platform for advanced cellular therapies austria

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Abstract Book

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Michael Comer – PACT Chairman

September 2016

Dear Colleagues and Delegates,

Welcome to our PACT Symposium "Designer Cells Go Clinic"! It would seem that sometimes it is extremely difficult to imagine why government and funding agencies remain so difficult to convince that "research" is an investment for the future health and well-being of our planet. While budgets for 'defence and security purposes' remain and increase disproportionately to all else, funding clinical research bridging the gap between fundamental thinking and its application remains a formidable challenge. Furthermore, in these days of exorbitant medical costs and restricted health budgets, it is apparent that the unit value of funding for research in medicine and the basic sciences continues to decrease dramatically per capita. Indeed, many universities have problems to cover the costs of their teaching responsibilities let alone for innovative or entrepreneurial creativity, where the "grey matter" is kept busy trying to "juggle" financial support in order to make ends meet. It is therefore (said cautiously) "refreshing" that in spite of this imposed corset to the inquiring mind we still manage to make breakthroughs. These sometimes take a little longer time for "im-pact" than expected and the ideas created in the lab or the clinic until they actually become an advanced therapeutic (or diagnostic) medical procedure are often not well represented or "marketed".

This month (September 7th 2016), the Körber-Prize (for scientific achievement similar in standards to Nobel) was awarded to Prof. Dr. Hans Clevers a stem cell biologist and medical doctor for his work (from 2009) on intestinal stem cells and their ability to grow and establish "mini-organs". Derived from the patient these can be used to specifically test potential medications, for example in cancer therapy and often thereby replacing or reducing animal experimentation and at the same time moving towards a "personalised" medical approach. Furthermore, staying with oncology aspects, the so called CAR T-Cell Therapy is another important development where the patients' own immune system is "activated" by taking cells and genetically modifying them to recognise and "kill" cancer cells in order to treat conditions like acute lymphoblastic leukaemia or othersThe process is known as adoptive cell transfer (ACT) and has shown very encouraging results in, albeit to date, relatively small clinical studies. This ACT-procedure will be addressed also during the course of this Symposium.

Other issues become apparent too within the framework of our topic namely, "advanced cellular therapies" in the clinical applications, for example regulatory affairs and/or ethical considerations which, will also be presented. The PACT-Scientific Organising Committee welcomes you to this; your "Platform" addressing the theme of your chosen scientific and clinical endeavours and creativity in the realisation of **"Designer Cells Go Clinic"**. We are looking forward to your participation, profound interest and the ensuing lively discussions.
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MYOBLASTS IN CLINICAL TRIALS – WHAT LESSONS DID WE LEARN ABOUT THE FUTURE OF CELL THERAPIES?

Rainer Marksteiner

Innovacell Biotechnologie AG, Innsbruck

Cell therapy has emerged as a new and promising option in the treatment of many diseases, which are related to tissue damage or degeneration. Over last decades enormous progress have been made in the technologies related to cell isolation, expansion and differentiation but it is still not the part of everyday clinical practice.
Both the clinical and non-clinical development programs required for the approval process for myoblasts as new treatment possibility for incontinence is a good example to demonstrate many challenges faced by developers in the field of cell therapy.
The regulatory pathway for standard pharmaceuticals is well defined and established but unfortunately it is not fitting in parallel to the characteristics of cellular products. A very small number of approved ATMP products on the market can be seen as a reason, since the new European regulation came into force. The vast experience, knowledge and important lessons have been obtained from clinical programs and are used to develop strategies in the approval process to avoid the fear that cellular therapies will be only limited to small clinical trials and will never attain the status of main stream treatments.

Rainer.Marksteiner@innovacell.com

MSC FOR GVHD: MULTICENTRE TRIALS AND TRIBULATIONS

Michael Cross

Department of Haematology and Oncology, University of Leipzig

Graft versus Host Disease (GvHD) is major cause of morbidity and mortality in patients receiving allogeneic haematopoietic stem cell transplantation, with around 1000 new cases every year requiring treatment in Germany. Although 30-50% of these can be controlled by standard first-line steroid therapy, there are insufficient options for those that are steroid resistant.
Mesenchymal stromal cells suppress cellular immunity *in vitro*, suppress graft rejection *in vivo* and were first reported to alleviate steroid-resistant GvHD more than 10 years ago. In the meantime, a large number of studies have attempted to define and characterize this activity. However, variability in the results raise important questions concerning the mechanisms of action of MSC and the effects of source, culture protocol, product specification as well as patient inclusion criteria.
Rethrim is a phase III, randomized, double blind, placebo controlled, multi-centre phase III trial coordinated by the University of Leiden and designed to approach some of these issues by providing a robust test of MSC to restore tissue regeneration in patients with acute visceral GvHD. The target is to include 150 patients in 6 treatment centres using standardised inclusion criteria, product preparation, quality control and treatment regimens, with centralised quality control and data analysis. This has necessitated extensive international harmonisation between participating centres and their respective regulatory authorities. The Rethrim project includes an intensive characterization of individual products

in an attempt to identify variables and candidate markers that can be used for prospective potency testing in the future.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 643580.

crossm@medizin.uni-leipzig.de

FROM PLURIPOTENT TO HEMATOPOIETIC STEM CELLS – HOX PROTEINS PAVE THE WAY

Hannes Klump[1], Nadine Teichweyde[1], Lara Kasperidus[1], Sebastian Carotta[2], Valerie Kouskoff[3], Georges Lacaud[4], Peter Horn[1].

[1]University Hospital Essen, Institute for Transfusion Medicine, Virchowstrasse 179, 45147 Essen, Germany
[2]Boehringer Ingelheim RCV, Dr Boehringer-Gasse, 1120 Vienna, Austria
[3]Cancer Research UK Stem Cell Haematopoiesis Group and [4] Cancer Research UK Stem Cell Biology Group, Cancer Research UK Manchester Institute, University of Manchester, United Kingdom

Generation of hematopoietic stem cells (HSCs) from pluripotent stem cells, *in vitro*, holds great promise for regenerative therapies. To date, the generation of HSCs remains challenging and has primarily been achieved in mouse cells by overexpression of the homeotic selector protein HOXB4. The exact cellular stage at which HOXB4 first unfolds its hematopoiesis-promoting activity, *in vitro*, is not yet known. However, its identification is a prerequisite for unambiguously identifying the molecular circuits controlling HSC development, because the activity of HOX proteins is highly cell- and context dependent. To identify this cell population, we retrovirally expressed HOXB4 in differentiating mouse embryonic stem cells (ESCs). Through the use of *Runx1*$^{(-/-)}$ ESCs containing a Doxycycline-inducible *Runx1* coding sequence, we uncovered that HOXB4 promotes the formation of hematopoietic cells by forcing hemato-endothelial precursors, the so-called hemangioblasts, towards the hematopoietic fate. HOXB4 may be paradigmatic for the activities of all HOX4 paralogues, as ectopic expression of the other orthologues HOXA4, C4 and D4 similarly enhanced hematopoietic development of differentiating ESCs, *in vitro*. Taken together, HOXB4 (and presumably the other HOX4 paralogues) acts as a cell fate determinant in hemangioblasts promoting the development of hematopoietic stem and progenitor cells from differentiating pluripotent stem cells, *in vitro*.

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hannes.klump@uk-essen.de

UNIQUE NEWLY DISCOVERED MUSE CELLS MAY LEAD TO THE PARADIGM SHIFT OF STEM CELL THERAPY

Mari Dezawa, MD, PhD,

Department of Stem Cell Biology and Histology, Tohoku University Graduate School of Medicine, Sendai, Japan

Multilineage-differentiating stress enduring (Muse) cells are naturally existing unique endogenous stem cells that are non-tumorigenic and are pluripotent-like because they express pluripotency markers, can generate cells representative of all three germ layers from a single cell and are able to self-renew and to spontaneously differentiate into cells compatible to the tissue after homing into damaged tissue. Whenever they are injected intravenously, they can escape from being trapped in the lung and spleen, unlike mesenchymal stem cells (MSCs), and efficiently home into damaged tissue, suggesting that robust repair can be delivered by simple intravenous injection of naïve Muse cells. Such unique functions of Muse cells were demonstrated in animal models of stroke, partial hepatectomy, skin ulcer of diabetes mellitus and muscle degeneration. They do not have to be "induced," or genetically manipulated, to be pluripotent or be purposive cells before transplantation as required with some other cell varieties - they already display inherent pluripotent-like properties after isolation and, with their acquired properties of purposive cells, Muse cells spontaneously repair damaged sites based on their unique mechanisms.
They can be collected as cells positive for SSEA-3, a surface marker for pluripotent stem cells, from readily accessible sources such as the bone marrow (~0.03% of the total mononucleated cell population), and from cultured fibroblasts (several %), as well as from the dermis and adipose tissue. Thus, they are expected to be practical cells for clinical application. Recently, Muse cells are shown to circulate in peripheral blood in healthy donors, and the number increases in stroke patients in an acute phase, suggesting that endogenous Muse cells are mobilized into peripheral blood to repair tissues while their number is not sufficient to recover, and that supply of exogenous Muse cells is expected to deliver statistically meaningful functional recovery. Overall, results suggest that Muse cells are a feasible source for cell-based approaches and may safely provide clinically relevant regenerative effects compatible with the 'body's natural repair systems'.

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mdezawa@med.tohoku.ac.jp

FROM CELLULAR SENESCENCE TO CIRCULATING miRNA AS BIOMARKERS IN BONE DISEASES

Johannes Grillari[1,7], Sylvia Weilner[1,2,7], Elisabeth Schraml[1], Ursula Heilmeier[3], Peter Pietschmann[4], Heinz Redl[5,7], Thomas Link[3], Fabian Schröder[6], Klemens Vierlinger[6], Regina Grillari-Voglauer[1,7], Matthias Hackl[2,7]

[1]Christian Doppler Laboratory on Biotechnology of Skin Aging, Department of Biotechnology, BOKU - University of Natural Resources and Life Sciences Vienna, [2]TAmiRNA GmbH, [3]University of San Francisco, [4]Medical University Vienna, [5]LBI for Experimental and Clinical Traumatology, [6]Austrian Institute of Technology, [7]Austrian Cluster for Tissue Regeneration

Damage to cells and tissues is one of the driving forces of aging and age-related diseases. To counteract this functional decline, various repair systems are in place. Among these are adult stem cells that self-renew and differentiate thus maintaining homeostasis and regeneration of tissues and organs. However, not only their functionality declines with age, but also the systemic environment of the elderly negatively impacts on them, either by circulating factors that inhibit stem cell functions, or by the absence of factors needed to support it. One organ notably affected by the reduced differentiation capacity of stem cells with age is the skeleton leading to slow bone healing or low trauma bone fractures.
Here, we report that circulating microvesicles impact on the osteogenic differentiation capacity of mesenchymal stem cells in a donor-age dependent way. While searching for factors mediating the inhibitory effect of microvesicles on osteogenesis, we identified miR-31 as a crucial component as secreted by senescent endothelial cells, known to increase during aging *in vivo*. We demonstrate that endothelial miR-31 is secreted by senescent cells within microvesicles and are taken up by mesenchymal stem cells where it inhibits osteogenic differentiation.
These results prompted us to look more broadly at miRNAs differentially circulating in diabetic and non-diabetic osteoporosis fracture patients, and indeed, identified signatures of miRNAs with predictive power superior to the gold standard of DXA measurements. Several of these miRNAs interfere with osteogenic differentiation, indicating a causal role in the pathobiology of osteoporosis. Besides their potential as plasma-based biomarkers for aging and osteoporotic fracture, they might also be indicative for a systemic environment that does not favour cell based therapies, whenever osteogenesis is a limiting factor. Finally, these miRNAs represent therapeutic tools and targets in the context of bone diseases.

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Johannes.grillari@boku.ac.at

REPAIR PROCESSES AT THE PROTEIN LEVEL – AN EXPLORATORY WHOLE-PROTEOME PROFILING APPROACH TO FOLLOW UP TENDON AND CARTILAGE HEALING

Iris Ribitsch[1], Andrea Bileck[2], David Kreil[3], Julie Rosser[1], Eva Haltmayer[1], Manuela Miksic[1], Simone Gabner[4], Monika Egerbacher[4], Christopher Gerner[2], Florien Jenner[1]

[1]Equine Clinic, Vetmeduni Vienna, Vienna, AUT.
[2]Institute of analytical Chemistry,University Vienna, Vienna AUT.
[3]Chair of Bioinformatics, BOKU, Vienna, AUT.

[4] Institute of Anatomy, Histology and Embryology, Vetmeduni Vienna, Vienna, AUT.

Tendon and cartilage tissue does not regenerate but repairs with biomechanically inferior scar tissue. Better understanding of the mechanisms behind tendon and cartilage repair would provide pivotal information to tailor regenerative therapies inducing regeneration rather than scar formation: Standardized cartilage and tendon lesions were surgically induced in 2-4 year old sheep. Full thickness 7mm diameter cartilage lesions were drilled into the cartilage of the medial femoral condyle. The lateral femoral condyle served as sham operated control. Microfracture of the subchondral bone plate was performed. Tendon lesions were inflicted to the Achilles tendon using a 3mm biopsy punch. Samples were harvested according to the three stages of healing 3 days, three weeks and 5 months. Exploratory whole-proteome profiling was performed to identify key factors involved in adult healing. Protein fractions were digested with Lys-C and trypsin. Peptides were seperated by Nano Flow Liquid Chromatography (Dionex 3000 UHPLC) and analysed by mass spectrometry (Thermo QEXACTIVE orbitrap). Data were evaluated using Proteome Discover (Thermo) and MaxQuant software (free software by M.Mann). For quantification of proteins, reaction monitoring using triple quadrupole mass spectrometry (Agilent 6490) was employed. Peptides were quantified referring to isotope labeled internal standards. When comparing secretomes from injured samples with normal tissue samples, fold-control values greater than 2^{11} and p-values smaller than 10^{-11} were achieved, indicating robust and sensitive methodology and allowing analysis of the changes in the inflammatory response, growth factor expression and extracellular matrix compared to healthy cartilage and tendon tissue over the three healing phases.

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iris.ribitsch@vetmeduni.ac.at

SCAFFOLD CONCEPTS FOR CARTILAGE REGENERATION

S Nürnberger[1,2], X Monforte Vila[3], D Hanetseder[3], B Rieder[3], S Wolbank[2], H Redl[2], A Teuschl[3]

[1]Department of Trauma Surgery, Medical University of Vienna, Vienna, AT.
[2]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, AT.
[3]Department of Biochemical Engineering, University of Applied Sciences Technikum Wien, Vienna, AT.

The success of cartilage regeneration by tissue engineering depends on the differentiation potential of the applied cells and the properties of the cell-carrying biomaterial. The major requirements of the latter are sufficient stiffness to withstand the varying loads during joint movement right after transplantation as well as traction forces on the material by cells. Cells should be protected from overload and only a reduced amount of mechanical stress transferred as chondrogenic stimulus. We present natural and synthetic polymers as well as decellularized tissue with promising properties as biomaterials for cartilage regeneration.
Silk is a natural polymer with high mechanical stiffness, slow degradation times and due to its inert character for cells of great interest for *in vitro* models. As gels it had a chondroinductive influence on bovine chondrocytes without the requirement for growth factors, most likely due to the dense encapsulation of the cells. Porous silk sponges allowed regular cell distribution and long term culture of several weeks without shrinkage. It resisted compression

significantly better than common commercial scaffolds. Synthetic polyphosphazene-based scaffolds also have a good compressive strength and allow various chemical adaptations. Functionalized with glutathione the scaffolds improved the chondrogenic differentiation potential of adipose derived stromal cells. Despite encouraging results with those two materials, we additionally follow a strategy based on decellularized homologous cartilage tissue as scaffold material for cartilage repair. Several protocols were tested to remove cells and glycosaminoglycans from articular cartilage to avoid body reactions and increase the porosity of the dense matrix.
All three materials, silk, polyphosphazenes and decellularized cartilage showed promising properties, either as *in vitro* system to mimic articular cartilage, to investigate mechanical influences on cells or for translational studies for *in vivo* application.

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sylvia.nuernberger@meduniwien.ac.at

PERIPHERAL BLOOD MONONUCLEAR CELL SECRETOME FOR TISSUE REPAIR

Lucian Beer[1,2], Michael Mildner[4], Mariann Gyöngyösi[5], Hendrik Jan Ankersmit[2,3,6]

[1]Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna,
Austria
[2]Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical
University of Vienna, Austria
[3]Head FFG Project 852748 "APOSEC
[4]Department of Dermatology, Medical University of Vienna, Vienna, Austria
[5]Division of Cardiology, Medical University of Vienna, Vienna, Austria
[6]Department of Thoracic Surgery, Medical University Vienna, Vienna, Austria

For almost two decades, cell-based therapies have been tested in modern regenerative medicine to either replace or regenerate human cells, tissues, or organs and restore normal function. Secreted paracrine factors are increasingly accepted to exert beneficial biological effects that promote tissue regeneration. These factors are called the cell secretome and include a variety of proteins, lipids, microRNAs, and extracellular vesicles, such as exosomes and microparticles. The stem cell secretome has most commonly been investigated in pre-clinical settings. However, a growing body of evidence indicates that other cell types, such as peripheral blood mononuclear cells (PBMCs), are capable of releasing significant amounts of biologically active paracrine factors that exert beneficial regenerative effects. The apoptotic PBMC secretome has been successfully used pre-clinically for the treatment of acute myocardial infarction, chronic heart failure, spinal cord injury, stroke, and wound healing. In this review we describe the benefits of choosing PBMCs instead of stem cells in regenerative medicine and characterize the factors released from apoptotic PBMCs. We also discuss pre-clinical studies with apoptotic cell-based therapies and regulatory issues that have to be considered when conducting clinical trials using cell secretome-based products. This should allow the reader to envision PBMC secretome-based therapies as alternatives to all other forms of cell-based therapies.

Keywords: PBMC, regenerative medicine, tissue regeneration, paracrine, secretome

hendrik.ankersmit@meduniwien.ac.at

TRANSITION OF STEM CELLS TO THE CLINIC - ETHICAL ISSUE

Peter Kampits

Danube University Krems, Austria

CLINICAL STUDIES WITH ATMPs – PRACTICAL ISSUES

Martin Brunner

Department of Clinical Pharmacology and Ethics-Committee, Medical University of Vienna, Austria

Advances in the development of ATMPs have led to an increasing number of clinical studies submitted to Ethics-Committees for review and approval. This results in new challenges for all involved parties that need to be considered and will be discussed.

martin.brunner@meduniwien.ac.at

AN UPDATE ON EUROPEAN REGULATORY ACTIVITY AND EXPERIENCE

Ilona Reischl

BASG - Federal Office for Safety in Health Care
AGES - Austrian Agency for Health and Food Safety

Scientific activity has greatly increased in recent years and fueled an increasing number of clinical trials in Europe. In parallel, regulatory discussions are ongoing on the European level to ensure information of developers on framework requirements and to further develop the framework itself through guidance development. These activities will be reviewed briefly.
The classification of ATMPs will be reviewed briefly and linked to recent issues encountered in the context of ATMP development.

ilona.reischl@ages.at

INVESTIGATING THE ROLE OF MECHANOSENSORS IN CELL FUNCTION AND IN CARDIAC DISEASES

Giancarlo Forte[1,2]

[1]Center for Translational Medicine, International Clinical Research Center (CTM-ICRC), Brno,
Czech Republic
[2]Department of Biomaterials Science, Institute of Dentistry, University of Turku, Turku, Finland

The term “mechanosensing” describes the ability of the cell to perceive mechanical cues arising from the surrounding milieu and transduce them into a biological response. This feature resides in the peculiar properties of protein complexes to either rearrange their molecular structure or to modify their localization and activity in response to changes in the mechanics of the environment. The mechanosensing of the cells is historically associated to the ability of the focal adhesions (FAs) to rearrange and transduce ECM cues or to the capacity of YAP/TAZ proteins to shuttle to the nucleus and participate in the transcription of cell- and stage-specific genes. Heart failure - the end stage condition for which cardiac muscle is not able to produce the pumping force necessary to provide adequate blood flow to

the organism – can be described as the mechanical failure of the muscle. As such, defects or modifications in the extracellular matrix (ECM) composition and mechanics and/or in the mechanobiology apparatus can result in aberrant signalling and changes in tissue-specific cell function independently of genetic background. Our laboratory highlighted that YAP/TAZ proteins respond to dynamic modifications of the mechanics and nanostructure of the ECM during the acquisition of cardiac phenotype in vitro. Moreover, we recently unveiled the mechanism by which YAP protein controls the mechanics of the cell by promoting the assembly of FAs. These data shed a new light on the onset and progression of cardiac pathologies and are likely to be pivotal in the definition of new treatments for such conditions.

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giancarlo.forte@fnusa.cz

CHIMERIC ANTIGEN RECEPTOR T CELLS – KILLING CANCER BY DESIGN

Stanley R. Riddell[1,2], Daniel Sommermeyer[1], Lingfeng Liu[1], David Maloney[1,2], Cameron Turtle[1,2]

[1]Program in Immunology, Fred Hutchinson Cancer Research Center, [2]Department of Medicine, University of Washington, Seattle, WA.

The adoptive transfer of T cells that are genetically modified to express chimeric antigen receptors (CARs) targeting CD19 has resulted in complete remissions in advanced B cell malignancies. At this stage, the field has focused on the contributions of signaling domains in the CAR to enhance anti-tumor function. Our studies have analyzed the contribution of distinct T cell subsets and conducted the first studies of CAR-T cells in B cell malignancies in which the CD4 and CD8 T cell composition of the cell product is uniform in all patients. This resulted in high rates of complete remission in ALL, NHL, and CLL and provided insight into CAR-T cell dose/response and dose/toxicity relationships. These insights are being applied to targets expressed on common epithelial cancers with CAR-T cells. We have extended the principles of receptor design to include sequences that facilitate optimal signaling, in vivo tracking and elimination, and clinical manufacturing. Incorporation of a Strep-tag II sequence in various locations of the extracellular domain of the CAR provides a universal intrinsic marker for measuring CAR expression, tailors spacer length and flexibility to optimize CAR function, and enables rapid selection of highly purified CAR-T cell products for immediate in vivo administration or their large scale in vitro expansion. This approach can be applied for CARs with different scFvs and provides a target for antibody mediated elimination of CAR-T cells in in vivo models. StrepTactin is used in clinical cell processing, and this novel CAR design facilitate cGMP manufacturing with shorter culture times.

Supported by the grants from the National Institutes of Health, USA and the Washington State Life Sciences Development fund.

sriddell@fredhutch.org

CAR T-CELLS: CLINICAL APPLICATION – GETTING STARTED

Ulrich Jäger

Clinical Division of Hematology and Hemostaseology, Department of Medicine I, Medical University of Vienna, Austria

The introduction of CAR T-cell immunotherapy into the treatment of leukemia and lymphoma has revolutionized our thinking of immunotherapy in hematologic malignancies. First clinical results are encouraging. This new treatment is associated with a number of clinical and logistic challenges. Practical application of CD19 CAR T-cell treatment in the setting of a cancer centre is presented together with some clinical results.

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ulrich.jaeger@meduniwien.ac.at

MOLECULAR BASIS FOR THE TEMPORARY ACTIVATION OF ENDOGENOUS CARDIAC STEM CELLS IN THE DISEASED HEART

Georg Weitzer

Department of Medical Biochemistry, Medical University of Vienna, Max F. Perutz Laboratories, Vienna Biocenter, A1030, Vienna, Austria

Development of the mammalian heart starts at the beginning of gastrulation and leads to the first functional organ in the embryo within a relatively short time. The four-chambered heart grows by hyperplasia until one week *post partum* and further on by hypertrophy of existing cardiomyocytes until adulthood. Cardiac progenitor and stem cells are supposed to emerge from the primitive mesoderm and give rise to all heart cells during embryogenesis. They persist throughout adulthood in the heart muscle and sometimes even increase in number in the ageing or failing heart. Despite the presence of adult cardiac stem cells and the capacity of post-mitotic cardiomyocytes to re-enter the cell cycle, regeneration of wounded hearts has never been observed in mammalian species.
Regulations of cardiac stem cell self-renewal and differentiation to cardiac cells are regulated by a plethora of intrinsic and extrinsic cellular and molecular cues during embryogenesis, and they seem to persist in adult cardiac stem cells in an attenuated or dormant state. Understanding the molecular mechanisms guiding the life of embryonic cardiac stem cells opens the possibility to intervene and temporarily reactivate adult cardiac stem cells. Temporary proliferation and directed differentiation of endogenous cardiac stem cells might contribute to the healing process after myocardial infarction or chronic degenerative heart diseases.
Here I will introduce clonally derived cardiac stem cell lines as a model system to study molecular regulation of stem cell self-renewal and differentiation in the absence of extrinsic ectodermal and endodermal cellular influences. Additionally, the roles of two non-transcription factor proteins, desmin and SPARC, in the transcriptional regulation of cardiomyogenesis in cardiac stem cells will be discussed.

georg.weitzer@univie.ac.at

TRANSLATION OF TISSUE ENGINEERED HEART REPAIR

Wolfram-Hubertus Zimmermann

Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Georg-August University

Tissue engineered heart repair aims at offering a solution to a fundamental shortcoming in the present state-of-the-art in heart failure therapy, i.e., limited endogenous or induced regeneration of the heart after injury. Restoring muscle contractility by integration of tissue engineered myocardium is the ultimate goal. The following challenges have been overcome over the past 20 years: (1) proof-of-concept for feasibility, safety, and efficacy of engineered allografts in several rodent models, (2) construction of human engineered heart muscle (EHM), (3) demonstration of EHM scalability towards clinical demands, (4) development of a GMP-compatible EHM production process. A key challenge for clinical translation is the demonstration of proof-of-concept for feasibility and safety of engineered allografts in a relevant large animal model. In order to meet regulatory demands, our group has focused on

the Rhesus macaque model. Bioequivalence studies suggest similar function in human and Rhesus macaque EHM *in vitro*. The necessary steps towards the completion of a pivotal non-human primate study as a surrogate for a human Phase I trial will be discussed.

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w.zimmermann@med.uni-goettingen.de

PLASTICITY OF NEONATAL HEARTS

Bernhard J. Haubner, Johanna Schneider, Ulrich Schweigmann, Wolfgang Dichtl, Corinna Velik-Salchner, Jörg-Ingolf Stein, Josef Penninger

Department of Internal Medicine III (Cardiology and Angiology), Innsbruck Medical University; IMBA, Vienna

Cardiac remodeling and subsequent heart failure remain critical issues after myocardial infarction (MI) despite improved treatment and reperfusion strategies. Recently, complete cardiac regeneration has been demonstrated in fish and newborn mice following apex resection or cardiac infarctions. Two key issues remain to translate findings in model organisms to future therapies in humans: what is the mechanism and can cardiac regeneration indeed occur in newborn humans?
We report the case of a newborn child suffering from a severe myocardial infarction due to coronary artery occlusion. The child developed massive cardiac damage as defined by serum markers for cardiomyocyte cell death, electrocardiograms, echocardiography, and cardiac angiography. Remarkably, within weeks after the initial ischemic insult, we observed cardiac recovery, which translated into long-term normal heart functions.
These data show that humans have also the intrinsic capability to repair myocardial damage and cardiac regeneration.

bernhard.haubner@i-med.ac.at

HOW TO IMPLEMENT INNOVATION: CLINICAL TRIALS OF CAR T-CELL THERAPIES IN AUSTRIA

Florian Göbel, Niklas J. Münchmeier, Sonja Hinteregger, Heidemarie Pircher-Reif, Ivo Winiger-Candolfi

Novartis Pharma GmbH, Austria

Use of Chimeric Antigen Receptor (CAR) modified T cells targeting CD19 (CTL019) as a cell-based immunotherapy have demonstrated positive results in early phase trials with high complete response rates in hematological malignancies, including relapsed/refractory (r/r) acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphomas (NHL).
The CTL019 therapy relies on genetically modified autologous T-cells and the underlying manufacturing process involves leukapheresis, lentiviral cell transduction, cell expansion and application of modified cells into patients. Several steps are performed at different sites including shipment to/from the FDA-approved GMP site for cell therapy production in the US. In addition, high quality standards, regulations for genetically modified organisms and logistics make this a highly complex process for Phase II and Phase III clinical trials. CTL019 trials have successfully been started by Novartis in Austria in close collaboration with academic centres.
This talk will highlight how to implement sophisticated clinical trial protocols (such as those for CTL019) in Austria including pitfalls and challenges. In addition, the latest clinical data will be reviewed with an outlook on the opportunities of CAR therapies in the light of modern immunotherapy.

florian.goebel@novartis.com

LOST IN TRANSLATION: WHICH STEM CELLS CAN BE USED FOR WHAT APPLICATION?

Dirk Strunk, MD

Experimental and Clinical Cell Therapy Institute, Spinal Cord Injury and Tissue Regeneration Center, Paracelsus Medical University, Salzburg

More than one million patients so far received conventional hematopoietic stem/progenitor cell (HSPC) transplantation (HSCT) producing engraftment and hematopoietic reconstitution in most and cure in many. Over the past decade feasibility and safety data have also been obtained for a rapidly increasing portfolio of advanced cell therapies (ATMPs) mostly using non-hematopoietic stem/progenitor cells (SPC) from various tissues (with or w/o additional gene therapy or tissue engineering). Several ATMPs including mesenchymal stem/progenitor cell (MSPC) therapies already showed partial evidence of target organ regeneration despite lack engraftment. This has been interpreted as indicating a predominantly paracrine mode of ATMP action mediated by soluble factors and extracellular vesicle cargo. The underlying mechanisms are still poorly understood.
Additional progress in this field is limited by the substantial lack of insight into the pathways orchestrating human tissue and organ regeneration. It is still not clear which cell types are capable of regenerating what kind of usually complex tissue damage. For most treatment conditions the required cell number, timing and mode of application still need to be identified. Also the established knowledge regarding histocompatibility that enabled millions of

successful conventional homologous cell and organ transplantations is usually not considered accordingly in contemporary ATMP strategies as are the sophisticated immune suppressive regimens ordinarily required to facilitate allogeneic transplantation. As a consequence we can just speculate whether stable engraftment and subsequent firm integration of somatic SPC is possible after substantial cell manipulation ex vivo and if it could precipitate more profound long-term benefits. A note of caution has also been sounded concerning the rapid expansion of aggressive commercialization of a steadily growing spectrum of unproven 'stem cell treatment' options.
In this talk, the case of MSPC will illustrate the so far rare examples for both – successful engraftment and prospective potency assay development. Ease of MSPC isolation and expansion from virtually any organ multiplies with the presumably multipotent differentiation capacity resulting in thousands of hypothetic applications. Whether MSPC of skeletal origin can safely regenerate soft tissue and, vice versa, adipose-derived cells can really form new bone or marrow reticulum, is just one example of many challenging questions which urgently need to be answered before proceeding with hundreds of small scale clinical trials. Current regulation requiring precise documentation of cell identity, purity, safety may help to advance the field if combined with established transplant registries and with sorely missing obligatory follow-up records. Mechanistic molecular studies adding in depth insight into the tightly orchestrated interplay of various cell types during regeneration will further help to select promising clinical strategies. Answering some of the questions addressed above will definitively also nurture the novel induced pluripotent stem cell (iPSC) field where the same issues will be coming up, once basic problems regarding GMP-compliant cell derivation, propagation and stable 100% efficient and reproducible differentiation have been solved. Eventually, well-designed prospective randomized and precisely controlled multicenter clinical trials will require a significant rise of research funding from private and public sources to answer the key questions.

dirk.strunk@pmu.ac.at

THE CHALLENGING WAY FROM COMPASSIONATE USE TO APPROVED ATMP STUDY

René Geyeregger, Christine Freimüller, Nelli Frank, Julia Stemberger, Elke Zipperer, Gerhard Fritsch, Inge Hirsch, Susanne Karlhuber, Ulrike Pötschger, Michaela Artwohl, Beate Avanessian, Marion Graupner, Susanne Matthes-Leodolter

Dept. of Clinical Cell Biology and FACS-Core Unit, Children´s Cancer Research Institute

Cytomegalovirus (CMV) and human adenovirus (HAdV) infections following allogeneic stem cell transplantation are related to mortality rates between 30-60%. Because antiviral treatments show limited efficacy and are associated with substantial toxicity, we have developed an adoptive immunotherapy using stem cell donor-derived virus-specific T-cells.
We used PBMCs isolated from 100ml of donor-blood which were substantially manipulated by stimulating the cells with peptides and cytokines for 12 days in a G-REX bioreactor under GMP-compliant conditions. Furthermore, we reported the first-in-man use of peptide-stimulated HAdV-specific T-cell lines under compassionate use conditions.
During the clinical phase I/II trial, we expect to treat about 20-30 patients with persistent CMV or HAdV viremia with our short-term expanded virus-specific T-cell lines.
However, before starting a clinical trial with an ATMP, regulatory barriers have to be overcome. I will talk about challenges that emerge for a researcher when trying to get the manufacturer license and study approvals, including shortcomings assessed by the

regulatory authorities.
Additional challenges such as writing an investigator medicinal product dossier (IMPD), labelling of the IMP, transport logistics, data management and validation of analytical procedures according to EU-Pharmacopeia will also be discussed.

rene.geyeregger@ccri.at

EX VIVO GENE/CELL THERAPY: FROM NAMED PATIENT PROJECT TO PHASE I/II CLINICAL TRIAL

Elisabeth Mayr[1], Johann W Bauer[2], Eva M Murauer[1], Josef Koller[2], Sophie Kitzmüller[1], Martin Laimer[2], Laura De Rosa[3], Elena Enzo[3], Sonia Carulli[4], Graziella Pellegrini[3], Michele De Luca[3]

[1]EB House Austria, Research Program for Molecular Therapy of Genodermatoses, Department of Dermatology, Paracelsus Medical University Salzburg, Austria
[2]Department of Dermatology, Paracelsus Medical University Salzburg, Austria
[3]Center for Regenerative Medicine "Stefano Ferrari", University of Modena and Reggio Emila, Italy
[4]Holostem Terapie Avanzate S.r.l., Modena, Italy
[5]Institute of Pathology, Paracelsus Medical University Salzburg, Austria

We report on the development of a clinical study for patients suffering from dystrophic epidermolysis bullosa. Epidermolysis bullosa is a genodermatosis caused by mutations in genes encoding structural proteins, which mediate intraepidermal adhesion and dermo-epidermal anchorage within the basement membrane zone of epithelial tissues. Reduction, absence or malfunction of these proteins impair the functional and structural integrity of skin and mucous membranes and result in prototypic hyperfragility and blistering along even modest mechanical trauma. Current strategies of treatment are supportive and symptomatic. Curative molecular therapy is implicated to be achieved by substituting the mutated gene with a wild type copy. In our approach autologous human stem cells are corrected with full length cDNA and expanded into skin sheets *ex vivo*. The skin sheets are then transplanted onto large chronic wounds, erosions or stressed areas of the patients skin to achieve a local correction of the genetic defect. The first patient in Austria was treated with this *ex vivo* gene therapy approach in 2014 within a named patient project. The treatment resulted in a regenerated, self-renewing epidermis, which has been resistant to shear force over a follow up period of 1 year. Histologically, a normal and fully differentiated epidermis was observed, and electron microscopy revealed an appropriate morphology of the basement membrane zone in the transplanted area.
The positive results of this named patient project lead to the development of a clinical study, in which up to 12 patients shall be enrolled to be treated within the next 3 years.

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el.mayr@salk.at

P01 L-MIMOSINE AND HYPOXIA ENHANCE ANGIOPOIETIN LIKE-4 PRODUCTION INVOLVING HIF-1ALPHA IN DENTAL PULP-DERIVED CELLS

Hermann Agis[1, 2], Umar Alhujazy[1, 2], Klara Janjić[1, 2], Andreas Moritz[1, 2]

[1]Department of Conservative Dentistry and Periodontology, School of Dentistry, Medical University of Vienna, Vienna, Austria
[2]Austrian Cluster for Tissue Regeneration, Vienna, Austria
Authors are listed in alphabetical order

Hypoxia-based strategies are a novel approach with high potential for regenerative endodontics to stimulate angiogenesis. However, the impact of hypoxia on the dental pulp is not fully understood. Angiopoietin like-4 (ANGPTL-4) is a signaling factor that is involved in angiogenesis. We hypothesized that the hypoxia mimetic agent L-mimosine (L-MIM) and hypoxia stimulate the production of angiopoietin-like-4 in the dental pulp.
Monolayer cultures and spheroid cultures of human dental pulp-derived cells (DPC) were treated with L-MIM or hypoxia. Furthermore, tooth slice cultures were treated with L-MIM and hypoxia. The production of ANGPTL-4 was assessed at mRNA and protein levels using qPCR and immunoassays, respectively. To assess the involvement of HIF-1 signaling, inhibitor studies with echinomycin were performed.
In monolayer cultures of DPC, L-MIM and hypoxia increased the production of angiopoietin like-4 at mRNA and protein levels. Also in spheroid cultures, L-MIM and hypoxia increased angiopoietin-like-4. The increase of ANGPTL-4 was inhibited by echinomycin in monolayer cultures. In the tooth slice cultures, ANGPTL-4 follows the same trend.
Our results suggest that L-MIM and hypoxia lead to an increase in ANGPTL-4 production in DPC, involving the HIF-1 pathway. The potential role of this response of the dental pulp to hypoxia and the impact on pulp regeneration will be assessed in future studies.

hermann.agis@meduniwien.ac.at

P02 OSTEOARTHRITIS-ON-A-CHIP: 3D HYDROGEL CHONDROCYTE CULTURE TO ESTABLISH A NOVEL DISEASE MODEL FOR EFFICACY TESTING OF FUTURE THERAPIES

Barbara Bachmann [1,2], Julie Rosser [2], Iris Ribitsch[2], Florien Jenner F[2], Peter Ertl [1]

[1]Faculty of Technical Chemistry, Vienna University of Technology (TUW), Vienna, Austria
[2]Equine Clinic, University of Veterinary Medicine Vienna, Vienna, Austria

Articular cartilage shows limited potential for regeneration. Advances in maintaining human tissues on microfluidic platforms has led to growing interest in the development of microphysiological systems: organs-on-chips. We have integrated 3D-hydrogel based primary chondrocytes on-a-chip to establish an injury model to gain insights into onset and progression of osteoarthritis and to monitor inflammation-induced cellular responses similar to those seen in osteoarthritis:
Articular cartilage was harvested from healthy adult animals. Chondrocytes were isolated, stained with CellTracker™ Dye and 3D fibrin matrix was prepared mixing 100 mg/mL fibrinogen solution with 4 U/mL thrombin yielding a 20% fibrin gel, cell density 700 cells/mm^3. Microchips were manufactured by replica molding of Polydimethysiloxane and layering the

polymer between glass substrates. Surfaces were activated using a plasma discharge system and bonded at 70°C.
To characterize the transport properties of biomolecules within the gel on-chip a diffusion assay with fluorescently-labelled bovine serum albumin was performed, showing a gradient formation inside the hydrogel. Cells were viable in 3D culture for a period of 21 days as assessed by staining with cell tracker dyes. Morphologic evaluation showed up to 99 % of cells inside the hydrogel maintained spherical morphology throughout culturing.
Gradient formation is similar to physiologic conditions of cartilage. Cells remained viable for extended time suggesting high potential for in vivo-like cultivation. Chondrocytes seemed to align in cartilage-like cell clusters called chondrons. Further phenotypically characterization is planned using qPCR, GAG Assay and ELISA in the context of inflammatory conditions and mechanical stress.

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iris.ribitsch@vetmeduni.ac.at

P03 LEUKOTRIENE RECEPTOR INHIBITION MOBILIZES HUMAN STEM CELLS *IN VIVO*

Gabriele Brachtl[1,*], Anna Hochreiter[1], Volker R. Jacobs[2], Richard Schäfer[3], and Dirk Strunk[1]

[1]Experimental and Clinical Cell Therapy Institute, Paracelsus Medical University
[2]Department of Gynecology and Obstetrics, Paracelsus Medical University, Salzburg, Austria
[3]Cell Therapeutics & Cell Processing, Institute for Transfusion Medicine and mmunohematology, German Red Cross Blood Donor Service
*Presenting author

Leukotrienes are important response mediators. Their receptors are expressed on various cell types including immune cells, hematopoietic stem/progenitor cells (HSPCs), mesenchymal stem/progenitor cells (MSPCs) and endothelial progenitors. Leukotriene receptor antagonists are used for asthma treatment but were also shown to modulate HSPC migration and promote neurogenesis. Aiming to better understand injury-induced and drug-induced stem/progenitor cell mobilization, we asked whether cysteinyl-leukotriene receptor antagonism influences circulating stem cells.
Blood samples from five healthy adults taking an LTD4 antagonist daily for one week were analyzed at four time-points, defining baseline, early (4-6h after 1st medication), intermediate (day 3-4) and late (day 7) mobilization by complete blood count and high-resolution 10-color flow cytometry focusing on HSPCs, MSPCs and endothelial colony-forming progenitor cells (ECFCs). Human bone marrow aspirate and umbilical cord blood served as technical positive controls.
We found a significant mean 1.9-fold mobilization of lineage^{NEG}/CD34^{+}/CD38^{-}/CD45RA^{-}/CD90^{+} hematopoietic stem cells (HSCs; baseline=79±18; day seven=147±36/mL; mean±SEM) after seven days of drug intake. There were no significant differences in total CD34^{+} and various HPCs analyzed (multipotent progenitors, common myeloid progenitors, megakaryocyte/erythrocyte progenitors, granulocyte/macrophage progenitors, common lymphoid progenitors and lymphoid-primed multipotent progenitors) or in leukocyte count, subsets (neutrophils, monocytes, lymphocytes) or lymphocyte subsets (T cells, B cells, NK cells) before day seven. No detectable levels of MSPCs or ECFCs were mobilized by leukotriene inhibition (detection threshold 10^{-6}).

While inhibition of leukotrienes induced no significant changes in the composition of mature blood cells and their progenitors, it may have a previously unobserved mobilizing effect on the most immature pluripotent HSCs.

gabriele.brachtl@pmu.ac.at

P04 EFFECTS OF LOW LEVEL LIGHT THERAPY ON ENDOTHELIAL CELLS AND VASCULOGENESIS

Sabrina Rohringer[1,2], Wolfgang Holnthoner[1,2], Sidrah Chaudary[1,2], Paul Slezak[1,2], Eleni Priglinger[1,2], Martin Strassl[3], Heinz Redl[1,2], Peter Dungel[1,2]

[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria,
[2]Austrian Cluster for Tissue Regeneration, Vienna, Austria,
[3]Laser Consult Austria e.U., Salzburg, Austria

Low level light therapy (LLLT) receives increasing interest in the fields of wound healing and angiogenesis. Endothelial cells play an important role in angiogenic processes. The aim of this study was to compare the effects of pulsing LED light of three different wavelengths on endothelial cells and vasculogenic processes in vitro. The effects of pulsed LED light on proliferation and migration of human umbilical vein endothelial cells (HUVEC) were investigated in several 2D and 3D cell culture models. Cells were treated with either blue (475 nm), green (516 nm) or red (635 nm) LED light. 2D proliferation was determined at given time points by manual counting. 2D migration was assessed by scratch assays, 3D migration was evaluated by Cytodex bead assays. The vasculogenic potential of HUVEC in co-culture with adipose-derived stem cells (ASC) was determined by analysing network formation in a 3D model after 4 days. Stimulation with both red and green LED light significantly increased HUVEC proliferation, 2D migration was enhanced by green light. The 3D migration was significantly enhanced by green and red light. In the 3D fibrin co-culture model, HUVEC elongation as precursor of vasculogenesis was enhanced by green and red light during the first 4 days. Both red and green light enhanced proliferation, migration and vasculogenesis processes while blue light was ineffective. Further studies have to focus on light-induced intracellular signaling in order to optimize this promising, alternative application in tissue regeneration and wound therapy.

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peter.dungel@trauma.lbg.ac.at

P05 GENETICALLY MODIFIED EQUINE MESENCHYMAL STEM CELLS ALLOWING ON-DEMAND TRANSGENE EXPRESSION

Simone Gabner[1], Juraj Hlavaty[1], Karsten Velde[2], Matthias Renner[3], Florien Jenner[2], Monika Egerbacher[1]

[1]Institute of Anatomy, Histology and Embryology, University of Veterinary Medicine, Vienna, Austria
[2]Equine University Hospital, University of Veterinary Medicine Vienna, A-1210 Vienna, Austria
[3]Division of Medical Biotechnology, Paul-Ehrlich-Institut, D-63225 Langen, Germany

Mesenchymal stem cells (MSCs) have great potential for regenerative medicine because of their unique paracrine, differentiation and self-renewal abilities. To further reinforce the potency of MSCs, they can be expanded and genetically manipulated under standard cell culture conditions, making them an attractive target for cellular gene therapy. Hence, the development of systems allowing transgene expression, particularly regulated by natural disease-induced substances is highly desirable and represents a promissing field for further preclinical research.
Bone marrow-isolated equine MSCs were transduced with an HIV-1 based lentiviral vector expressing genes encoding either for the luciferase or interleukin-1 receptor antagonist protein (IRAP) under control of an inducible NFkB-responsive promoter. The marker gene expression (luciferase activity) or IRAP production was analysed in infected cells stimulated with different concentrations of IL-1β or TNFα.
Dose-dependent increase in luciferase as well as IRAP expression was found upon cytokine stimulation of infected MSCs. TNFα was a more potent inductor of transgene expression compared to IL-1 and did not cause negative feedback loop for promoter stimulation in IRAP-expressing MSCs. Maximum transgene expression was obtained after 48 hours of stimulation. Repeated cycles of induction allowed on-off modulation of transgene expression. Inducibility of the NFkB-responsive promoter was retained also in chondrocytes differentiated from vector-infected MSCs.
On demand transgene expression can be induced in genetically modified equine MSCs using naturally occuring inflammatory cytokines.

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juraj.hlavaty@vetmeduni.ac.at

P06 retracted

P07 ISOLATION AND CHARACTERIZATION OF ENDOTHELIAL EXTRACELLULAR VESICLES FROM CELL CULTURE SUPERNATANTS

Carina Hromada[1], Severin Mühleder[1], Heinz Redl[1], Wolfgang Holnthoner[1]

[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Austrian Cluster for Tissue Regeneration, Vienna, Austria

Once considered as cell debris with no biological function, extracellular vesicles (EVs) that include microparticles and exosomes have recently aroused great interest in the scientific community due to their importance in intercellular communication in both physiological and pathological conditions such as tissue regeneration, cardiovascular diseases and immunity. Here, we aimed to establish a differential centrifugation protocol to separate microparticles and exosomes, and showed that the use of different rotors and centrifugation parameters strongly influences particle yield as assessed by flow cytometry and nanoparticle tracking analysis, thereby highlighting the importance of standardized isolation and characterization protocols to assure reproducibility and study-to-study comparability in the promising and rapidly growing field of EV research. Moreover, a potential influence of extracellular vesicles on the formation of tube-like structures during co-culture of endothelial cells with adipose-derived stem cells (ASCs) was investigated, and evaluation of changes in the particle profiles revealed a decline in endothelial microparticles over time, the formation of which was presumably hampered by the release of certain factors from ASCs. Since the exact mechanisms of EV-mediated cell-to-cell communication still remain to be investigated, a deeper understanding of the complex and ambivalent, but highly interesting role of these vesicles in the next few years is warranted, which will allow the exploration of the numerous possible clinical applications of EVs.
Supported by a grant from FFG “Femtech for students”

Carina.Hromada@trauma.lbg.ac.at

P08 GENERATION OF ALIGNED SKELETAL MUSCLE-LIKE TISSUE BASED ON THE APPLICATION OF STRAIN TO A 3D FIBRIN SCAFFOLD

Babette Maleiner[1,4], Philipp Heher[2,3,4], Andreas Teuschl[1,4], Heinz Redl [2,3,4], D. Rünzler[1,4], Christiane Fuchs[1,4]

[1] Department of Biochemical Engineering, UAS Technikum Wien, Vienna, AUSTRIA
[2] Trauma Care Consult GmbH, Vienna, AUSTRIA
[3] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology/AUVA Research Center, Vienna, Austria
[4] The Austrian Cluster for Tissue Regeneration, Vienna, Austria

Fibrin is a natural hydrogel which is considered a suitable scaffold material for skeletal muscle tissue engineering. Its mechanical properties are tunable and can therefore be modulated to mimic the stiffness of native skeletal muscle tissue. Furthermore, fibrin hydrogels respond to mechanical stimulation with fibril alignment along the axis of strain, which allows for guided cellular patterning. We developed a 3-D *in vitro* culture system in combination with a bioreactor (MagneTissue) to apply mechanical stimulation to myoblasts embedded in fibrin scaffolds. After a strain regime of 10 % for 6 hours and 3 % for 18 hours on 6 consecutive days, mature myotubes in terms of sarcomeric patterning, width and length were observed. Additionally, static strain dramatically increased cellular alignment, resulting in a parallel array of myotubes. We further investigated the effects of static strain on myogenesis by tracking gene expression levels of myogenic markers. Static strain was found to enhance expression of the determination markers MyoD and Myogenin as well as the contractile markers Troponin T and MHC. Recent experiments have shown that both, cyclic as well as static strain have a positive influence on muscle maturation. On the morphological level, immunofluorescence confocal microscopy confirmed that, after mechanical stimulation for up to 9 days, both stimuli increased alignment and maturity of the muscle-like tissue. This

novel bioreactor system provides a versatile tool for skeletal muscle tissue engineering, as the nature and extent of stimulation can be individually adjusted using custom-made software.

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babette.maleiner@technikum-wien.at

P09 AUTOMATION AND MINIATURIZATION OF IN VITRO CELL CULTURES AND TISSUES USING MICROFABRICATION

Mario Rothbauer and Peter Ertl

Faculty of Technical Chemistry, Vienna University of Technology, Austria

The global trend towards more evidence-based medicine, closed-meshed clinical testing and personalized therapies has pushed the development of advanced *in vitro* diagnostic strategies in the last decade. Among others the application of microfluidic devices in medical research has fostered our understanding of the functioning of the human body. Microfluidics is vital for cell analysis because it is the only technology capable of simulating the physiological environment of cells and cell assemblies to investigate cellular transport mechanisms and cell proliferation events in the presence of test reagents, temperature or shear force gradients. The recent advance in microfabrication techniques has further enabled to integrate the third-dimension of cell biology into microfluidic devices, also called organ-on-a-chip technology, to study cell-to-cell interactions under reproducible measurement conditions. Here we present three organ-on-a-chip systems with the aim of studying the biological aspects of (i) the placenta barrier integrity under high blood pressure conditions, (ii) inflammatory reactions at the joint during rheumatic arthritis and (iii) neural degeneration rates in the midbrain to monitor the onset and progression Parkinson's diseases.

mario.rothbauer@tuwien.ac.at

P10 STEPWISE MATURATION OF HUMAN iPS CELLS INTO IMMUNOSUPPRESSIVE MESENCHYMAL STEM/PROGENITOR CELLS

Scharler C[1], Ketterl N[1], Höfler M[1], Brachtl G[1], Schallmoser K[2], Strunk D[1]

[1]Experimental and Clinical Cell Therapy Institute, Spinal Cord Injury and Tissue Regeneration Center, Paracelsus Medical University, Salzburg, Austria
[2]Department of Transfusion Medicine, Spinal Cord Injury and Tissue Regeneration Center, Paracelsus Medical University, Salzburg, Austria

Human mesenchymal stem/progenitor cells (MSPCs) are tested in multiple clinical trials evaluating their immunomodulatory and regenerative capacity. Restricted availability and limited life cycle of MSPCs confine clinical applicability and mechanistic studies that are needed to better understand their mode of action.
In this study induced pluripotent stem cell (iPSC) lines were generated from healthy bone marrow (BM)-MSPCs and umbilical cord blood (UCB)-MSPCs to optimize GMP-compliant

iPSC differentiation and study mechanisms of iPSC-derived mesenchymal progenitor cells (i-MPCs) function.
MSPCs were established under animal serum-free conditions. After primary large scale culture, purity and identity of the cells were characterized by clonogenicity (CFU-F), immune phenotype and three-lineage differentiation capacity, before reprogramming into iPSCs. The differentiation of iPSCs along mesenchymal lineage was initiated in commercially available mesoderm induction media. Further differentiation into i-MPCs was accomplished in media containing 10% pooled platelet lysate (pHPL). Small molecules targeting signaling pathways involved in stem cell self-renewal, immune modulation and cell adherence were added to promote differentiation into functional i-MPCs. Immune phenotype, clonogenicity, differentiation capacity and immune modulatory potential of i-MPCs were compared to their parental MSPCs.
We differentiated iPSCs into $CD73^{+}/CD105^{+}/Tra\text{-}1\text{-}81^{-}$ MSPC-like cells lacking immune suppressive competence. Small molecules modified the MSPC-like phenotype of i-MPCs. Additional passaging was required for reaching full immunosuppressive competence comparable to their parental MSPCs. Our data extend published knowledge by showing that the complete immune phenotype and functional repertoire of i-MPCs can be established in a stepwise order under animal serum-free GMP-compliant conditions to comply with the functionality of their parental MSPCs.

cornelia.scharler@pmu.ac.at

P11 ADIPOSE TISSUE-DERIVED THERAPEUTIC CELLS – TOWARDS A NON-ENZYMATIC PROCEDURE

Steffenhagen C[1,2], Priglinger E[1,2], Wurzer C[1,2], Nuernberger S[2,3,4], Suessner S[5], Wolbank S[1,2], Redl H[1,2]

[1] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, 1200 Vienna, Austria
[2] Austrian Cluster for Tissue Regeneration
[3] Bernhard Gottlieb University Clinic of Dentistry, Universitätsklinik für Zahn-, Mund- und Kieferheilkunde Ges.m.b.H, Vienna, Austria
[4] Medical University of Vienna, Department of Trauma Surgery, Vienna, Austria
[5] Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria

Human adipose tissue is an attractive and abundantly available source of adult stem cells applicable in regenerative medicine and tissue engineering. Hence, prerequisite for the translation into clinics is the production of the stromal vascular fraction (SVF) under good manufacturing practice (GMP). A number of companies developed systems aiming for a closed, sterile, safe and reproducible cell isolation process limiting risk for contaminations and unpredictability of the cell material. However, many of these systems are based on enzymatic digestion with collagenase which is the most expensive part of the isolation process, complicates regulatory authorization and may have negative impacts on cell potency and efficacy. Therefore we compared classical enzymatic cell isolation methods with reduced enzyme concentration methods and a new non-enzymatic isolation method regarding cell yield, identity and potency. Our results demonstrate that enzyme treatment is necessary for applications which require large cell numbers, since reduction of enzyme concentration to 30%, 10% and 0% result in smaller cell yields. In contrast, the viability of the isolated cells as determined via cellular ATP decreased with increasing collagenase

concentration. Further we found that a closed system incorporating non-enzymatic treatment steps is suitable to isolate therapeutically relevant subpopulations such as endothelial progenitor cells (CD45-/CD31+/CD34+), pericyte-like cells (CD45-/CD31-/CD146+), and supra-adventitial cells (CD45-/CD31-/CD146-/CD34+) with high cellular ATP content and elevated differentiation potency. Cells derived from our new non-enzymatic method showed stronger potential to form tube-like structures. Our findings support the concept of using non-enzymatic closed systems which allow the isolation of therapeutically active cells in a one-step procedure.

Carolin.Steffenhagen@ trauma.lbg.ac.at

P12 EXTRACORPOREAL SHOCK WAVE THERAPY IN SITU – A NOVEL APPROACH TO OBTAIN AN ACTIVATED FAT GRAFT

Steffenhagen Carolin[1,2], Priglinger Eleni[1,2], Peterbauer Anja[2,3], Wurzer Christoph[1,2,4], Redl Heinz[1,2,4], Sandhofer Matthias[5], Wolbank Susanne [1,2]

[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Linz/Vienna 1200, Austria
[2]Austrian Cluster for Tissue Regeneration, Vienna 1200, Austria
[3]Red Cross Blood Transfusion Service of Upper Austria, Linz 4010, Austria
[4]Liporegena GmbH, Breitenfurt 2384, Austria
[5]Austrian Academy of Cosmetic Surgery and Aesthetic Medicine, Linz 4020, Austria

One of the mainstays of facial rejuvenation strategies is volume restoration which can be achieved by autologous fat grafting. In our novel approach, we treated the adipose tissue harvest site with extracorporeal shock wave therapy (ESWT) in order to improve the quality of the regenerative cells in situ. The latter was demonstrated by characterizing the cells of the stromal vascular fraction (SVF) in the harvested liposuction material regarding cell yield, ATP content, proliferative capacity, surface marker profile and differentiation potential. While SVF cell yield was only slightly enhanced, viability and ATP concentration of freshly isolated cells as well as proliferation doublings after 3 weeks in culture were significantly increased in the ESWT compared to the untreated group. Likewise, cells expressing mesenchymal and endothelial/pericytic markers were significantly elevated concomitant with an improved differentiation capacity towards the adipogenic lineage. Hence, in situ ESWT might be applied in the future to promote cell fitness and adipogenesis within the fat graft for successful facial rejuvenation strategies with potential long-term graft survival.

Carolin.Steffenhagen@ trauma.lbg.ac.at

P13 IN-DEPTH CHARACTERIZATION OF VITAL HUMAN AMNIOTIC MEMBRANE

Banerjee A[1,2#], Weidinger A[1,2#], Lindenmair A[1,2], Hennerbichler-Lugscheider S[2,3], Kozlov AV[1,2], Eibl J[4], Redl H[1,2], Wolbank S[1,2]

# equal contribution
1 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingenstraße 13, 1200 Vienna, Austria
2 Austrian Cluster for Tissue Regeneration, Austria
3 Red Cross Blood Transfusion Service of Upper Austria, Krankenhausstraße 7, 4017 Linz, Austria
4 Bio-Products & Bio-Engineering AG, Schottenring 10, 1010 Vienna, Austria

For over a century, the human amniotic membrane (hAM), the innermost fetal membrane, has been used in clinics for tissue repair mainly in devitalized form. Now, it is becoming more and more evident that resident cells of vital hAM substantially contribute to tissue regenerative and healing processes. Optimized application of vital hAM for clinical settings requires investigation of its basic properties on cellular, sub-cellular and extracellular level.
On the sub-cellular level, mitochondria have recently been shown to play an important role for tissue regeneration. This study aimed to characterize specific properties of two sub-regions of the hAM connected to mitochondrial activity.
We found higher mitochondrial respiration and higher ATP levels in the placental sub-region of fresh hAM. Inhibition of the ATP synthase led to elevated lactate levels in placental amnion, indicating increased glycolysis. Interestingly, this switch was not observed in the reflected amnion.
Despite higher respiratory activity, we found lower levels of intracellular reactive oxygen species (ROS) in placental amnion, however, higher levels of extracellular ROS, suggesting different regulatory mechanisms in the two sub-regions.
Regarding long-term cultivation of the hAM, measurement of mitochondrial activity showed that distension seems to be necessary to sustain cellular viability, which should impact banking strategies of the hAM.
Taken together, we found distinct metabolic differences of placental and reflected amnion. These differences should be taken into account for an optimized clinical application of the vital hAM, as an alternative to current devitalized applications.

asmita.banerjee@trauma.lbg.ac.at

P14 TARGETING HCMV-INFECTED FIBROBLASTS WITH BI-SPECIFIC CAR-T CELLS

Charlotte Brey, Julia Proff, Florian Full, Armin Ensser, Wolfgang Holter and Manfred Lehner

Children´s Cancer Research Institute, University of Natural Resources and Life Sciences

We investigate the possibility of an HLA-independent T cell therapy of infections with human cytomegalovirus (HCMV) and developed a chimeric antigen receptor (CAR) for targeting HCMV glycoprotein B (gB). This CAR contains an IgG1-Fc spacer domain, which is known to interact with Fc receptors (FcRs) and to abrogate persistency and efficacy of CAR-T cells in preclinical tumour models. We speculate, however, that in our context this Fc-domain could be beneficial by enabling additional targeting of HCMV-encoded FcRs. When we investigated

fibroblasts three days after infection with HCMV we found strong expression of gB on the cell surface and high capacity for binding of IgG1, indicating expression of HCMV-FcRs. T cells modified with the gB-specific CAR were strongly activated by these HCMV-infected target cells and efficiently inhibited further HCMV-infection by secretion of IFN-γ and TNF. By performing blocking experiments we could demonstrate that the HCMV encoded FcRs enhanced the activation of the CAR-T cells and, hence, the secretion of the inhibitory cytokines. In order to exploit this fact therapeutically, we now ask whether we can specifically target HCMV-FcRs separately from endogenous human FcRs. Such specific targeting might be accomplished by using mutated Fc variants, since HCMV-FcR and human extracellular FcRs have different binding sites within the Fc domain. This possibility is investigated in current experiments. In summary our data show that CAR-T cells can inhibit HCMV-infection by secretion of IFN-γ and TNF, and that designing bi-specific CARs containing mutated Fc spacers might be attractive for enhancing CAR function.

charlotte.brey@ccri.at

P15 YAP ACTS AS A MECANOTRANSDUCER BY CONTROLLING FOCAL ADHESION ASSEMBLY

Jorge Oliver De La Cruz[1], Giorgia Nardone[1], Jan Vrbsky[1], Cecilia Martini[1], Jan Pribyl[2], Petr Skládal[2], Martin Pešl[1,3], Guido Caluori[1] Nicola Maria Pugno[4,5,6], Gorazd Bernard Stokin[1], Giancarlo Forte[1,7].

[1] Center for Translational Medicine, International Clinical Research Center (CTM-ICRC), Brno, Czech Republic
[2] CEITEC MU, Masaryk University, Brno, Czech Republic
[3] Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[4] Laboratory of Bio-inspired & Graphene Nanomechanics, Department of Civil, Environmental and Mechanical Engineering, University of Trento, Trento, Italy
[5] Center for Materials and Microsystems, Fondazione Bruno Kessler, Povo, TN, Italy
[6] School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London, United Kingdom
[7] Department of Biomaterials Science, Institute of Dentistry, University of Turku, Turku, Finland

The extracellular matrix (ECM) surrounding the cells offers mechanical cues that are determinant in crucial functions such as proliferation, differentiation and apoptosis, therefore directing tissue development and organogenesis. Among the different intracellular systems described as mechanosensors for their ability to perceive modifications in ECM mechanics and coordinate cell response, two molecular pathways have been intensively characterized: the focal adhesion (FA) complexes that link integrin signalling and actin cytoskeleton, and the Hippo pathway through its main effector YAP, which nuclear localization and consequent cotranscriptional activation are tightly regulated. The latest models describe a hierarchical relationship among them where FA and Rho/ROCK pathways control the Hippo pathway activation through a FAK–Src–PI3K cascade and/or G-protein coupled receptors.
In this study, taking advantage of micropatterned surface and single cell analysis, we demonstrate that YAP nuclear accumulation is regulated by the cell area and independently of FA formation. Moreover, we further determine a novel feedback axis by which YAP acts downstream of RhoA GTPase controlling the FA assembly. Through its cotranscriptional activity, YAP activates the expression of key FA proteins involved in stabilizing the

interphase between the integrins and F-actin cytoskeleton. In consequence, depletion of YAP induces an impairment in cell shaping, migration, and biophysical parameters such as cell tension and adhesion force. Altogether, our results indicate that YAP functions not only as a mechanosensor but also as a mechanotransducer by orchestrating FA assembly and subsequently cell-ECM interface.

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oliver.jorge@fnusa.cz

P16 HUMAN AMNIOTIC MEMBRANE – UNREALIZED SOURCE OF PULMONARY SURFACTANT?

Angela Lemke[1,2], Anton Klotz[1,2], Andrea Lindenmair[1,2], Sylvia Nürnberger[1,4], Simone Hennerbichler[2,3], Heinz Redl[1,2], Susanne Wolbank[1,2]

[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria
[2]Austrian Cluster for Tissue Regeneration, Austria
[3]Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria
[4]Bernhard Gottlieb University Clinic of Dentistry, Vienna, Austria

Pulmonary surfactant reduces surface tension which prevents the collapse of alveoli and is therefore essential for lung function. It is composed of a lipid-protein complex, amongst others of pulmonary proteins A, B, C and D (SP-A to –D) and is produced by alveolar epithelial type 2 cells (AEC2). All SPs have been found in amniotic fluid, and are an indication for fetal pulmonary maturity. So far the origin of SPs was not clearly determined, though they were supposed to be secreted by the fetal developing lung.
The human amniotic membrane (hAM) is the innermost layer of the fetal membranes enclosing the amniotic fluid. It contains epithelial (hAEC) and mesenchymal cells exhibiting stem cell characteristics, which makes it as medical waste material to a promising alternative for regenerative medicine.
In our study we aimed to investigate whether we can draw a link between hAM and amniotic fluid-derived pulmonary surfactant.
We discovered intracellular surfactant protein expression via Flow Cytometry and Immunohistochemistry/Immunofluorescence staining not only directly after isolation but over several passages (hAECs) or weeks (hAM) of incubation. Moreover we could show with ELISA that SP-D is actively secreted into cell culture medium.
All in all we could show that hAM is likely to be one of the producers of pulmonary surfactant found in the amniotic fluid. Since it is a very promising alternative for regenerative medicine, our results could imply a new therapeutical approach, such as for respiratory distress syndrome (RDS), which needs to be further investigated.

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susanne.wolbank@trauma.lbg.ac.at

P17 KLF15 AS A NOVEL SENSOR OF CELL-CELL AND CELL-MATRIX INTERACTION

Fabiana Martino[1], Stefania Pagliari[1], Ana Rubina Perestrelo[1], Jorge Oliver De La Cruz[1], Jan Vrbský[1], Giancarlo Forte[1,2]

[1] Center for Translational Medicine (CTM), International Clinical Research Center (ICRC), St. Anne´s University Hospital, Brno, Czech Republic
[2] Department of Biomaterials Science, Institute of Dentistry, University of Turku, Finland

Cell responsiveness to extracellular matrix (ECM) composition and mechanics has been the subject of several investigations so far, showing that these parameters are critical for cell maintenance and function.
Modifications in the mechanics and nanostructure of the ECM parallel the onset of a number of pathologies (i.e.: cardiac pathologies, tumor invasion, neurodegenerative diseases). Such remodeling processes are likely to impair the activity of tissue-specific cells and thus organ function.
How mechanosensing system transduces these mechanical cues into the activation of specific genetic programs remains poorly understood.
Here we demonstrate that Kruppel-like-factor 15 (KLF15) expression in normal human dermal fibroblasts (NHDFs) is affected by cell density, with its nuclear localization being sensitive cell-to-cell interaction. The nuclear localization of the zinc finger DNA-binding protein is not affected by serum depletion, thus pointing to cell-to-cell contact as main determinant of KLF15 nuclear exclusion.
Taking advantage of micropatterned arrays design to control single cell surface and adhesion area, we show that KLF15 localization is independent from cell shape, polarity and the availability of ECM adhesion sites, while being directly correlated to cell surface area.
Cell spreading determines the transmission of ECM tension via cell cytoskeleton.
Indeed, when a remodeling of the cytoskeleton is induced, a modification in KLF15 distribution and expression can be achieved. This data is corroborated by the sensitivity of the protein to substrate stiffness.
Altogether, these results strongly point at KLF15 as a novel nuclear sensor of mechanical cues arising from the ECM or surrounding cells.

fabiana.martino@fnusa.cz

P18 FLUORESCENCE BASED REPORTER CELLS RAPIDLY IDENTIFY AND DISTINGUISH FUNCTIONAL CHIMERIC ANTIGEN RECEPTORS (CARS)

Julian Rydzek[1], Sabrina Jutz[2], Judith Leitner[2], Lars Wallstabe[1], Thomas Nerreter[1], Johannes Huppa[3], Hermann Einsele[1], Peter Steinberger[2], Michael Hudecek[1]

[1] Medizinische Klinik und Poliklinik II, Universitätsklinikum Würzburg, Germany
[2] Institut für Immunologie, Zentrum für Pathophysiologie, Infektiologie und Immunologie, Medizinische Universität Wien, Austria
[3] Institut für Hygiene und Angewandte Immunologie, Zentrum für Pathophysiologie, Infektiologie und Immunologie, Medizinische Universität Wien, Austria

Adoptive immunotherapy with chimeric antigen receptor (CAR) -modified T-cells is under intense pre-clinical and clinical investigation involving new CAR designs and target antigens.

Here, we present a novel reporter cell line enabling high-throughput testing and identification of functional CAR designs for pre-clinical validation and clinical translation.
Triple parameter reporter (TPR) were derived from Jurkat cells and modified with three inducible reporter genes, each expressing a distinct fluorophore under control of the T-cell transcription factors NF-κB, NFAT or AP-1. TPR cells were transduced with CAR constructs, co-cultured with target antigen-expressing stimulator cells and reporter gene-activation was analyzed by flow cytometry.
We transduced TPR cells with ROR1-specific CARs and detected highest NF-κB and NFAT reporter gene-induction after 24 hours of co-culture with ROR1-expressing stimulator cells. Further, TPR cells enabled the identification of a functional receptor out of a panel of ROR1 CARs with different spacer length consistent with our observations using primary T-cells *in vitro*. Of note, we also compared the activation of 2nd and 3rd generation ROR1 CARs with defined co-stimulatory domains and detected stronger induction of NF-κB in CARs providing 4-1BB rather than CD28 co-stimulation whereas a 3rd generation CAR did not result in stronger reporter gene-activation.
Our data demonstrate the potential of TPR cells to evaluate CARs based on the induction of key T-cell transcription factors. We are currently integrating TPRs into the testing of novel CAR designs to accelerate development of CARs with optimal anti-tumor function and safety.

rydzek_j@ukw.de

P19 REVERSAL OF PREMATURE AGING MARKERS AFTER BARIATRIC SURGERY

Ebenbauer B, Hohensinner PJ, Kaun C, Prager M, Wojta J, Rega-Kaun G

Department of Cardiology, Medical University of Vienna, Vienna, Austria

Background: Obesity is considered to be a major risk factor in developing cardiac disease. In addition, obese patients suffer from a premature aging phenotype including increased secretion of senescence associated secretory proteins (SASP) and reduced telomere length compared to healthy controls.
Purpose: The aim of our study was to determine, if bariatric surgery and the resulting weight loss could reverse the previously observed premature aging phenotype.
Methods: We enrolled 76 patients undergoing bariatric surgery. Blood samples were taken before and 12 months after surgery.
Results: Overall, patients showed a significant drop of body mass index. In addition plasma levels for IL6 and PAI-1 were significantly reduced after surgery. For the anti-inflammatory protein IL37, we did not find a significant increase after bariatric surgery. However, we found increased plasma levels for IL10. In addition, telomere length on average increased by 58% in the patient cohort. The telomere increase was accompanied by a reduction in the telomere oxidation index indicating reduced oxidative stress for the telomeric region. This is further supported by an inverse correlation of telomere length with telomere oxidation at both time points.
Conclusion: Our data indicate a significant reduction of the SASP IL6 and PAI-1 in plasma 12 months after bariatric surgery. In addition we observed an increase in telomere length in this setting. However, given the reduction in oxidative stress at telomeric regions we speculate that the increased telomere length is not due to active elongation but due to reduced breakage caused by telomere oxidation.

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benjamin.ebenbauer@meduniwien.ac.at

P20 SHOCK WAVE TREATMENT POSITIVELY INFLUENCES CARDIOMYOGENESIS IN AN ENERGY-DEPENDENT MANNER

Christiane Fuchs[1,2,*], Dorota Szwarc[1,2,*], Anna M. Weihs[1,2] and Dominik Rünzler[1,2]

[1] University of Applied Sciences Technikum Wien, Department of Biochemical Engineering, Vienna, Austria
[2] Austrian Cluster for Tissue Regeneration, Austria
[*] authors contributed equally to this work

In the past years, accumulating evidence for the positive effects of shock wave treatment (SWT) on myocardial regeneration has gained increasing attention of clinicians as a possible alternative treatment after myocardial infarction (MI). Previous experimental and clinical studies have shown that SWT significantly improves systolic function, the number of blood vessels and myocardial blood flow. Although there is evidence that SWT can improve regeneration of the myocardium after MI to some extent, the underlying mechanisms, however, are not entirely understood. Therefore, our aim was to investigate the effects of SWT on *in vitro* cardiomyogenesis using embryonic stem cell-derived embryoid bodies and

cardiovascular progenitor cell-derived cardiac bodies as 3-D cardiac model systems to elucidate the underlying molecular mechanisms.We analyzed expression levels of cardiac markers and signaling pathways involved in mechanotransduction, proliferation and differentiation. We could show an energy-dependent effect of SWT on signaling pathways which play a role in mechanotransduction and differentiation. We observed that, within a time frame of 24 hours, the ERK and rS6 signaling pathway were induced upon SWT, with an initial rise of ERK activation which was followed by rS6 activation. Moreover, on the gene expression level, SWT significantly up-regulated lineage-specific and cardiac markers compared to untreated controls. Our intention is to provide clinicians with a solid base to pave the way for SWT into the clinics as an alternative or additive therapeutic approach in the treatment of MI.

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christiane.fuchs@technikum-wien.at

P21 MECHANOSENSING SYSTEM CONTROLS CARDIAC CELL MATURATION AND FUNCTION THROUGH YAP/TAZ PROTEINS

Stefania Pagliari[1], Vladimir Vinarsky[1], Ana Rubina Perestrelo[1], Fabiana Martino[1], Giancarlo Forte[1,2]

[1] Center for Translational Medicine (CTM), International Clinical Research Center (ICRC), St. Anne´s University Hospital, Brno, Czech Republic
[2] Department of Biomaterials Science, Institute of Dentristy, University of Turku, Finland

The composition and arrangement of extracellular matrix (ECM) components In adult cardiac tissue plays a key role in the organization and alignment of synchronously and functionally beating cardiomyocytes.
Following Heart failure (HF) - the end stage of cardiac pathologies in which cardiac muscle is not able to pump adequate blood to the organism – critical changes in mechanics and nanotopography occur along with the composition of cardiac ECM.
The Hippo signaling pathway effectors YAP/TAZ have been shown to be sensitive to changes in ECM mechanical properties while influencing stem cell shape, homeostasis and differentiation.
Here we show that in adult cardiac progenitor cells (CPCs), the intracellular localization of YAP/TAZ can be controlled by modifying substrate mechanics and nanotopography. Importantly, YAP/TAZ seem also to be involved in CPC fate decision, by controlling the switch between the endothelial and contractile phenotype.
Moreover, when human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were challenged with a number of inotropic compounds and pharmacological regulators, a perturbation in YAP/TAZ localization and transcriptional activity was noticed, along with the acknowledged effect on contractility and beating rate of cardiac cells. Surprisingly, YAP/TAZ displacement in these cells resulted in a deterioration of the contractile apparatus. Given the role of YAP/TAZ in promoting adult cardiomyocyte survival, the connection between their activity and contractile cell function appears of interest.

To understand how cardiac cell mechanosensors can influence cell maturation and function as well as their involvement in cell responses after injuries might be useful for the design of novel therapeutic strategies for cardiac pathologies.

stefania.pagliari@fnusa.cz

P22 DECELLULARIZED HEART EXTRACELLULAR MATRIX AS A TOOL TO INVESTIGATE THE MOLECULAR BASIS OF CARDIOVASCULAR DISEASES

Ana Rubina Perestrelo[1,2], Vladimír Vinarský[1,2], Jorge Oliver de la Cruz[1,2], Víta Žampachová[2], Stefania Pagliari[1,2], Vladimír Horváth[3], Diana S. Nascimento[4], Perpétua Pinto-do-Ó[4], Giancarlo Forte[1,2,5]

[1] Center for Translational Medicine, International Clinical Research Center (CTM-ICRC), Brno, Czech Republic
[2] St. Anne's University Hospital Brno (FNUSA), Brno, Czech Republic
[3] Cardiovascular and Transplant Surgery, Heart Failure Treatment and Transplant Programs (Cardio 1), Brno, Czech Republic
[4] Instituto de Engenharia Biomédica, Instituto de Investigação e Inovação em Saúde (INEB-i3s), Universidade do Porto, Portugal
[5] Department of Biomaterials Science, Institute of Dentristy, University of Turku, Finland

The complex design of cardiac extracellular matrix (ECM) provides a unique 3D environment for cardiomyocytes to exert their contractile function in physiological conditions. The derangement of ECM integrity occurring during the onset and progression of cardiac diseases drives a modification in the nanotopography and mechano-physical properties of the ECM itself, thus impairing cardiac cell contractility and organ function.
Our group demonstrated that slight dynamic modifications in substrate mechanics and nanotopography affects cardiac cell maturation and function and that cell response to such conditions is mediated by their mechanosensing apparatus. Additionally, immunohistochemistry analysis of infarcted mouse hearts and biopsies obtained from cardiac patients confirms that the dramatic structural changes occurring as a consequence of myocardial infarction (MI) and during the progression of cardiac pathology towards heart failure modulate cardiac cell mechanosome.
Cardiac decellularized tissues obtained from physiological and pathological conditions feature the three dimensional cues, mechanical properties, chemical complexity and the native organization of heart tissue and thus are here proposed as a model to set up *in vitro* tools to investigate the molecular determinants of cell-ECM interaction in cardiac pathologies. In this study, we demonstrate that second harmonic generation imaging and scanning electron microscopy can be implemented to obtain high-resolution 3D maps of cardiac dECM nanotopography. Furthermore, preliminary cell-dECM interaction experiments suggested that physiological and pathological conditions can be reproduced *in vitro* by these biological scaffolds. Altogether our data show that cardiac dECMs are powerful and reliable toolboxes to monitor nanostructure remodelling and cells' functional properties in a cardiac-like niche.

ana.rubina.perestrelo@fnusa.cz

P23 IMPROVED WOUND HEALING BY TOPICAL APPLICATION OF THE SUPERNATANT OF APOPTOTIC PERIPHERAL MONONUCLEAR BLOOD CELLS IN GENETICALLY DIABETIC DB/DB MICE

Traxler D, Keibl C, Lidinsky D, Marsh LM, Nuernberger S, Hartinger J, Berger T, Simader E, Nemec L, Mildner M, Redl H, Olschewski A, Gabriel C, Ankersmit HJ, Slezak P

Christian Doppler Laboratory for Diagnosis and Regeneration of Cardiac and Thoracic Diseases, Medical University of Vienna, Austria
Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria
Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria
Department of Dermatology, Medical University of Vienna, Austria
Department of Experimental Anaesthesiology, Medical University of Graz, Austria
Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria
Division of Thoracic Surgery, Department of Surgery, Medical University of Vienna, Austria

Diabetic foot ulcer is a severe complication of diabetes for which no sufficient therapeutic option can be offered. The supernatant of apoptotic PBMCs (APOSEC), has previously shown beneficial effects in tissue regeneration and wound healing, but has not been tested in a model of diabetic wound healing.
We used a full-thickness wound healing model in genetically diabetic *db/db* mice. APOSEC (three concentrations) or control were topically applied to the wound site for ten consecutive days. Wound size was measured until day 25 by tracing the wound on acrylic foil and using a stereoscopic camera for 3D wound measurement (circumference, surface area). Masson's trichrome, Weigert's elastic stain and immunohistochemical stainings (CD31, CD45, K10) were performed.
We demonstrated that topical application of APOSEC enhances wound healing in genetically diabetic mice. Wound circumference, wound surface area and wound size assessed planimetrically were significantly reduced in mice treated with APOSEC at day 18. Additionally a significant dose dependency could be shown. Histopathological analyses revealed no differences between APOSEC and control in angiogenesis, percentage of $CD45^+$ cells, collagen or elastic fibre deposition in the wound zone. Continuous K10 staining was observed only in APOSEC treated mice.
We showed that topical administration of APOSEC significantly enhances wound healing in diabetic mice, even though histopathological analyses did not exhibit any differences. Chronic diabetic ulcer displays a severe burden. Hitherto no adequate therapy to address this problem exists. The supernatant of apoptotic PBMCs show a promising potential for tissue regeneration in diabetic wound healing.

denise.traxler-weidenauer@meduniwien.ac.at