factors enabling reprogramming of adult blood cells back into induced-HSC state.

Our recent data show that HLF's transcriptome effects in primary cells include enriched sets of stem-cell and leukemia genes, along with a reduction of myeloid-differentiation genes. Hence, Hlf seems to enhance both normal and malignant sets of genes. Moreover, as the gene harbors several spliced variants, Hlf's canonical form seems to be the predominant one in HSCs. Suitably, the canonical Hlf had an expanded expression in primary murine cells in several in vitro experiments over time, while an alternative variant exhibited the opposite phenomenon.

By understanding molecular mechanisms of a key regulator we will shed new light on normal and malignant stem cells, providing novel ways to fulfill their broad potential.

P18 TITLE

Concomitant loss of Phf6 and Tet2 leads to myelodysplastic syndrome in mouse

AUTHORS

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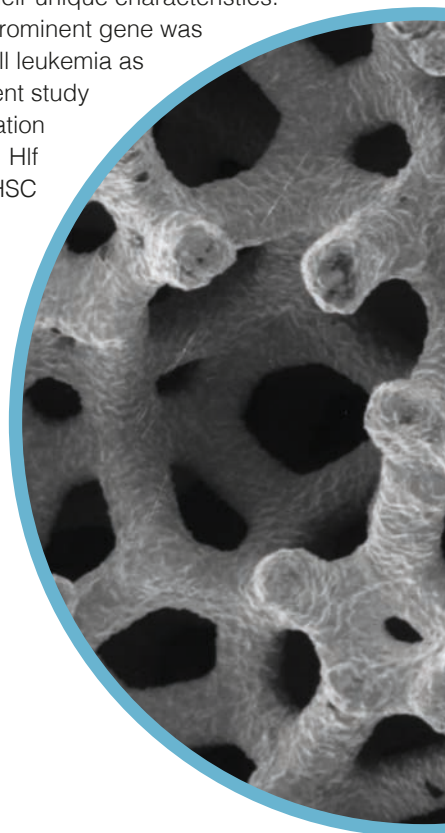
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ABSTRACT TEXT

PHF6, located on the X chromosome, is coding for a putative chromatin protein that has a potential role in regulating transcription. Recent genomic studies in patients with hematological malignancies have revealed that inactivating somatic mutations in the PHF6 genes occur frequently in patients with adult primary T-cell acute lymphoblastic leukemia and, to a lesser extent, in those of acute myeloid leukemia (AML) and myelodysplastic disorders (MDS). However, it is currently unknown how PHF6 regulates normal hematopoiesis and how inactivation of PHF6 contributes to leukemogenesis. To get insight into this, we investigated the consequence of downregulating Phf6 expression in mouse hematopoietic stem cells (HSCs). Allogenic transplantation of Phf6 knockdown (KD) hematopoietic stem cell (HSC) induced neither AML nor T-ALL, but showed severe defect in repopulation activity of HSCs. Our in vitro proliferation assay also revealed that Phf6KD negatively affected the proliferation of long-term HSCs, but not on that of short-term HSCs, indicating that Phf6 function is specifically required for the transition from long-term to short-term HSC. These results also suggest that Phf6 deletion alone is not sufficient to induce overt acute leukemia, thereby implying an importance of co-occurring mutation in leukemogenesis. To test this, we knocked down the Phf6 gene in the Tet2^{-/-}-HSC, since PHF6 mutations co-occur with TET2 mutations in patients with myeloid malignancies. We found that transplantation of Phf6KD-Tet2^{-/-}-HSC induced fatal MDS characterized by the myelodysplasia and pan-cytopenia in the recipients.

Our findings demonstrate that PHF6 has tumor suppressor activity in myeloid malignancies and highlight the cooperative effect of concurrent gene mutations in the pathogenesis of myelodysplastic disorders.



P19 TITLE

Cardiac Stem Cells - In pursuit of cardiac regeneration therapies (Poster)

Cardiac progenitor cells in juvenile pigs differentiate towards cardiac lineage - In pursuit of cardiac regeneration therapies (Abstract)

AUTHORS

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ABSTRACT TEXT

Introduction: Heart failure lists as a growing cause of mortality worldwide and with current therapies only being symptomatic an ever-increasing demand for more definitive therapies is in demand.

Stem cell research has shown promising results with the identification as well as clinical application of several cardiac progenitor cells (CPC) and sparked hope of a new therapy on the rise.

Still, controversy exists regarding their isolation and differentiation and in addition most studies have been conducted in rodents questioning extrapolation of results to humans.

We have, for the first time, isolated, proliferated and differentiated CPC's from juvenile pigs and in addition introduced a new CPC enrichment step during isolation.

Methods: CPC's are isolated from the ventricle in the porcine heart using Collagenase/Trypsin digestion and Percoll-gradient centrifugation. Cells are subsequently cultivated in Matrigel coated dishes and differentiation is initiated by 5-azacytidine and controlled by varying the serum concentrations.

Gene and protein expression of heart specific genes and proteins (Troponin I and Cardiac Actin a.o.) are conducted during proliferation and differentiation using RT-PCR and Western blotting.

Results: The cardiac genes: Troponin I, Cardiac Actin, GATA-4, Connexin-43, MYH7 were all found expressed in the isolated CPC after differentiation. In addition, gene expression of smooth muscle actin and Von Willebrand was detected implying differentiation towards smooth muscle and endothel tissue.

None of the above-mentioned genes were detected at protein level using Western blot.

Conclusion: Our newly isolated CPC differentiate towards cardiac lineage but further investigation is needed to document their differentiation into mature cardiomyocytes.

P20 TITLE

Tissue engineering of muscle/meat: a futuristic project

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ABSTRACT TEXT

During the coming years meat intake will increase dramatically due to increased population and middle class people. Meat production is not in particular environmental friendly especially production of beef. Thus, meat production faces serious problems because it uses large amounts of land, fossil energy, fresh water and energy, and pollute with nutrients. A significant emission of Green House Gasses occurs. In addition, welfare problems, risks of meat scandals like Salmonella, Foot and Mouth diseases, MRSA, BSE, and occur occasional, and finally medicine residues and resistance to antibiotics may occur. This calls for the development of new sustainable foods including stem cell based tissue engineered meat (also called cultured meat or in vitro meat). Thus, Life Cycle Assessment analysis (LCA) suggests that in vitro meat is an environmentally friendly way of producing meat and requires less energy, causes a lower GHG emission (-96%) and less use of land and water compared to conventional produced meat dependent on meat animal type. An environmental friendly cultured meat rest on 4 premises:

1) Growing stem cells from meat producing animals, which can proliferate (without differentiating) 2) That stem cells efficiently and fast differentiate to muscle fibre, which contain all nutrients which are present in conventional produced meat 3) Use of growth- and differentiation media which does not contain animal proteins. 4) Organisation of muscle fibres in a 3-D structure. In August 2013 the first burger was produced as cultured by Prof. Mark Post (Maastricht University), however, the production steps need to be optimized through research.

POSTER ABSTRACTS

P21 TITLE

Mechanical stimulation for muscle tissue engineering

AUTHORS

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ABSTRACT TEXT

Although several mechanical cues are involved in the regulation of myogenic differentiation, the relevance of mechanical stimulation for muscle tissue engineering remains poorly understood. Here, we describe a novel methodology that allows imparting well-controlled mechanical stimulation patterns to cells cultured on flexible substrates, with the aim of driving the arrangement and differentiation of skeletal and smooth myogenic precursors. By means of computer-based simulations, parts of a commercial cell-stretching device were modified to generate a ring-shaped strain field. Skeletal myoblasts and aortic smooth muscle cells (ASMCs) were cultured on flexible-bottomed 6-well plates. Upon confluency, cells were induced by their respective differentiation media and subjected to cyclic strain for 24 h. Differentiation was compared to non-stretched controls after 1 and 7 days of induction by means of microscopy and qPCR. After 1 day, while the passive cells remained randomly oriented, the mechanically stimulated cells aligned perpendicularly to the radial direction of the wells forming a ring pattern on the culture surface. After 7 days, the skeletal precursors differentiated into arrays of mature myotubes, keeping the initial alignment pattern. However, the ASMCs gradually lost the initial alignment gained after mechanical stimulation, suggesting that active strain is needed to maintain the orientation of these cells. In both cell types, gene transcription analysis by qPCR revealed an enhanced rate of differentiation in response to mechanical stimulation. These results highlight the importance of mechanical cues for skeletal and smooth myogenic differentiation, which may be crucial for the success of future muscle tissue engineering paradigms.

P22 TITLE

Bioactive Nanofibrous Scaffold for Craniofacial Tissue Engineering

AUTHORS

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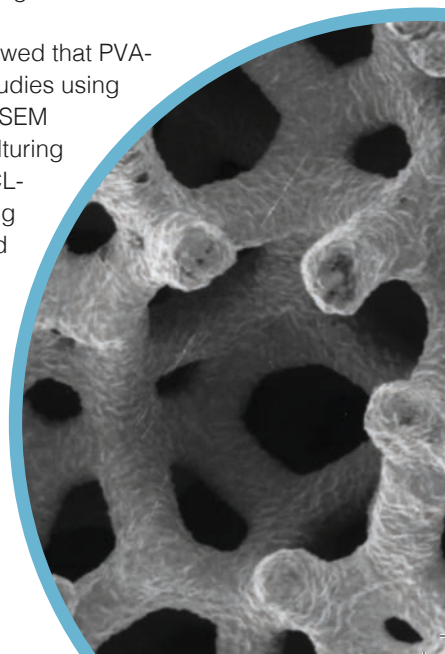
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ABSTRACT TEXT

Bone tissue engineering for craniofacial region is considered challenging owing to its physiologic and anatomical complexities. A porous bioactive scaffold promoting osteogenesis and angiogenesis is required for clinical applications. We have developed an electrospun polyvinyl alcohol (PVA) poly-caprolactone (PCL) - triphasic bioceramic(HAB) scaffold to biomimic native tissue and we tested its ability to support osteogenic differentiation of stromal stem cells (MSC) and its suitability for regeneration of craniofacial defects.

Physiochemical characterizations of the scaffold, including contact angle measurements, showed that PVA-PCL-HAB was more hydrophilic than PCL alone or PCL-HAB combined ($P < 0.05$). Ion release profile studies using ICP-OES of the scaffold showed release of calcium and silica ions, required for initiation of bioactivity. SEM and EDS analyses revealed apatite formation on stimulated body fluid immersed scaffold samples. Culturing human adult dental pulp stem cells (DPSC) and human bone marrow derived MSC seeded on PVA-PCL-HAB scaffold showed enhanced cell proliferation and in vitro osteoblastic differentiation. Cell-containing scaffolds were implanted subcutaneously in immune deficient mice. Histologic examination of retrieved implant sections stained with H&E, Collagen Type I and Human Vimentin antibody demonstrated that the cells survived in vivo in the implants for at least 8 weeks with evidence of osteoblastic differentiation and angiogenesis within the implants. Our results suggest that PVA-PCL-HAB scaffold can support growth and osteoblast differentiation of MSC and thus should be considered for clinical application in fields of dentistry and orthopaedics.



P23 TITLE

Role of PSEN1 in neuronal differentiation and Alzheimer's – modeling human neurodegenerative diseases in a dish

AUTHORS

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ABSTRACT TEXT

Alzheimer's disease (AD) is the most common type of dementia and targets the cerebral cortex and certain subcortical regions. The familial (FAD) form shows early-onset symptoms and it is attributed to mutations in presenilin (PSEN) 1 and 2 and amyloid precursor protein (APP) genes. PSENs are constituents of the gamma-secretase complex that cleaves APP and it correlates with increased production of A β 42 and senile plaque formation. The sporadic (SAD) type is the late-onset form for which no causative genetic background has been identified except for apolipoprotein E (APOE) gene.

Human induced Pluripotent Stem Cells (hiPSC) allow the generation of patient-specific derived neurons, providing an in vitro tool for studying neurodegenerative disorders. We have generated PSEN1-hiPSCs carrying the L150P mutation from two affected brothers. These PSEN1-hiPSCs were carefully characterized in regards to their pluripotent potential by quantitative PCR and immunocytochemistry for key pluripotency genes and proteins. Furthermore, their differentiation potential was explored by embryonic body formation and differentiation of these into all three germ layers. Neuronal differentiation and characterization was performed in PSEN1 lines and isogenic controls, which have been generated via CRISPR-Cas9 technology, and age-matched controls. To address functional impairment of PSEN1-hiPSC neurons we performed apoptosis and autophagy assays. Furthermore ELISA assays were performed in the PSEN1 lines, clearly showing an increase in A β 42 over A β 40, compared to isogenic controls.

Electrical and calcium-signaling in PSEN1-derived neurons will also be evaluated considering its reported involvement in AD pathogenicity in parallel with mitochondria studies that are also related to "Ca²⁺ hypothesis" in AD.

P24 TITLE

Determining Factors Responsible for the Regenerative Capacity of the Stromal Vascular Fraction

AUTHORS

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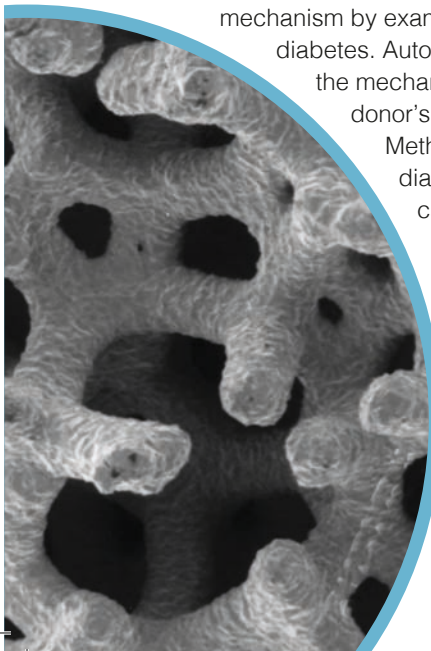
ABSTRACT TEXT

We hypothesize that the difference between SVF from diabetic rats and healthy rats is reflected in the regenerative capacity of the SVF.

Aim: We aim to optimize the stromal vascular fraction (SVF) based correction of erectile dysfunction (ED) in a cavernous nerve crush injury rat model. To that end, we investigate the consequences of the diabetic state of the SVF--donor rat on the quality and regenerative capacity of the SVF. Furthermore, we aim to determine candidate proteins crucial for the regenerative mechanism by examining proteomic differences between the two groups. **Background:** ED is a frequent complication of diabetes. Autologous stem cell therapy with SVF cells is a promising future treatment for ED. Little is known about the mechanism by which the SVF performs its regenerative effect and studies regarding the impact of the SVF--donor's health status and the regenerative capacity of SVF, are scarce.

Method: In vitro studies are conducted to characterize the potential differences between SVF cells from diabetic and healthy rats. Additionally, the rat model for ED is used to determine the in vivo regenerative capacity of the two SVF populations. Finally, proteomic differences may reveal proteins crucial for the regeneration mechanism.

Perspectives: Stem cell therapy with SVF holds great potential as a treatment of diabetic ED. However before this option for treatment can be a clinical reality, further knowledge regarding the mechanism and SVF quality is crucial in order to optimize regenerative effects and to outline the prognosis of the treatment.



POSTER ABSTRACTS

P25 TITLE

Murine miR-302 as a potential stem cell marker

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ABSTRACT TEXT

Stem cells have some unique properties including self-renewal and multipotency which are also shared with cancer stem cells (CSCs). MicroRNAs are short non-coding RNAs that post-transcriptionally regulate gene expression either by degradation or by translation inhibition of their mRNA targets. MiRNAs are involved in many biological processes. MiR-302s/367 gene is a polycistronic miRNA cluster including miR-302b/c/a/d and miR-367. The stem cell specific transcription factors Oct4, Sox2 and Nanog drive miR-302 expression. Furthermore, the miR-302 promoter is a direct target of the Wnt/ β -catenin signaling pathway. The miR-302s/367 gene is overlapping with the LA related protein 7 (LARP7) gene, encoded by the opposite DNA strand. MiR-302 has been shown to repress mRNAs required for differentiation and can induce pluripotency in somatic cells. The purpose of this study is to produce miR-302-EGFP reporter mice, which will enable us to evaluate the mmiR-302 gene expression pattern during embryogenesis and in adult mouse tissues. Moreover, as mmiR-302 is expressed in stem cells, we hypothesize that we can trace CSCs through EGFP expression in tumor cells. We have found that mmiR-302 is expressed in murine embryonic stem cells, teratomas and in mammary gland tumors. Compared to housekeeping genes like HPRT, the miR-302 gene is low expressed. We have performed 'Rapid Amplification of cDNA Ends' (RACE) for the 5' and 3' ends of miR-302. In contrast to the human miR-302 gene which has 3 exons, the mouse gene only consists of two exons with miR-cluster located in the intron. Sequencing of ES cell and teratoma derived cDNA showed splicing variants and alternative polyadenylation.

P26 TITLE

Investigation into the interactions of primary limbal epithelial stem cells and hT-CEpi using tissue engineered cornea

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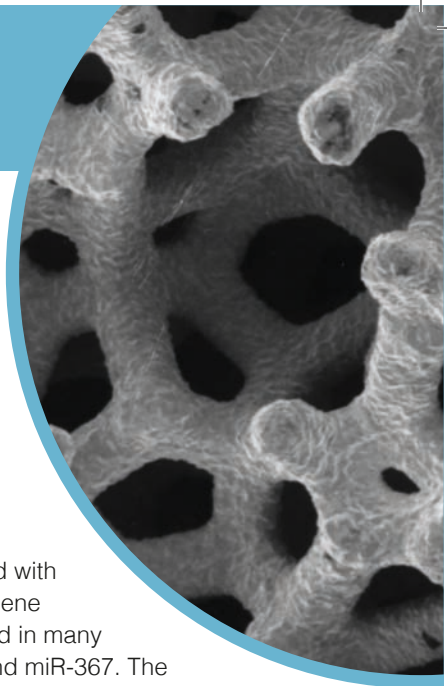
ABSTRACT TEXT

Introduction: Though knowledge of the compositional make up and structure of the limbal niche has progressed exponentially during the past decade, much is yet to be understood. Identifying the precise profile and role of the stromal make up which spans the ocular surface may inform researchers of the most optimum conditions needed to effectively expand LSCs in vitro.

Methods: The corneal stroma was tissue engineered in vitro using both limbal and corneal fibroblasts embedded within a tissue engineered 3D collagen matrix. The effect of these two different fibroblasts on LSCs and hTCEpi corneal epithelial cell line were then subsequently determined using phase contrast microscopy, histological analysis and PCR for specific stem cell markers. The study aimed to develop an in vitro model which could be used to determine whether limbal, as opposed to corneal fibroblasts, maintained the stem cell phenotype of LSCs and hTCEpi cell line.

Results: Histological analysis of the tissue engineered cornea showed a comparable structure to that of the human cornea, though with limited epithelial stratification. PCR results for epithelial cell markers of cells cultured on limbal fibroblasts showed reduced expression of CK3, a negative marker for LESC's, whilst also exhibiting a relatively low expression level of P63, a marker for undifferentiated LSCs.

Conclusion: We have shown the potential for the construction of a tissue engineered human cornea using a 3D collagen matrix and described some preliminary results in the analysis of the effects of varying stroma consisting of limbal and corneal fibroblasts, respectively, on the proliferation of stem cell phenotype of primary LSCs and hTCEpi corneal epithelial cells.



POSTER ABSTRACTS

P27 TITLE

Modeling Frontotemporal Dementia using Neurons from Human Induced Pluripotent Stem Cells

AUTHORS

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ABSTRACT TEXT

Individuals carrying a microtubule-associated protein tau (MAPT) R406W mutation develop a form of Frontotemporal dementia, which clinically resembles Alzheimer's disease. Neurons derived from iPSCs have potential to improve in vitro models for neurodegenerative diseases. In our study, neurons were derived from five iPSC lines from patients with a MAPT R406W mutation and five control individuals. The ten neuronal cultures exhibited clear neuronal morphology with neurite outgrowth. Real-time PCR showed up-regulation of neuronal marker genes and immunocytochemistry demonstrated the appearance of β III-tubulin, microtubule-associated protein 2, and tau positive cells. Western blot showed the presence of the embryonic tau isoform (3RN0). Depolarisation of the membrane potential by increasing the extracellular potassium levels and stimulation with neurotransmitters (GABA or glutamate/glycine) resulted in a considerable increase in cytosolic calcium as detected by fluorescent probes. One line was further analysed by image analysis demonstrating spontaneous calcium spikes, and by patch clamp recordings showing voltage-gated Na⁺ and K⁺ currents in a subpopulation of the cells. Overall, the ten neuronal cultures did indeed exhibit neuronal marker expression and functional characteristics although encompassing a relatively immature neuron population. Investigation for disease phenotypic difference in the five MAPT versus five control neurons showed differences in total tau protein levels in standard growth conditions, whereas no differences in tau subcellular localisation or phosphorylation levels (p199 and p396) have been identified so far. Moreover, media supplement withdrawal resulted in stronger responses (decreases in total tau levels) in neurons derived from MAPT carriers supporting the presence of a disease phenotype.

P28 TITLE

Effects of fetal bovine serum, human platelet lysate and hypoxia on adipose tissue-derived stromal cell proliferation and senescence

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ABSTRACT TEXT

Background: Adipose tissue-derived stromal cells (ASCs) are an attractive source for cell-based therapies due to their regenerative and immune modulatory properties. ASCs are expanded in vitro prior to autologous or allogenic treatment. However, expansion at inadequate culture conditions might result in low proliferation rates, quiescence or senescence.

Aim: To investigate the effects of fetal bovine serum (FBS), pooled human platelet lysate (hPL) and hypoxia on ASCs with emphasis on cell cycle regulation, proliferation, quiescence and senescence.

Methods: ASCs are expanded in FBS- or hPL- supplemented media, at atmospheric or hypoxic oxygen tensions. Effects of culture conditions on ASCs will be evaluated based on proliferation rates, senescence-associated (SA) β -galactosidase activity, and expression of genes involved in growth factor receptor signaling, proliferation, cell cycle regulation and senescence.

Results: ASCs expanded in hPL exhibit high proliferation rates, low expression of cell cycle inhibitors and negativity for SA β -galactosidase activity. Hypoxia has no effect on proliferation rates, cell cycle inhibitor expression levels or SA β -galactosidase activity in hPL-expanded ASCs. ASCs expanded in FBS exhibit low proliferation rates, high expression levels of cell cycle inhibitor p21, and increasing SA β -galactosidase activity with increasing passage. Hypoxia increases proliferation rates and decreases p21 expression in FBS-expanded ASCs.

Conclusion: ASCs expanded in FBS appear to exhibit a basal level of cell cycle arrest evident from passage 1, which partly is reversed by hypoxia. In contrast, hPL promotes high ASC proliferation rates, regardless of oxygen tension. This indicates that hPL fully supports ASC proliferation, in contrast to FBS.

POSTER ABSTRACTS

P29 TITLE

Efficacy of human SVF and ASCs for improving flap survival in a rat model.

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ABSTRACT TEXT

Introduction: Flap necrosis due to limited vascular supply is an ongoing challenge in plastic surgery. In the last decade especially adipose-derived stromal cells have emerged as a possible solution to improve flap survival. The cells can be used freshly isolated as the stromal vascular fraction (SVF) or after culture-expansion of the adipose-derived stromal cells (ASCs). The aim of this study was to compare the efficacy of human SVF and ASCs for improving flap survival in a rat model.

Methods: Lipoaspirates from three human female donors were used for isolation of SVF. 36 male Sprague Dawley rats were randomized to three groups. A caudally based random flap measuring 2x7cm including a triangular area at the tip was elevated on the dorsum of each rat (flap 15cm²). The flaps were injected with 5 x 10⁶ SVF cells, 5 x 10⁶ ASCs or phosphate buffered saline. Seven days later flap survival was analyzed with standardized photography and flap skin was fixed for histologic analysis to examine capillary density and stem cell retention in the flap.

Results: The mean survival rates \pm SD were 55.0 \pm 7.2%, 50.4 \pm 9.1% and 45.7 \pm 9.5% for the SVF, ASC and control group respectively (SVF group significantly better than control group $p < 0.05$). Histology results will be presented at the conference.

Conclusions: Flap survival was most improved with SVF treatment. The clinical effect was modest and could represent the physiologic limits of stem cell induced angiogenesis in an acute setting. Future research should focus on which cellular subpopulation of SVF that is responsible for this effect.

P30 TITLE

Modeling frontotemporal dementia-3 neurons derived from patient-specific IPS cells

AUTHORS

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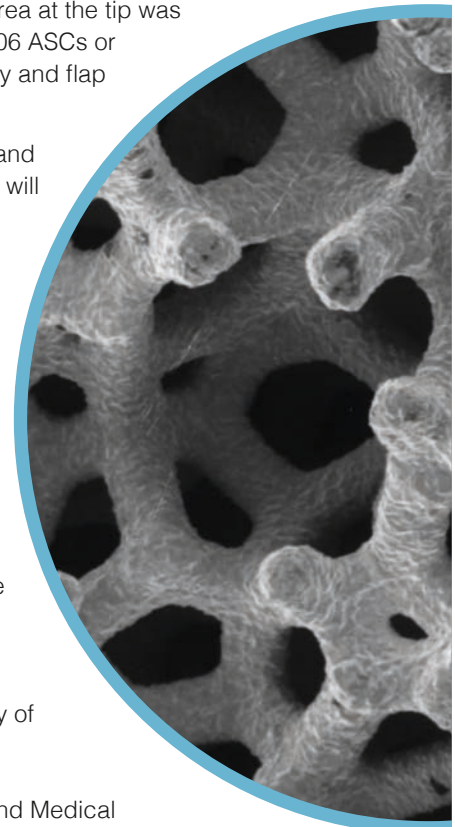
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ABSTRACT TEXT

The charged multivesicular body protein 2B (CHMP2B) has been identified as a dominant gene mutation for endo-lysosomal dysfunction observed in frontotemporal dementia linked to chromosome 3 (FTD-3) patients. Thus, different disease models of the FTD-3 have been developed, such as mouse and Drosophila, both showing dominant phenotype. Although, recent study has resulted in new progress using mice carrying forebrain-specific expression of mutant CHMP2B, the mechanistic involvement of the endo-lysosomal dysfunction in pathological processes still remains fundamentally unexplored. In the present study, we aim at constructing a disease model directly based on human induced pluripotent stem cells (hiPSCs) from FTD-3 patients and age-matched healthy control individuals. Using a patient hiPSC cell line we have derived neural progenitor cells (NPCs) expressing NPC marker NESTIN and the forebrain region-specific markers FOXG1, OTX2 and PAX6. Subsequently, we matured these NPCs to glutamatergic cortical neurons expressing MAP2AB, TUJ1, TAU, VGLUT1, TBR1 and CTIP2. Those neurons will be tested for disease phenotype using known late-stage endosome marker through immunocytochemical staining. Transmission electronic microscopy will also be applied to show the distribution of aberrant endosomes. Furthermore, neural differentiation and comparison with hiPSCs derived from different patients carrying the same mutation and age-matched healthy controls will show whether there is an abnormal expression of the CHMP2B protein in FTD-3 patient neurons. Taking together these experiments will provide valuable insight into FTD disease modeling, with a particular focus on the CHMP2B mutation, and establish a platform for elucidating the mechanism that leads to neuronal dysfunction in early-onset dementias.



P31 TITLE

Reprogramming of MCADD patient fibroblast into induced pluripotent stem cells using a lentivirus-based gene delivery method

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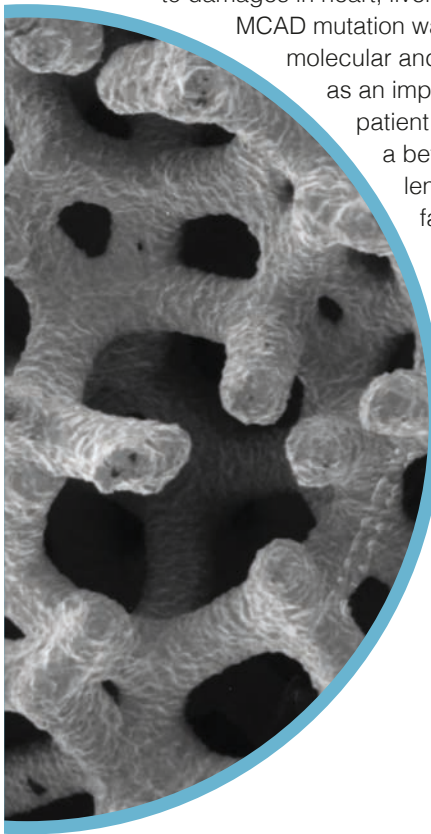
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ABSTRACT TEXT

Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCADD) is one of the most common defects in fatty acid oxidation. MCADD is frequently caused by a prevalent homozygous mutation (Lys329Glu). This mutation causes metabolic disorders, which eventually lead to damages in heart, liver and skeletal muscle systems. Previous understanding of the molecular and cellular consequence of MCAD mutation was mainly based on studies of dermal fibroblasts in vitro. However, dermal fibroblasts cannot fully mimic molecular and cellular effects resulted from the MCAD mutation. Induced pluripotent stem cells (iPSCs) have evolved as an important cell model for the investigation of disease pathogenesis. Thus, iPSCs derived from MCADD patient fibroblasts, followed by differentiating into functional cardiac, hepatic and muscular cells, will serve as a better cellular model for the understanding of MCADD pathogenesis. In this study, we have established a lentivirus-based reprogramming method, which delivers a polycistronic cassette of the four transcription factors (OCT4, KLF4, SOX2, C-MYC). To enable the real-time monitoration of reprogramming process and the silencing of the exogenous genes, the polycistronic cassette is linked to a red fluorescent gene dTOMATO by an IRES element. With this method, we have successfully derived MCADD iPSC lines carrying the Lys329Glu mutation as validated by Sanger sequencing. We have observed that the reprogramming efficiency of MCADD fibroblasts was much lower than the normal dermal fibroblasts. The MCADD-iPSC lines are being differentiated into functional cardiomyocytes, hepatocytes and skeletal muscle cells. These functional lineages will be further subjected to mitochondrial functioning assay (mitochondrial morphology, mitochondrial membrane potential, mitochondrial superoxide levels), proteomics, and transcriptomics analysis. These results will provide a promising alternative to elucidate the physiological and molecular effects of MCADD mutation.

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