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The Fountain of Youth Symposium
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Abstract Book

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Michael Comer – PACT Chairman

April 2015

It is just over a year ago that we held the first PACT Symposium where the "Cell" was presented not only as the fundamental unit of life but also illustrating its potential to act as a potent and regenerative therapeutic agent. This year's Symposium will seek to address not only the use of cell-based therapies from the huge theoretical benefits that may be postulated but also demonstrate how this may be translated to practical patient treatments in the description and presentation of so called "bench to bedside" scenarios. Of course this is by no means a "one way street" and equally important is the reverse, where the results from the "clinic" return to the laboratory for analytical and critical evaluation and for eventual modification or adjustment to the scientific approach for the particular medical condition but always with the patient as the centre of focus.

The human fascination for "forever young" has long been a desire documented in history, among other sources in **The Fountain of Youth** "a spring that supposedly restores the youth of anyone who drinks or bathes in its waters." Tales of such a fountain have been recounted across the world for thousands of years, appearing in writings by Herodotus (5th century BCE) and may other stories and legends including the restorative powers of the waters in the mythical land of "*Bimini*" described in the folklore of the indigenous habitants of the Caribbean. The regenerative or "anti" ageing prospects still remain as an experimental possibility of the science that we are engaged in.

However, the PACT Scientific Organizing Committee would not only like to consider the previous aspects but would also like to honour the research efforts of our more youthful researchers in particular in moving the frontiers of the possible to the probable by giving our younger colleagues the platform for the discussion of their results in the face of their peers and how to best proceed into the future. The form of the scientific programme reflects the new organisation of the PACT membership's efforts to address the challenges of advanced cellular therapies in a more structured approach under various potential disease classification topics.

There will be keynote talks followed by presentations of topical results and discussion.

We hope therefore that the following abstracts will not only provide a logical record of our efforts and provide food for thought but also act as a stimulation to novel ideas that will advance the frontiers of knowledge in our challenge to relegate many, if not all diseases and conditions of suffering to the annals and archives of medical science.

BONE REPAIR: FROM TISSUE ENGINEERING TO REGENERATIVE MEDICINE

Ranieri Cancedda, Fiorella Descalzi, Claudia Lo Sicco, Maddalena Mastrogiacomo, Roberta Tasso

Universita' di Genova & IRCCS AOU San Martino - IST, Istituto Nazionale per la Ricerca sul Cancro, Largo Rosanna Benzi 10, 16132, Genova, Italy

Since the end of the last century, major progresses have been made with regard to the transplant of “ex vivo” expanded autologous stem/progenitor cells, in most cases associated to a biomaterial carrier. However, with few exceptions, this therapeutic approach never really took-off. There are scientific aspects, such as vascularization of large size implants, identification of the “optimal” source of cells and the “optimal” biomaterial carrier that require further investigations. Moreover, additional bottlenecks are: i) the logistic of collecting from patients, expanding in culture and returning the cells to the surgical theater; ii) the high cost of the culture procedure within the GMP facilities required by the strict rules defined by National and European Regulatory Agencies.
Several decades of stem cell research have progressively uncovered within developed organs a stunning capacity for endogenous regeneration and repair, mediated by specialized stem and progenitor cells residing in their native constitutive tissues. However, tissues with low regenerative capacity, or with an age-related decline in progenitor populations, require external stimulation and/or exogenous stem cells to catalyze the repair process. Compelling experimental evidence suggests that, in many cases, the reported contribution of these cells in the regeneration of damaged organs in vivo depends on the release of paracrine factors (trophic factors) interacting with endogenous progenitors and other cells, including cells of the native immunoresponse (neutrophils and macrophages) and endothelial and cell tissue progenitors present at or migrated to the site of injury.
We propose, an “off the shelf” product, obtained by the integration of a biomaterial scaffold with a Platelet Lysate (PL), as source of growth factors in the “right” composition and in the “right” concentrations as well as with stem cell conditioned culture medium or released microvesicles.
The identification of a rare population of cells present in the peripheral blood of healthy mice that actively participates in the tissue repair/regeneration process will be also presented. In particular it will be reported that the injury signals are sufficient to (i) specifically direct the recruitment and these Circulating Healing (CH) cells; (ii) promote their differentiation and appropriate integration in the regenerative microenvironment. CH cells were identified by an innovative flow cytometry strategy as small cells not expressing CD45 and lineage markers. The analysis of their global transcriptome revealed their uniqueness when compared to other cells characterized by varying stemness degree. Moreover, CH cells presented a high expression of key pluripotency-associated genes and positive selective markers of the epiblast developmental stage.

ranieri.cancedda@unige.it

THE AGED SYSTEMIC ENVIRONMENT AND BONE METABOLISM

Sylvia Weilner, Elisabeth Schraml, Heinz Redl, Peter Pietschmann, Johannes Grillari

Evercyte, BOKU Vienna, Austria

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. A case in point is the characteristics of adult stem cells to self-renew and to differentiate that is essential for 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. 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 extracellular vesicles (EVs) 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 EVs on osteogenesis, we identified hsa-miR-31-5p (miR-31) as a crucial component. Furthermore, we demonstrate that miR-31 is present at elevated levels in the plasma of elderly and of osteoporosis patients. A potential source of its secretion, senescent endothelial cells, known to increase during aging in vivo, were identified. We demonstrate that endothelial miR-31 is secreted by senescent cells within EVs 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 osteoporosis patients and, indeed, identified a signature of 4 miRNAs with a predictive power for fracture risk that is superior to that of bone mineral density measurements.

sylvia.weilner@evercyte.com

A NOVEL BIOREACTOR FOR THE GENERATION OF HIGHLY ALIGNED 3D SKELETAL MUSCLE-LIKE CONSTRUCTS THROUGH PATTERNING OF FIBRIN AND APPLICATION OF STATIC STRAIN

Philipp Heher[1,2,3], Babette Maleiner[2,4], Johanna Prüller[1,2,4], Andreas Teuschl[2,4,5], Josef Kollmitzer[4,6], Susanne Wolbank[1,2,3], Heinz Redl[1,2,3], Dominik Rünzler[2,4], Christiane Fuchs[2,4,5]

[1] Trauma Care Consult, Vienna, Austria
[2] Austrian Cluster for Tissue Regeneration, Vienna, Austria
[3] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology/AUVA Research Center, Vienna, Austria
[4] Department of Biochemical Engineering, UAS Technikum Wien, Vienna, Austria
[5] City of Vienna Competence Team Bioreactors, UAS Technikum Wien, Vienna, Austria
[6] Higher Technical Institute HTL –TGM, Department for Biomedical- and Health-Engineering, Vienna, Austria

The generation of biomimetic skeletal muscle constructs is still one of the fundamental challenges in skeletal muscle tissue engineering. With the notion that structure strongly dictates functional capabilities, a myriad of cell types, scaffold materials and stimulation strategies have been combined. To further optimize muscle engineered constructs, we have developed a novel bioreactor system (MagneTissue) for rapid engineering of skeletal

muscle-like constructs that should resemble native muscle in terms of structure, gene expression patterns, and maturity.
Murine myoblasts embedded in fibrin, a natural hydrogel that serves as extracellular matrix, are subjected to static mechanical strain via magnetic force transmission. We identify mechanical strain as a trigger for cellular alignment concomitant with the patterning of the scaffold material into highly organized fibrin fibrils. This ultimately yields myotubes with a more mature phenotype in terms of sarcomeric patterning, width and length. On the molecular level, a faster progression of the myogenic gene expression program is evident as myogenic determination markers *MyoD* and *Myogenin* as well as the Ca^{2+} dependent contractile structural marker *TnnT1* are significantly upregulated when strain is applied.
The major advantage of the MagneTissue bioreactor system, compared to most previously published systems, is that the generated tension is not exclusively relying on the strain generated by the cells themselves but its ability to subject the constructs to individually adjustable strain protocols. In future work, this will allow implying mechanical stimulation with different strain regimes in the maturation process of tissue engineered constructs and elucidating the role of mechanotransduction in myogenesis.

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heherp@technikum-wien.at

ENHANCED FORMATION OF MECHANICALLY FUNCTIONAL ENGINEERED BONE BY HYPERTROPHIC CHONDROCYTES

Jonathan C. Bernhard[1], Ming Li[2], Sarindr Bhumiratana[1], Andreas Teuschl[3], Heinz Redl[4], Gordana Vunjak-Novakovic[1]

[1]Columbia University, New York, USA; [2]Peking University, Peking, China; [3]University of Applied Sciences Technikum Wien, Vienna, Austria; [4]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria.

In vivo there are two types of bone forming cells, osteoblasts and hypertrophic chondrocytes. Osteoblasts are utilized to repair small defects and are responsible for the near constant remodeling of bone. Hypertrophic chondrocytes, a mature state of the chondrocyte, are utilized in initial bone development and large bone fracture repair.
With the recent advancement of hypertrophic chondrocyte differentiation from stem cell sources, the aim of the presented study was to compare the capacity for bone tissue engineering of the two cell types. Based on their role in bone development, we hypothesized that the derived hypertrophic chondrocytes would produce a more suitable engineered bone construct, defined by mechanical properties and deposition of the bone template.
Silk scaffold-based, tissue engineered constructs were seeded with human bone marrow derived stem cells and cultured for five weeks in differentiation media. Differentiation media promoted either hypertrophic chondrocyte differentiation or osteoblast differentiation. Upon harvest at five weeks, the hypertrophic chondrocyte group showed significantly higher deposition of bone mineral, as calculated by quantitative analysis with micro-computed tomography. Histological sections verified these results through Von Kossa stains, while also demonstrating enhanced production of collagen type I and bone sialoprotein. Mechanically testing of the two treatment groups resulted in a significant, five-fold increase in the elastic modulus of the hypertrophic chondrocyte constructs compared to the osteoblast constructs. The ability of hypertrophic chondrocytes for robust generation of bone matrix and

an increase in mechanical stability supports the utility of this pathway for future bone tissue engineering projects.

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jcb2219@columbia.edu

AGE MATTERS – MOLECULAR MECHANISMS CONTRIBUTING TO TENDON SENESCENCE

Renate Gehwolf[1,2], Wagner Andrea[1,2], Christine Lehner[1,2], Herbert Tempfer[1,2], Amy D. Bradshaw[3], Hans-Christian Bauer[1,2,4], Andreas Traweger[1,2]

1 Institute of Tendon and Bone Regeneration; Paracelsus Medical University – Spinal Cord Injury and Tissue Regeneration Center Salzburg, Austria
2 Austrian Cluster for Tissue Regeneration, Vienna, Austria
3 Gazes Cardiac Research Institute, Medical University of South Carolina, Charleston, USA
4 University Hospital of Salzburg, Department of Trauma Surgery and Sports Injuries, Salzburg, Austria

The ability of tendons to withstand stress generally decreases with age, often resulting in increased tissue degeneration and tendinopathies. However, the underlying molecular and cellular mechanisms remain poorly characterized. Therefore, we aimed to identify genes showing an altered expression in tendons from mice of two different ages. Suppression-subtractive-hybridization-screen comparing cDNA libraries generated from pooled RNAs extracted from Achilles tendons of mature-adult (3months) and old (18months) female C57BL/6 mice was conducted. Subsequently, the differential expression of the identified genes was validated by qRT-PCR. Selected genes were then further analysed by immunohistochemistry and immunoblotting. To investigate age-related alterations in the collagenous extracellular matrix we applied SHG-microscopy and TEM.
Differential screening identified approx. 90 differentially expressed genes in young-mature versus old tendons. The majority of these genes encodes for ECM-proteins (e.g. decorin, biglycan, fibromodulin, lysyl-oxidase, Sparc, collagen type-1 and type-3). As evidenced by qRT-PCR the mRNA levels of these genes were down-regulated in old tendons as well as in tendon derived stem/progenitor cells isolated from old mice. The impact of Sparc on tendon senescence was further analysed in mature-adult and old Sparc-/- mice as well as in age-matched wild type animals. Employing SHG-microscopy and TEM we further observed age-related changes in the collagenous structure of these tendons.
The decreased expression of ECM-proteins and modulators thereof in old tendons in combination with structural changes is potentially associated with an increased risk of tendon injury in the elderly, since structure and composition of the tendon are directly related to its function.

renate.gehwolf@pmu.ac.at

COMPARISION OF DIFFERENTIATION AND PROLIFERATION CAPABILITIES OF ADULT AND FETAL MESENCHYMAL STEM CELLS

Daniel Zodl and Florian Sefcik[2], Iris Ribitsch[1], Simone Gabner[3], Andrea Pichler Wallace[2], Monika Egerbacher[3], Florien Jenner[1]

[1] Equine Clinic, University of Veterinary Medicine Vienna, Vienna, Austria; [2] Department of Biochemistry, HBLVA Rosensteingasse, Vienna, Austria; [3] Institute of Anatomie, Histology and Embryology, University of Veterinary Medicine, Vienna, Vienna, Austria

Both in human and veterinary medicine there is a strong trend towards a new form of therapies utilizing self-regeneration mechanisms enhanced with live cells and cell products. For example, mesenchymal stem cells (MSCs) are used to improve regeneration of equine tendon tissue. However, though re-injury rates in equine patients were significantly reduced and a better histologic architecture could be achieved, MSC application has not led to the degree of tendon regeneration seen in fetal tendon lesions. Fetal tendons have the inherent ability to regenerate completely and without loss in resilience. This property is lost as animals grow older.
There is some evidence that fetal cells transplanted into an adult organism may still retain their regenerative potential. The presented study therefore aimed at characterizing fetal bone marrow derived MSCs by comparing their *in vitro* properties to MSCs obtained from adult individuals. Differentiation capacity towards the adipogenic, osteogenic and chondrogenic lineage, proliferation properties and surface markers were compared.
It was found that fetal MSCs differentiate faster and better into osteoblasts and chondrocytes, but differentiate far less into adipocytes than adult MSCs. Additionally, fetal MSCs multiply 73.8% faster than their adult counterparts (population doubling/day: fetal: $\bar{x}$=1.001 s=0.168 adult: $\bar{x}$=0.576 s=0.175 p-value=3.9%).
It is not completely understood why fetal MSCs do not differentiate into adipocytes, however this finding is in accordance to the results of other research groups (Ragni et al. 2013). The better proliferation and differentiation properties of fetal cells may make therapies using these cells much more efficient and therefore desirable.

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daniel.zodl@live.at

ADAPTION OF A VASCULARIZED MENISCUS MODEL AS A POTENTIAL MODEL FOR EQUINE MENISCUS REGENERATION – PRELIMINARY RESULTS

Iris Ribitsch,[1] Jenny Reboredo,[2] Antje Kremer,[2] Nicole Ade, [1,4] Julia Dürr,[1,3] Hanna Schöpper[3], Johann P. Schrammel,[1] Christian Peham,[1] Monika Egerbacher,[3] Florien Jenner,[1] Heike Walles, [2]

[1] Equine Clinic, University of Veterinary Medicine Vienna, Vienna, Austria; [2] Chair of Tissue Engineering and Regenerative Medicine, Würzburg, Germany; [3] Institute of Anatomie, Histology and Embryology, University of Veterinary Medicine, Vienna, Vienna, Austria; [4] Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

Meniscal injuries are a common cause of arthritis, degeneration of the meniscus and articular cartilage. In order to study and improve meniscal treatments a model for meniscus regeneration is required. A 3D meniscus model is currently being adapted for horses.

A histological study with special emphasis on vascularisation patterns, collagen types and alignment was performed. Shore hardness, stiffness, energy loss during dynamic compressive loading and the impedance of the menisci during compressive loading were evaluated.
As a 3D model, eqMC and eqMSC were co-cultured embedded in a collagen type I hydrogel. As a secondary 3D approach, eqMC and eqMSC embedded in collagen type I hydrogels were co-cultured on an acellularized jejunum segment as a vascularized scaffold.
A shore hardness of 37.0–64.0 Shore A (mean: 50.5±8.6 Shore A) was determined. Stiffness yielded 592±222 N/mm for the center portion of the meniscus, respectively 561±312 N/mm for the outer portion of the meniscus (overall: 576±263 N/mm). Energy loss overall: 0.595±0.220 Nm.
Immunohistochemical analysis of eqMC confirmed the presence of typical fibrocartilage markers. Cell differentiation status in static co-culture systems was shown by immunohistochemical analysis.
The biomechanical tests allowed for distribution of force in an equine physiologic manner and good reproducibility independent of different meniscal sizes and shapes. Static co-culture of the two cell types results in differentiation of MSC into MC supporting 3D meniscus tissue modeling. The results reported here suggest the usage of acellularized jejunum segment as suitable matrix for 3D tissue engineering of a meniscus model.

iris.ribitsch@vetmeduni.ac.at

FROM EMBRYONIC DEVELOPMENT TO TISSUE ENGINEERING: DEVELOPING NEW STRATEGIES FOR CARTILAGE REPAIR

Roberto Narcisi, Mairéad A. Cleary, Pieter A.J. Brama, Martin J. Hoogduijn, Nesrin Tüysüz, Derk ten Berge, Gerjo J.V.M. van Osch

Erasmus MC, University Medical Center, Department of Orthopaedics, 3015 CN Rotterdam, The Netherlands.

Mesenchymal stem cells (MSCs) are good candidates for cartilage tissue engineering due to their capacity to proliferate and differentiate into chondrogenic cells. However, loss of chondrogenic potential during *in-vitro* expansion, and the propensity of the cartilage to undergo hypertrophic maturation impede their therapeutic application. Based on observations from cartilage development, we investigated the effect of Wnt signalling modulation of *in-vitro* cultured MSCs in order to generate stable cartilage *in-vivo*.
Flow cytometry analysis revealed that MSC extensively expanded with Wnt3a better maintain the expression of the stem cell markers CD90, CD105, CD166 and CD271. Wnt3a-treated cells proliferate faster and longer, express high levels of the proliferative associated genes *MYC* and *NMYC*, and an enhanced expression of the chondrogenic transcription factor *SOX9*. After expansion, transcript and immuno-histological analysis showed that Wnt3a-expanded MSCs chondrogenically differentiated with the addition of IWP2 (a Wnt inhibitor) displayed the highest accumulation of the cartilage specific markers collagen type-II and glycosaminoglycan, and the most evident reduction of the undesired hypertrophic genes, *COL10*, *MMP-13* and *ALP*. Interestingly, when the chondrogenic pellets generated by this culture combination were implanted subcutaneously in nude mice showed no signs of ossification, while the pellets generated in absence of IWP2 showed calcified matrix and blood vessel invasion. By maintaining potency during expansion and preventing hypertrophic maturation following differentiation, the modulation of Wnt signalling removes two major obstacles that impede the clinical application of MSCs in cartilage repair.

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r.narcisi@erasmusmc.nl

OSTEOCYTOGENESIS IN VITRO AND IN VIVO

Günter Lepperdinger[1,2], Sebastian Klepsch[2]

1. University Salzburg, Cell Biology & Genetics, Hellbrunnnerstr. 34, 5020 Salzburg,
2. University Innsbruck, Institute for Biomedical Aging Research, Rennweg 10, 6020 Innsbruck

Mesenchymal stromal cells (MSC) can be reliably isolated from human connective tissues. Naïve MSC can be culture-expanded and induced to form osteoblasts in a standardized manner. Further differentiation to gain osteocytes is however hard to achieve; also knowledge concerning the molecular mechanisms which specifically trigger and convey osteocyte differentiation is scarce.
Through a serendipitous observation while investigating the role of hyaluronan in MSC biology, we were able to unveil molecular mechanisms of osteocytogenesis. These involve specific stress response mechanisms regarding detoxification by means of glucuronidation, upregulation of O-glycosylation which grossly changes the cellular phospho-proteome, induction of stress granule formation and concomitant mRNA-binding thereby yielding differential regulation of translation of osteogenic transcription factors and the formation of poly-ADP–ribose (PAR) in the cytoplasm otherwise known to coordinate the nuclear DNA repair machinery. We could further show in 3D-mesenspheres that provoking this particular stress response pathway in the presence of osteogenic inducers promotes the calcification of MSC-derived microorganoids resulting in the formation of trabecular-like hard tissue structure bearing osteoblastic linings which engulf marrow-like stroma.
These observations could be further corroborated in histological sections of human bone in particular showing the presence of stress granule and PAR deposition in osteocytes residing in compact bone. We could further show accelerated healing of a bone defect model in rat calvaria by provoking this stress response pathway.

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guenter.lepperdinger@sbg.ac.at

STEM CELL THERAPY IN CARDIOVASCULAR MEDICINE - UPDATE 2015

Christoph Brenner

Department of Cardiology and Angiology, Medical University of Innsbruck

Cardiovascular diseases are a main cause of death in the Western world. Due to myocardial infarction or non-ischemic cardiac disorders in the aging population congestive heart failure has become a rising problem in our present health system. Additionally, western life style has led to a rising number of atherosclerotic diseases. However, till today curative treatment options for both cardiac and vascular diseases are limited. Current stem cell research provides promising therapeutic options for tissue regeneration and replacement in the future. While adult stem cell therapy mainly acts in a paracrine way, pluripotent stem cells have the potential to provide transplantable cardiac and vascular tissue.

mail@med.cbrenner.net

CLINICAL CARDIAC REGENERATION STUDIES: PAST, PRESENT AND FUTURE

Mariann Gyöngyösi

Division of Cardiology, 2nd Department of Internal Medicine, Medical University of Vienna, Austria

Almost twenty years ago, stem or progenitor cell therapy seemed to be a promising new approach to regenerate human myocardium, with the assumption that these cells can rescue ischemic myocyte damage, enhance vascular density and rebuild injured myocardium. Recent studies and meta-analyses, however, indicated, that the therapeutic success of these cells and applied methods for human cardiac regeneration are moderate or even not verifiable. As the quest for a frontrunner continues, alternative cell types, such as resident cardiac stem cells, adipose-derived or phenotypic modified cells have also been applied, aiming to increase both the number and the retention of the reparative cells in the myocardium. Two main delivery routes (intracoronary and percutaneous intramyocardial) of stem cells are currently used preferably for patients with recent acute myocardial infarction or ischemic cardiomyopathy. The most recent prospective individual patient data based meta-analysis including the largest randomized studies with intracoronary delivery of cells in patients with recent myocardial infarction showed no benefit of cell therapy on clinical or left ventricular functional outcome or cardiac remodeling. Currently, the European Commission support two human cardiac regeneration studies, the BAMI trial and the SCIENCE trial. The latter is just started, with the participation of the Medical University of Vienna, and involves patients with chronic ischemic heart disease, who will be randomized to receive allogeneous adipose-derived stem cells (GLP-production) intramyocardially. Besides increasing effort to enhance the human cardiac cell therapy success, pre-clinical experiments using tissue engineering or paracrine factors hold promise to regenerate injured human cardiac tissue.

mariann.gyongyosi@meduniwien.ac.at

CARDIOVASCULAR ANIMAL MODELS AND CELLULAR THERAPY

Bruno Podesser

Medical University of Vienna, Austria

bruno.podesser@meduniwien.ac.at

SECRETOME OF STRESSED PERIPHERAL MONONUCLEAR CELLS IS EFFECTIVE IN ACUTE AND CHRONIC CARDIAC ISCHEMIA IN THE PORCINE MODEL

Noemi Pavo, Dominika Macejovska, Katrin Zlabinger, Matthias Zimmermann, Lucian Beer, Dietmar Pils, Michael Mildner, Mariann Gyöngyösi, Hendrik Jan Ankersmit

Department of Cardiology, Medical University of Vienna

Cell-assisted modification of immunomodulatory mechanisms aiming to attenuate myocardial injury are currently under investigation. Intravenous injection of secretome of irradiated apoptotic peripheral blood mononuclear cell (PBMC) suspensions restored cardiac dysfunction in a rat acute myocardial infarction (AMI) model. To study the effects in the porcine model cell culture supernatants derived from irradiated apoptotic PBMC (APOSEC) were collected from pigs. After the induction of closed chest reperfused AMI, APOSEC was either injected intravenously as a single infusion dose during the coronary occlusion phase of AMI, or percutaneously intramyocardially in the border zone of AMI in a chronic phase of LV dysfunction.
The administration of APOSEC in the acute phase of AMI resulted in a reduction of scar tissue formation, and higher values of ejection fraction (57.0 vs. 40.5%, $p<0.01$), cardiac output (4.0 vs. 2.4 l/min, $p<0.001$) and a reduced extent of infarction size (12.6 vs. 6.9%, $p<0.02$) as determined by magnet resonance imaging (MRI). In the chronic AMI model, injection of APOSEC significantly decreased infarct size ($p < 0.05$) and improved cardiac index and myocardial viability. Transcriptome analysis revealed downregulation of caspase-1, tumor necrosis factor and other proinflammatory genes in the APOSEC-affected areas. The sustained alteration in gene expression one month post treatment proved the long-acting effects of cell-free therapy with paracrine factors.
In conclusion, intravenous or intramyocardial injection of APOSEC attenuates ischemia induced myocardial remodeling in experimental acute or chronic AMI models. This effect is probably due to the activation of pro-survival signaling cascades in the ischemic-injured cardiomyocytes.

noemi.pavo@meduniwien.ac.at

DISSECTING MECHANISMS OF MURINE NEONATAL CARDIAC REGENERATION

Bernhard J. Haubner, Martyna Adamowicz-Brice, Luigi Tortola, Claire Morgan, Tim Aitman, Josef M. Penninger

Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Dr. Bohrgasse 3, 1030 Vienna, Austria

Recently, we demonstrated complete cardiac regeneration in a neonatal mouse model of myocardial infarction. This regenerative potential of the heart is lost within the first week of life. The key mechanisms that promote regeneration at the perinatal stage remained entirely unclear. Therefore, we used our protocol of left anterior descending artery (LAD) ligation in combination with consecutive FACS analysis 36 hours and 5 days later to analyze the inflammatory response in neonatal vs. one-week old mice. Moreover, we performed an in-depth analysis of the transcriptome of sham versus myocardial infarction treated hearts. LAD ligation induced a robust inflammatory response within the first 36 hours irrespective of the timepoint of myocardial infarction. Importantly, we found a significant difference in the lymphoid compartment. Whereas newborn mice presented a significant increase of γδT-cells upon LAD ligation, no change was evidenced in the one-week-old animals. Remarkably, five days after the initial ischemic injury, we observed complete clearance of the leukocyte infiltrate in the neonatal heart. In contrast, hearts that were LAD ligated on postnatal day seven showed prolonged cardiac inflammation beyond the fifth day post injury. Furthermore, RNA-sequencing and KEGG pathway analyses of neonatal hearts imply pathways in cancer, MAPK signaling, and focal adhesion as mechanistic players. In summary, neonatal hearts demonstrate rapid clearance of the ischemia induced infiltrate and their transcriptome is enriched in genes of mitosis and DNA replication. Together, these differences support the fast recovery of newborn mice following a complex ischemic cardiac damage.

bernhard.haubner@imba.oeaw.ac.at

THE STEM CELL SECRETOME: THE STORY OF MSCs

Michael B. Fischer[1,2]

[1] Clinic of Blood Group Serology and Transfusion Medicine, Medical University of Vienna
[2] Center for Biomedical Technology, Danube University Krems, Austria.

Introduction: MSCs are multipotential adult progenitor cells that have the capacity to differentiate along the mesenchymal lineage to form cartilage, adipose, marrow-stroma, and bone tissue and because of their capacity to secrete trophic factors that contribute to repair via the promotion of vascularization and the inhibition of cell death MSCs have a therapeutic effect in tissue and organ repair.
Current knowledge: MSCs can produce a range of growth factors and cytokines, which inspired the designation of these cells as *'injury drugstore'*.
MSCs have an intrinsic affinity for sites of tissue injury, as MSCs respond to chemokines, such as SDF-1, MCP-3, CXCL9, CXCL16, CCL20, CCL25, and HGF. These mediators can either act locally or recruit MSCs from the bloodstream. MSCs are then activated by the surrounding microenvironment and respond to local cues by secreting a site-specific array of bioactive molecules. These molecules act to modulate the MSC microenvironment, reduce inflammation, and establish a regenerative milieu by repairing inflammation compromised vessels.
MSCs are capable not only to stimulate angiogenesis but also vasculogenesis by secreting large amounts of vascular endothelial growth factor (VEGF) that attracts vascular endothelial progenitor cells. Together with these progenitors this local reaction attracts pericytes that are necessary to stabilize newly formed capillaries and prevent vessel pruning (disambiguation).
For clinical application of MSCs there are two principal routs to introduce MSCs into the body: (1) local delivery into the tissue and (2) systemic delivery. Local delivery can be defined either by a specific type of delivery such as cells embedded in a scaffold or injected intraperitoneal, intramuscular, and into cardiac tissue. In contrast, systemic delivery is defined by the vascular route of venous or arterial application. The optimal method of transplantation will depend on whether or not MSCs perform best when present at sites of injury and inflammation. In theory, if MSCs can exert their effect distally by secreting cytokines into the circulation it may not be necessary for the cells to be located at the specific injury site and the systemic effect could be achieved using MSCs as reservoir of bioactive molecules ready for delivery.
Conclusion: This lecture will highlight the functional capabilities of MSCs, discuss clinical trials using MSCs for the treatment of a range of diseases and will show that the therapeutic efficacy of delivered MSCs will increase dramatically if MSCs can be made to engraft more efficiently by directing them to the site(s) of the lesion(s) to be treated.

michael.fischer@donau-uni.ac.at

BONE-CONDITIONED MEDIUM

Reinhard Gruber, Jianbo Peng, Kosaku Sawada, Matthias Zimmermann, Guenther Schuldt Filho, Jordi Caballé-Serrano, Dieter Bosshardt, Daniel Buser

Laboratory of Oral Cell Biology, School of Dental Medicine, University of Bern, Bern, Switzerland

Bone grafting is among the most frequently performed surgical procedures worldwide.

Surprisingly little is known about the role of bone grafts serving as a source for soluble molecules that modify bone regeneration. The question arises if freshly prepared bone chips release soluble molecules that targets cells involved in bone regeneration. We analyzed the conditioned medium of freshly prepared porcine bone chips. The bone-conditioned medium (BCM) was first subjected to proteomic analysis. The cellular response to BCM was determined by genomic analysis revealing a strong expression of TGF-β-regulated genes such as IL11, PRG4, ADM and PTX3. Autoclaving abolished the activity of BCM, but not demineralization, radiation, or exposure of the bone chips to antiseptic solutions. Another round of bioassays was based on osteoclastogenesis with murine bone marrow cultures. In this model, heated BCM had a moderately positive effect on osteoclastogenesis. Together, we provide pioneer data on the composition and activity of BCM in vitro. These findings suggest that cortical bone chips release soluble molecules that can target mesenchymal and hematopoietic cells. This knowledge can be translated into the supplementation of biomaterials with BCM to more closely mimic autografts, the “gold standard” in bone grafting.

reinhard.gruber@meduniwien.ac.at

ANALYSIS OF APOPTOTIC MONONUCLEAR CELL SECRETOME: CONTENT, FUNCTION, GMP PRODUCT, AND PRECLINICAL EVIDENCE

Lucian Beer[1,2], Matthias Zimmermann[1,2], Andreas Mitterbauer[1,2], Adolf Ellinger[3], Florian Gruber[4,5], Marie-Sophie Narzt[4,5], Maria Zellner[6], Mariann Gyöngyösi[7], Sibylle Madlener[8], Elisabeth Simader[1,2]; Christian Gabriel[10], Michael Mildner[4]*, Hendrik Jan Ankersmit[1,2]*

[1] Department of Thoracic Surgery, Medical University of Vienna, Austria
[2] Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical University of Vienna, Austria
[3]Department of Cell Biology and Ultrastructure Research, Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria.
[4]Department of Dermatology, Research Division of Biology and Pathobiology of the Skin, Medical University Vienna, Austria
[5] Christian Doppler Laboratory for Laboratory Biotechnology of Skin Aging, Medical, Austria
[6]Centre of Physiology and Pharmacology, Institute of Physiology, Medical University of Vienna, Austria
[7]Department of Cardiology, Medical University Vienna, Vienna, Austria
[8]Molecular Neuro-Oncology Research Unit, Department of Pediatrics and Adolescent Medicine and Institute of Neurology, Medical University of Vienna, Austria
[9]University Hospital Würzburg, Department of Cardiothoracic Surgery, Germany
[10]Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria

Stressed peripheral blood mononuclear cells (PBMCs) release paracrine factors with regenerative capacity, and the stressed PBMC secretome enhances angiogenesis and wound healing in vivo and in vitro. Here, irradiated PBMCs differentially express genes for secreted proteins involved in pro-angiogenic and regenerative pathways, and Good Manufacturing Practices–compliant and experimentally prepared PBMC secretomes exert comparable cardioprotective effects in vivo. PBMCs are readily obtainable, making “cell-free” regenerative medicine feasible for clinical trials.

hendrik.ankersmit@meduniwien.ac.at

FROM CD362 TO CYNDACEL: THE RAPID DEVELOPMENT OF A NOVEL STROMAL CELL THERAPY

S J Elliman[1], L O'Flynn[1], MM Duffy[1], LM Deedigan[1], P Loftus[1], L. Watson[1], S. Alagesan[1], S D'Arcy[2], A Morrison[2], C Masterson[2], D O'Toole[2], SA Hanley[2], AE Ryan[2], MD Griffin[2], S. Patil[2], SFH de Witte[3], CC Baan[3], MJ Hoogduijn[3], PN Newsome[4] , V Wong[5], AW Stitt[5], A Viola[6] , HJ Anders[7], I Tavares[8], JG Laffey[2], T O'Brien[1,2], FP Barry[1,2]

[1]Orbsen Therapeutics Ltd and [2]Regenerative Medicine Institute, NUI Galway, University Rd., Ireland. [3]Nephrology and Transplantation, Department of Internal Medicine , Erasmus MC, Rotterdam, The Netherlands; [4]Department of NIHR Liver Biomedical Research Unit and Centre for Liver Research , University of Birmingham, UK. [5]Centre for Experimental Medicine, Queen's University Belfast, Belfast, BT12 6BA, Northern Ireland, UK. [6]Humanitas Clinical and Research Center, University of Milan, Italy. [7]Division of Nephrology, Ludwig-Maximilians-University of Munich, Germany. [8]Department of Experimental Biology, Faculty of Medicine, University of Porto, Portugal.

Orbsen is developing highly purified, antibody-selected CD362/Syndecan-2 positive mesenchymal stromal cells (MSCs) isolated either from human bone marrow (Cyndacel-M™), human umbilical cord (Cyndacel-C™) or human adipose tissue (Cyndacel-A™). Notably, antibodies to CD362 can also be used to purify MSC from rat, rabbit and horse bone marrow for the first time. Murine CD362$^+$MSC are perivascular, express gp38, PDGFRa and nestin and can be identified in bone marrow, lymph nodes, spleen, thymus and skeletal muscle. We demonstrate that may represent a functional component of the immuno-modulatory MSC. CD362 is a cell surface heparan sulfate proteoglycan that is stimulated by hypoxia and inflammatory cytokines. Notably, CD362 protein suppresses cytokine-mediated NFkB activation and IL-6 secretion. Anti-CD362 antibodies suppress T helper 17 cells activation, proliferation and IL-17 secretion. As part of the REDDSTAR and MERLIN EU FP7 consortia, we have tested Cyndacel-M™ and Cyndacel-C™ in preclinical models of inflammatory disease. Specifically, we report that a single low dose of intravenous Cyndacel-M™ was effective in reducing inflammation and sclerosis and improving kidney function up to 8 weeks post-administration in a chronic diabetic (db/db) model of nephropathy. Intravenous Cyndacel-M™ alleviates thermal and mechanical pain in a SD rat model of diabetic neuropathy. Topical Cyndacel-M™ improves wound healing in a NZ rabbit model of non-healing diabetic ulcers. Intravenous Cyndacel-C™ improves liver function in a murine CCL_4 model of liver inflammation and lung oxygenation in a SD rat model of acute lung injury. Orbsen will test the clinical safety of Cyndacel-M™ and Cyndacel-C™ in Phase 1/2 clinical trials starting in 2015.

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steve.elliman@orbsentherapeutics.com

COSTIMULATORY BLOCKADE – AN OPTION IN THE TREATMENT OF GLOMERULONEPHRITIS

[1]Katharina Artinger, [1]Alexander H. Kirsch, [2]Daniel J. Cooper, [1]Ida Aringer, [1]Corinna Schabhüttl, [3]Philipp Eller, [2]Peter J. Lane, [1]Alexander R. Rosenkranz, [1]Kathrin Eller

[1]Clinical Division of Nephrology, Department of Internal Medicine, Medical University of Graz, Graz, Austria
[2]Medical Research Council Centre for Immune Regulation, Division of Immunity and Infection, University of Birmingham, Birmingham, United Kingdom
[3]Intensive Care Unit at the Department of Internal Medicine, Medical University of Graz, Graz, Austria

Introduction: Being an emerging therapeutic option in organ transplantation, the blockade of costimulatory molecules is also seen as an attractive target in the treatment of autoimmune disease. The TNF superfamily-members CD30 and OX40 are involved in costimulatory pathways via propagation and survival of $CD4^+$ Th1 cells, thereby maintaining their different functions. Nephrotoxic serumnephritis (NTS) is a model of experimental immune complex glomerulonephritis in mice, known to be dependent on Th1 and Th17 cells. Since the blockade of CD30 and OX40 was shown to also abrogate the development of lethal Foxp3-deficient disease in mice, this blockade could also be a treatment option in glomerulonephritis.
Methods: NTS was induced in wildtype (WT) mice and mice deficient in CD30 and OX40. Additionally, NTS was induced in WT mice and antibodies against CD30-ligand and OX40-ligand or control IgG were injected intraperitoneally twice a week. The kidney phenotype was analyzed after 14 days.
Results: CD30/OX40-deficient mice developed significantly decreased albuminuria when compared to WT animals. Furthermore, levels of glomerulosclerosis as well as kidney infiltrating cells such as $CD4^+$ cells and macrophages were significantly decreased in CD30/OX40 deficient mice. In line to the data obtained from CD30/OX40 knock-out mice, antibody blockade resulted in decreased albumin/creatinine ratio as well as glomerulosclerosis 14 days after NTS induction when compared to control IgG treated mice.
Conclusion: Since not only knock-out animals, but also the treatment with antibodies against CD30-ligand and OX40-ligand lead to an improved disease phenotype, this blockade represents a potential treatment option in the therapy of glomerulonephritis.

katharina.artinger@medunigraz.at

HETEROGENEOUS SUSCEPTIBILITY FOR UREMIC MEDIA CALCIFICATION AND CONCOMITANT INFLAMMATION

Alexander H. Kirsch (1), Andrijana Kozina (2), Katharina Artinger (1), Corinna Schabhüttl (3), Walter Goessler (4), Ingeborg Klymiuk (5), Christian Gülly (5), Gerald A. Fritz (6), Saša Frank (2), Roxana Wimmer (1), Marianne Brodmann (3), Hans-Joachim Anders (7), Peter P. Pramstaller (8), Alexander R. Rosenkranz (1), Kathrin Eller (1)* and Philipp Eller (3)*

1. Department of Internal Medicine, Clinical Division of Nephrology, Medical University of Graz, Graz, Austria
2. Center of Molecular Medicine, Institute of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria

3. Department of Internal Medicine, Division of Angiology, Medical University of Graz, Graz, Austria
4. Institute of Chemistry-Analytical Chemistry, Karl-Franzens University of Graz, Graz, Austria
5. Center for Medical Research, Core Facility Molecular Biology, Medical University of Graz, Graz, Austria
6. Department of Radiology, Medical University of Graz, Graz, Austria
7. Renal Division, Medizinische Klinik und Poliklinik IV, Klinikum der Universität München-Innenstadt, Munich, Germany
8. Center for Biomedicine, European Academy of Bolzano/Bozen, Bozen, Italy

Background: End-stage renal disease is strongly associated with arterial calcification of the *Tunica media*, decreased vascular compliance, and sudden cardiac death. Here, we analysed distribution pattern of uremic media calcification and concomitant inflammation in mice and men.
Methods and Results: Uremic DBA/2 mice showed only minor calcifications in thoracic aortas, whereas there was overt media calcification in abdominal aortas, which was qualitatively and quantitatively evaluated by histology, mass spectrometry, and myography. The transcriptional profile and immunohistochemistry revealed induction of *Vcam1* expression by vascular smooth muscle cells in uremic abdominal aortas. Macrophages infiltrated the *Tunica media* of the abdominal aorta. Anti-inflammatory treatment did not improve uremic media calcification in our animal model. Arterial calcifications in patients with end-stage renal failure showed a similar distribution pattern in computed tomography scans, with higher calcium scores of the abdominal aorta when compared to the thoracic aorta.
Conclusion: Taken together, there was a similar heterogeneous pattern of calcification in both mice and humans, where the abdominal aorta was more prone to media calcification when compared to the thoracic aorta. In uremia, smooth muscle cells of the abdominal aorta showed a phenotypic switch to an inflammatory phenotype.

alexander.kirsch@medunigraz.at

TRANSPLANTATION TOLERANCE THROUGH MIXED CHIMERISM

Pilat Nina[1], Mahr Benedikt[1], Unger Lukas[1], Schwarz Christoph[1], Hock Karin[1], Wrba Fritz[2], Wekerle Thomas[1]

[1]Department of Surgery, Medical University of Vienna
[2]Institute for Pathology, Medical University of Vienna

Induction of donor-specific tolerance via the mixed chimerism approach is a potent tolerance approach which was shown to be successful in humans. Notably, although chimerism leads to longterm heart allograft survival and prevents acute rejection, allografts show signs of chronic rejection and tissue damage in some models. Recently, we could show that therapeutic administration of activated polyclonal Tregs improves outcome of heart allografts in mixed chimeras despite the need for less recipient conditioning. We hypothesized that Treg treatment prevents chronic rejection induced by minor(tissue specific)antigens.
Groups of mice treated with non-myeloablative (3Gy) TBI or a therapeutic Treg dose received a dose of fully-mismatched bone-marrow under the cover of costimulation-blockade. Multi-linage chimerism and deletion of donor-reactive Tcells was followed by flow-cytometry. Tolerance was assessed by skin/heart transplantation from either Balb/c (MHC+minor-mismatch) or B10.D2 (MHC-mismatch-only) donors.

Durable multilineage chimerism was achieved with both regimens, with substantially higher chimerism levels in the group receiving irradiation. Balb/c and B10.D2 skin/heart allografts from Treg treated mice were devoid of signs of chronic rejection, whereas there was a striking difference between Balb/c and B10.D2 grafts in the 3Gy TBI group (P=0.023). B10.D2 grafts resemble grafts from Treg chimeras, however Balb/c cardiac grafts present with interstitial fibrosis, vasculitis and myointimal thickening and skingrafts show massive leucocyte infiltrates and atrophy of epidermis.
We could show that chronic rejection in a non-myeloablative mixed chimerism model is caused by minor-antigen-mismatch. These data suggest that Treg treatment induced regulatory mechanisms are superior to models relying mainly on deletional tolerance mechanisms.

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nina.pilat@meduniwien.ac.at

IL-2 / ANTI-IL-2 COMPLEXES PRIMARILY EXPAND THYMUS DERIVED TREGS BUT ALSO STIMULATE OTHER LYMPHOCYTE POPULATIONS

Benedikt Mahr[1], Lukas Unger[1], Ulrike Baranyi [1], Karin Hock [1], Andreas Farkas[1], Christoph Schwarz [1], Nina Pilat[1], Thomas Wekerle[1]

[1]Institute Section of Transplantation Immunology, Department of Surgery, Medical University of Vienna

Regulatory T cells (Tregs) have become an attractive target for alleviating alloresponses. We previously found that the adoptive transfer of natural Tregs induces mixed chimerism and tolerance without the need for any cytoreductive conditioning in a costimulation blockade-based bone marrow transplantation (BMT) model. Regarding clinical translation it would be more reasonable to substitute the cell therapy by protocols that selectively expand and activate Tregs in vivo. Recently, it could be shown that interleukin-2 (IL-2) complexed with a monoclonal antibody against IL-2 (IL-2 complexes) efficiently expands Tregs in vivo.
IL-2 complexes were administered intraperitoneally into C57BL/6 mice on three consecutive days (d0, d1, d2). Selective surface markers were analyzed in blood, spleen, lymph nodes and thymus at distinct time points (d4, d6, d9) by flow cytometry. BMT Recipients received $20*10^6$ unseparated Balb/C bone marrow cells under costimulation blockade (anti-CD154mAb, CTLA4-Ig) and a short course of rapamycin. Seleceted groups were further treated with Treg therapy or IL-2 complexes. Mixed lymphocyte reaction were performed by culturing $4*10^5$ splenocytes from untreated mice or mice treated with IL-2 complexes with equivalent numbers of irradiated donor bone marrow cells.
Upon treatment with IL-2 complexes 90% of Foxp3 positive cells were Ki67 positive in secondary lymphoid organs and 70% of the Ki67 positive Tregs simultaneously expressed Helios and Neuropilin-1. The in vivo expanded Tregs were able to suppress polyclonal activated T cells in vitro and to prolong graft survival of BalbC hearts [MST: 7.8d vs. 14.5d] when adoptively transferred. However, the administration of IL-2 complexes inhibited bone marrow engraftment (4/6 vs. 0/8) despite the strong expansion of natural Tregs. The rapid bone marrow rejection was associated with an enhanced proliferative response of CD8 T cells and NK cells towards donor bone marrow cells in vitro.
These results reveal that IL-2 complexes specifically expand and activate natural Tregs that but also stimulate alloreactive CD8 T and NK cells that abrogate the beneficial effect of Treg expansion in a mixed chimerism model.

benedikt.mahr@meduniwien.ac.at

EVALUATION OF IMMUNE RESPONSES IN CANCER PATIENTS UNDER DENDRITIC CELL-BASED CANCER IMMUNOTHERAPY

Visus C, Klingenbrunner S, Fischhuber KM, Reitermaier R, Erhart F, Barresi C, Prymaka V, Lobmayr L, and Felzmann T.

Activartis Biotech GmbH, Zimmermannplatz 10, 1090 Vienna, Austria.

We have conducted a randomized phase II trial designed to demonstrate safety and feasibility and deliver first evidence for efficacy of a Dendritic Cells (DC)-based cancer vaccine on glioblastoma (GBM) patients, AV0113 (Audencit). We use lipopolisaccharide (LPS) and interferon-gamma (IFN-γ) for DC maturation, which enables interleukin (IL)-12 secretion resulting in type 1 immune polarization and a potent anti-tumor immune response. Based on the hypothesis that there are immune parameters that may predict the response to immunotherapy we performed a complete immunological evaluation in the peripheral blood of vaccinated patients and assessed correlation with clinical outcome.
We implemented a comprehensive immunologic monitoring in 42 GBM patients and 6 healthy volunteers including functional assays using IFN-γ and Granzyme B (GranzB) ELISPOT and broad phenotypic analysis comprising differentiation and activation markers of T cells, B cells, Natural Killer (NK)/NK-T lymphocytes (NKT) cells, T regulatory cells (Tregs), T helper (TH)1/2/17 and Myeloid Derived Suppressor Cells (MDSCs). In addition, we examined cytokine release using cytokine bead assays (CBA) and transcription factors by quantitative real time retro transcriptase polymerase chain reaction (qRT-PCR). A total of 147 immune related variables were screened and estimated potential correlation with overall survival and progression free survival.
A pre-existing anti-tumor type I immune response (high anti-tumor GranzB and IFN -γ production) detected at the time of diagnosis indicates potential success of our Cancer Immunotherapy (CIT) approach. Other immune parameters measured in peripheral blood before DC-CIT were positively associated with overall survival: CD14 (Monocytes), Plasma Blasts (B cells), NK cells and CD8+ T cells (cytotoxic T lymphocytes (CTLs)) or negatively associated with overall survival (activated CD4/Tregs). Furthermore, a predominant Type I specific anti-tumor immune response has been identified after DC vaccination in patients that favorably responded to our AV0113 CIT characterized by release of IFN-γ, GranzB and IL-2 as well as increased percentage of CTLs in the peripheral blood. An increase in MDSCs was on the contrary predictive of disease relapse in GBM patients.
The measurement of immune biomarkers in GBM patients before CIT can help vaccine development by utilizing this information to select patients that most likely will benefit from this type of treatment as well as immune monitoring responses and guidance to therapy.

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maria.visus-miguel@activartis.com

TUMOR-PRIMED NATURAL KILLER CELLS – FROM CLINICAL OBSERVATION TO COMMERCIAL DEVELOPMENT

Mark W Lowdell

Centre for Cell, Gene & Tissue Therapeutics, Department of Haematology, UCL Medical School,London, UK

Clinical trials of natural killer cells (NK) in cancer have been broadly disappointing but some notable exceptions have been published. One such trial from the Fred Hutchinson in the early 1990s in acute myeloid leukaemia patients was particularly remarkable. At the same time we reported two cases of endogenous autologous NK responses in AML which we associated with clearance of residual disease and long term disease free survival. These clinical observations led to a period of basic research which showed that AML patients have fully functional NK cells which, in approximately 40% of patients, achieve disease clearance and long term remission after chemotherapy. The patients who relapse after chemotherapy are those with AML blasts which are resistant to NK killing. Our basic research identified a new mechanism of priming of NK cell anti-tumour killing which rendered NK-resistant AML cells (and several other tumour types) sensitive to NK mediated lysis. Conversion of the priming method to GMP compliance led to a phase I/II academic trial as an advanced therapy medicinal product in AML patients at UCL and the subsequent product licensing to a US biopharmaceutical company. This required development of novel product quality and reproducibility assays to allow technology transfer of a highly complex process and the submission for regulatory approval in a second, substantially different domain. The challenges in moving from academic GMP manufacture to a CMO will be described and opened for general discussion.

m.lowdell@ucl.ac.uk

THE FINAL PUSH TOWARDS LICENSING OF DENDRITIC CELLS FOR CANCER IMMUNOTHERAPY

Thomas Felzmann[1], Maria-Carmen Visus-Miguel[1], Alexander M. Dohnal[2],

[1] Activartis Biotech GmbH, Vienna, Austria; [2] St. Anna Children's Cancer Research Institute, Vienna, Austria

The first dendritic cell (DC) -based cancer immunotherapy (CIT) received regulatory approval in 2010, but failed so far to develop into a practicable routine treatment. More advanced DC-CIT technologies are currently in late stage clinical development. Significant progress came from improved understanding of the DCs' immune regulatory function. In vitro, monocyte-derived DCs differentiate via distinct functional stages: in their steady-state, they contribute to maintaining tolerance; contact with microbial or damage-associated danger molecules switches them into a potently immune stimulatory mode of action; and finally, with one day delay the DCs assume an immune suppressive phenotype for terminating immune responses and preventing them from running out of control.
Consequently, an ideal DC-CIT will (i) use a danger signal such as lipopolysaccharide that turns on the DCs' immune stimulatory capacity; and (ii) prevent DCs from differentiating into their immune suppressive phenotype. This might be accomplished by treating patients with immune checkpoint (ICP) inhibitors, monoclonal antibodies interfering with the delivery of immune suppressive signals to activated T-cells. We chose to use small molecule kinase

inhibitors for blocking immune suppressive signalling in DCs, which need only to be present during the in vitro maturation but not given to the patients. This required the identification of the pathways that guide this final differentiation step. Blocking key signalling molecules of the JAK/STAT and MAPKAP kinase pathways greatly improved the DCs' capacity for triggering anti-tumour immunity. It appears reasonable to assume that these superior strategies will realise the full potential of DC-CIT in the near future.

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thomas.felzmann@activartis.com

TREATMENT OF OSTEOARTHRITIS WITH FRESHLY ISOLATED STROMAL VASCULAR FRACTION CELLS FROM ADIPOSE TISSUE

Jaroslav Michalek, Rene Moster, Zuzana Dudasova, Zlatuse Kristkova, Marek Cibulka, Michal Holek, Michal Jurik, Ladislav Lukac, Martina Capkova, Ales Chaloupka, Miron Petrasovic, Adas Darinskas and Kenneth Proefrock.

International Consortium for Cell Therapy and Immunotherapy, Masaryk University, Brno; Cellthera, Ltd., Brno, Czech Republic; Immunolita, Ltd., Vinius, Lithuania; Medissimo Hospital, Bratislava, Slovakia; Stem Cell Center, Phoenix AZ, USA.

Therapy of osteoarthritis relies on non-steroid analgesics, chondroprotectives and in late stages total joint replacement is considered a standard of care. We performed a pilot study using novel stem cell therapy approach that was performed during one surgical procedure. It relies on abdominal lipoaspiration and processing of connective tissue to stromal vascular fraction (SVF) cells that typically contain relatively large amounts of mesenchymal stromal and stem cells. SVF cells are injected immediately to the connective tissue immediately adjacent to the target joint. A total of 1128 patients have been recruited and followed for up to 42 months to demonstrate the therapeutical potential of freshly isolated SVF cells. At the same time, one to four joints (knees and hips) were injected with SVF cells per patient. A total number of 1769 joints were treated. Clinical scale evaluation including pain, non-steroid analgesic usage, limping, extent of joint movement and stiffness was used as measurement of the clinical effect. All patients were diagnosed with stage II-IV osteoarthritis using clinical examination and X-ray, in some cases MRI was also performed to monitor the changes before and after stem cell therapy. After 12 months from SVF therapy, at least 50% clinical improvement was recognized in 81%, and at least 75% clinical improvement in 47% of patients, respectively. Within 1-2 weeks from SVF therapy 72% of patients were off the non-steroid analgesics and most of them remain such for at least 12 months. No serious side effects, systemic infection or cancer was associated with SVF cell therapy. In conclusion, here we report a novel and promising therapeutical approach that is safe, cost effective, and relying only on autologous cells.

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michalek@cellthera.cz

CHALLENGES IN THE DEVELOPMENT OF A DENDRITIC CELL CANCER THERAPY IN AUSTRIA

Marianne Imhof[1&2], Wolfgang Huber[2], Martin Imhof[3]

[1]Life Research Technologies & [2]Cellmed Research GmbH, Schoenlaterngasse 11/13,1010 Wien; [3]Landesklinikum Lehrspital Korneuburg, Wiener Ring 3-5, 2100 Korneuburg

The PROCURE story started with medical doctors having a vision, an ingenious invention and the Austrian province of Lower Austria being ambitious in supporting innovative start-ups at the location of a future biotech cluster called “RIZ” standing for “regional centers of innovation” in Krems, approx. 60km from Vienna.
Many hurdles had to be taken to establish an authority approved GMP production facility with an approved production process for the innovative dendritic cancer therapy PROCURE. During the entire development phase of PROCURE so far enormous regulatory and financial challenges had to be overcome. This difficult task could be successfully approached by a highly motivated scientific and management team supported by extremely loyal investors, who share the basic vision of the medical doctors: to develop an efficient cancer vaccine basically without any serious side effects.
PROCURE, a mRNA and peptide double loaded mature dendritic cell therapy made it to the clinic. The phase I/IIa trial included 15 patients with advanced ovarian cancer enrolled 12 weeks after standard treatment comparing two intradermal vaccination schedules. Each patient was vaccinated with a maximum of 8 doses of $13*10^6$ double loaded DCs. Primary endpoint was safety, and the secondary endpoint was quality of life and analysis of immunological and clinical response. PROCURE has been shown to elicit highly significant cellular immune and exciting clinical responses against ovarian cancer.
Next steps include a phase IIb trial enrolling approximately 80 patients to proof clinical efficacy. Challenges remain the same, though the financial part seems more difficult to overcome.

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marianne.imhof@cellmed.eu

REGULATORY UPDATE - CURRENT ISSUES

Ilona G. Reischl

Austrian Medicines and Medical Devices Agency; Austrian Federal Office for Safety in Health Care

Scientific progress is rapid in field of Advanced Therapies, leading to innovative products reaching the clinical trial stage and marketing authorization. Equally, the regulatory oversight for these therapies is not stagnant and evolving with the experience gained with these products. Challenges for regulators and developers nevertheless remain and merit discussion. Recent and upcoming regulatory activities in Europe will be summarized in the presentation.

ilona.reischl@ages.at

P01 SUB-REGIONAL DIFFERENCES IN METABOLIC ACTIVITY OF HUMAN AMNIOTIC MEMBRANE – A RICH SOURCE OF STEM CELLS

Asmita Banerjee[1], Adelheid Weidinger[1], Andrea Lindenmair[1], Simone Hennerbichler[2], Heinz Redl[1], Andrey Kozlov[1], Susanne Wolbank[1]

1 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingenstraße 13, A-1200 Vienna, Austria
2 Red Cross Blood Transfusion Service of Upper Austria, Krankenhausstr. 7, 4017 Linz, Austria

The human amniotic membrane (hAM), the innermost fetal membrane, contains cells with stem cell characteristics with low immunogenicity, making it a suitable material for tissue engineering. For clinical application, profound knowledge of properties, differentiation capacity and quality of the applied material is a prerequisite. Previously, we have shown that hAM can be differentiated towards osteogenic, chondrogenic and Schwann cell-like lineages. Differentiation is a highly energy-consuming process. However, hAM can be partitioned in placental and non-placental regions, giving rise to the question, whether these sub-regions show differences in morphology and energy metabolism. Mitochondrial respiration, monitored by high resolution respirometry, Oroboros Instruments, was 4-fold higher in the placental region compared to the non-placental region. Interestingly, respiratory control ratio showed no differences, reflecting similar quality of mitochondria in both regions, suggesting higher numbers of mitochondria in the placental region. Thus, the placental region seems to be more capable in terms of energy production, but also more oxygen-dependent. In histological sections of hAM, stained with haematoxylin/eosin, epithelial cells of the placental region appeared cylindrical with decentralized nuclei, whereas epithelial cells of the non-placental region were flattened and more homogenous. Thus, the placental and non-placental regions show distinct differences in morphology and mitochondrial activity. Since mitochondrial activity is closely related to cell type and function, the question is whether regional differences reflect different cellular functions. If so, may this also play a role in functionality, differential potential and cell fate, and may hence impact therapeutic properties in clinical applications.

asmita.banerjee@trauma.lbg.ac.at

P02 LOW LEVEL LIGHT THERAPY BY REPULS IN CELL CULTURE MODELS

Sidrah Chaudary, Adelheid Weidinger, Heinz Redl, Peter Dungel

Ludwig Boltzmann Institute for experimental and clinical Traumatology
Cluster for Tissue Regeneration

Low-level light therapy (LLLT) by light-emitting diodes (LED) is used to reduce inflammatory reactions, neurological pain and to promote wound healing. Relux GmbH, Austria, markets REPULS, a high frequency red light radiation lamp, which emits cold pulsed (2.5Hz) LED light at 632nm. Fibroblasts and myoblasts play important roles in wound healing, in this setting often challenged by hypoxic conditions. Thus, the aim of this study was to investigate the effects of REPULS, on a myoblast and a fibroblast cell line under normoxic and hypoxic conditions.
C2C12 myoblasts and NIH3T3 fibroblasts were incubated at 21% O_2 (normoxia) or 1% O_2 (hypoxia) for 3h, after which they were illuminated for 10min with REPULS at 95 mW/cm^2. Cell proliferation, metabolic activity and cell viability were determined by BrdU and MTT

assays and flow cytometry. ATP production was measured via a luminescence assay and mitochondrial respiration analyzed on an Oroboros-2k oxygraph.
LLLT with REPULS significantly increased cell proliferation under normoxic conditions in both cell lines. NIH3T3 fibroblast, however, reacted more sensitive to light treatment. Cellular stress induced by hypoxia/reoxygenation (H/R) potentiated the positive effects. A significant decrease in ATP production induced by H/R was significantly recovered by LLLT in both cell lines. This was associated with enhanced mitochondrial activity in the light-treated groups compared to controls.
Our data demonstrate that pulsed red LED light by REPULS positively influences cellular metabolism and proliferation, especially when cells were challenged by hypoxia/reoxygenation. These findings may contribute to explain the impressive positive effects of REPULS in wound healing.

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sidrah.chaudary@trauma.lbg.ac.at

P03 SHOCKWAVE TREATMENT AUGMENTS PROLIFERATION AND IMPROVES WOUND HEALING VIA PURINERGIC SIGNALING LINKED ERK1/2 PATHWAYS

Christiane Fuchs[1,2], Anna M. Weihs[1,2], Andreas H. Teuschl[1,2], Joachim Hartinger [2,3], Paul Slezak[2,3], Rainer Mittermayr[2,3], Heinz Redl[2,3], Wolfgang G. Junger[3,4], Harald H. Sitte[5], Dominik Rünzler[1,2]

[1]University of Applied Sciences Technikum Wien, Department of Biochemical Engineering, Vienna, Austria
[2]The Austrian Cluster for Tissue Regeneration, Vienna, Austria
[3]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology/AUVA Research Center, Vienna, Austria
[4]Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States of America
[5]Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Austria

Shockwave treatment (SWT) accelerates poor wound healing, though the general underlying principles of this beneficial effect remain to be fully elucidated. Therefore the aim of this study was to identify signaling pathways involved in the proliferative and wound healing effect of SWT.
C3H10T1/2 mouse mesenchymal stem cells, a human Jurkat T cell line and primary human adipose derived stem cells were subjected to *in vitro* SWT and ATP release was assessed. Proliferation after SWT was determinded via cell-cycle phase distribution analysis (flow cytometry) and BrdU incorporation. Western blot analysis was performed to assess ERK1/2 activation. An *in vivo* rodent ischemic excision wound healing model was used to assess the dependency of the SWT wound healing effect on ERK1/2 signaling by using a MEK1/2 inhibitor.
SWT dose-dependently released ATP in all three cell types and significantly increased the amount of proliferating cells. Hydrolysis of released ATP with apyrase diminished the proliferative effect of SWT. Shockwaves induced significant pERK1/2 activation, which was prevented by the P2 receptor antagonist suramin as well as by ATP depletion. Our *in vivo* study confirmed that SWT induces wound healing in an ERK1/2 dependent manner.

We conclude that *in vitro* SWT releases cellular ATP, activating downstream ERK1/2 signaling via purinergic receptors, ultimately causing the proliferative effects of shockwave treatment. Our *in vivo* data confirm the ERK1/2 signaling pathway being essential in the SWT wound healing effect. Thus, this signaling cascade could be one of the underlying principles of the beneficial effects of shockwave treatment in wound healing.

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fuchsc@technikum-wien.at

P04 NUTRITION AFFECTS TENDON HEALING IN A RAT MODEL

Stefanie Korntner[1,2,3], Nadja Kunkel[1,2,3,4], Christine Lehner[1,2,3], Gehwolf Renate[1,2,3], Wagner Andrea[1,2,3], Peter Augat[5], Herbert Resch[4], Hans-Christian Bauer[1,2,3], Andreas Traweger[1,2,3], Herbert Tempfer[1,2,3]

1 Paracelsus Medical University, Institute of Tendon & Bone Regeneration, Salzburg, Austria
2 Paracelsus Medical University, Spinal Cord Injury and Tissue Regeneration Center, Salzburg, Austria
3 Austrian Cluster for Tissue Regeneration, Vienna, Austria
4 University Hospital of Salzburg, Department of Trauma Surgery and Sports Injuries, Salzburg, Austria
5 Institute of Biomechanics, Trauma Center Murnau, Germany

Human and rat tendon cells produce and secrete insulin upon glucose stimulation. Therefore, we hypothesize that nutritional glucose potentially affects tendon healing. In 60 female Lewis rats a unilateral full-thickness Achilles tendon defect was created and animals were randomly assigned to three dietary groups: a high glucose diet, a diet with low glucose/high fat and a control diet, for 2 weeks each. Newly formed tissue was significantly thicker for the high-glucose (4,26mm±0,29) and high-fat group (4,32mm±0,20; n=20) when compared to the control group (3,66mm±0,39; n=19). Gait analysis revealed a significantly increased Intermediate Toe Spread for animals receiving high glucose diet one week p.o. (0,49cm±0,07; n=20; $p<0.01$) when compared to the control (0,42cm±0,08; n=19) and high fat diet group (0,40cm±0,12; n=20). In comparison to intact Achilles tendon controls, biomechanical testing of the repair tissue showed a significant reduction in maximum tensile load for the control and fat diet group, whereas no significant decrease was obvious for the high glucose group (n=14/group). Further, the repair tissue from the glucose group was significantly stiffer (20,82N/mm±8.08; n=14) compared to the control group (15,07N/mm±4.32; n=14), resembling values determined for intact tendon tissue (20,63N/mm ±10,96; n=14). Finally, cell proliferation was approximately 3-fold higher in repair tissue of the glucose (9,79±0,96%) and fat (8,40±0,83%) diet group. In summary, tendon repair tissue quality is moderately affected by nutritional glucose. These findings may serve as basis for developing a dietary intervention accelerating tendon regeneration after trauma or chronic tendon diseases.

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stefanie.korntner@pmu.ac.at

P05 ARE TENDON-DERIVED STEM CELLS A BETTER SOURCE FOR BONE REGENERATION?

Nadja Kunkel[1,2,6], Andrea Wagner[1,6], Renate Gehwolf[1,6], Patrick Heimel[3,6], Herbert Tempfer[1,6], Stefanie Korntner[1,6], Peter Augat[4], Herbert Resch[2], Heinz Redl[3,6], Oliver Betz[5], Hans-Christian Bauer[1,6], Andreas Traweger [1,6]

[1] Institute of Tendon and Bone Regeneration, Paracelsus Medical University - Spinal Cord Injury & Tissue Regeneration Center Salzburg, Austria
[2] Department of Traumatology and Sports Injuries, Paracelsus Medical University Salzburg, Austria
[3] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria
[4] Institute of Biomechanics, Trauma Center Murnau, Germany
[5] Laboratory for Biomechanics and Experimental Orthopedics, Department of Orthopedic Surgery, Hospital Großhadern, Munich, Germany
[6] Austrian Cluster for Tissue Regeneration, Vienna, Austria

Despite significant advancements in bone tissue engineering applications, the clinical impact of bone marrow stromal cells (BMSCs) for the treatment of large osseous defects remains limited. Therefore, other cell sources are under investigation for their osteogenic potential to repair bone. In this study tendon-derived stromal cells (TDSCs) were evaluated in comparison to BMSCs to support the functional repair of a 5mm critical-sized, segmental defect in the rat femur.
Analysis of the trilineage differentiation capacity of TDSCs and BMSCs cultured on collagen sponges revealed an impaired osteogenic differentiation and mineral deposition of TDSCs *in vitro*, whereas chondrogenic and adipogenic differentiation was evident for both cell types. Radiographic assessment demonstrated that neither cell type significantly improved the healing rate of a challenging 5mm segmental femoral defect *in vivo*. Both, transplanted TDSCs and BMSCs led to the formation of only small amounts of bone in the defect area and histological evaluation revealed non-mineralized, collagen- rich scar tissue to be present within the defect area. Newly formed lamellar bone was restricted to the defect margins resulting in closure of the medullary cavity. Interestingly, in comparison to BMSCs, significantly more TDSC-derived cells were present at the osteotomy gap up to 8 weeks after transplantation and were also found to be located wihin newly formed lamellar bone, suggesting their capacity to directly contribute to *de novo* bone formation. To our knowledge this is the first study investigating the *in vivo* capacity of TDSCs to regenerate a critical-sized defect in the rat femur.

andreas.traweger@pmu.ac.at

P06 IDENTIFICATION AND CHARACTERIZATION OF A NOVEL TISSUE BARRIER: THE BLOOD-TENDON BARRIER

Lehner C (1,4), Gehwolf R (1), Ek J (2), Traweger A (1), Bauer H (3), Bauer H.C. (1, 4), Tempfer H (1)

1. Institute of Tendon and Bone Regeneration, Paracelsus Medical University, SCI-TReCS, Salzburg, Austria; 2. Institute for Neuroscience and Physiology, Department of Physiology, University of Gothenburg, Sweden; 3. Department of Organismic Biology, University of

Salzburg, Austria; 4. University Clinic for Trauma Surgery and Sports Injuries, Salzburg, Austria

Tissue barriers function as “gate keepers” between different compartments and are created by specialized membrane-associated proteins, located at the lateral plasma membrane of epithelial and endothelial cells. By sealing the paracellular space, such barriers impede the free diffusion of solutes and molecules across epithelial and endothelial monolayers, thereby creating an organ-specific homeostatic milieu.
Tendon cells originate from yet poorly described precursor cells and develop in a particular "niche" close to vascular walls. Degenerative processes, trauma and injury severely disturb the internal milieu of the niche. Concomitantly, pathological alterations including hyperproliferation, erroneous tendon cell differentiation, and calcification take place.
Even though the vasculature is considered to play a crucial role for tendon cell development, evidence of how this is accomplished has been lacking. Perfusion with defined tracer substances revealed that the blood-tendon-barrier impedes the passive transport of macromolecules (≥10kDa) from the blood stream to the surrounding tendon tissue, but is permeable to molecules <287D. The expression of barrier-related proteins, such as zonula occludens protein-1 (ZO-1), occludin, claudin-3, and claudin-5, in human and murine tendon vascular cells further corroborates the assumption that a restrictive tissue barrier acts at the blood-tendon interface.
The mechanisms and factors maintaining the internal milieu of the developmental niche in healthy tendons are not yet fully understood. Here, we describe a novel vascular structure, the blood-tendon barrier (BTB), which separates tendon tissue from the systemic circulation in intact human and murine tendons. The role of this structure in tendinopathy, tendon regeneration and tendon development remains to be elucidated.

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christine.lehner@pmu.ac.at

P07 TISSUE BANKING IMPROVES OSTEOGENIC DIFFERENTIATION POTENTIAL OF HUMAN AMNIOTIC MEMBRANE

Andrea Lindenmair[1,2,3], Katharina Aichinger[2,3], Karin Witzeneder[2,4], Sylvia Nürnberger[2,5], Johann Eibl[6], Christian Gabriel[2,4], Heinz Redl[1,2,3], Susanne Wolbank[1,2,3]

1 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Linz/Vienna, AUVA Research Center, Austria
2 Austrian Cluster for Tissue Regeneration, Austria
3 Trauma Care Consult, Vienna, Austria
4 Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria
5 Bernhard Gottlieb University School of Dentistry, Vienna, Austria
6 Bio-Products & Bio-Engineering AG, Vienna, Austria

Human amniotic membrane (hAM) represents an abundantly available biomaterial being applied in many surgical procedures. Furthermore, it is consistently gaining access to new areas in tissue engineering and regenerative medicine. Since hAM represents a pre-formed sheet of stem cells there is no need for additional carrier material. Being able to differentiated hAM *in toto* without any prior cell isolation and further going towards tissue banks xenogeneic-free differentiation conditions of hAM are required. Therefore, fetal calf serum

(FCS) in the osteogenic differentiation medium was substituted by human alternatives, i.e. human platelet lysate (hPL) and human serum (huS).
8mm punch biopsies of hAM were cultured under osteogenic conditions substituting 10% FCS by 5 or 10% huS or hPL compared to controls. Differentiation was characterized by activity of alkaline phosphatase, calcium content and mineralization. Furthermore, the setting was upscaled by clamping hAM pieces in cell crowns and co-cultured with human endothelial cells.
Mineralization was observed under all osteogenic conditions. Activity of alkaline phosphatase (ALP) and calcium content of hAM biopsies was even increased using human alternatives. Best results were gained supplementing the differentiation medium with 10%huS or 5%hPL. Comparing ALP and the calcium content from biopsies and cell crown pieces, levels were higher in biopsies suggesting an important impact of mobility of hAM. Moreover, endothelial cells were successfully co-cultured with osteogenically induced hAM resulting in constant or even increased viability levels of the construct. Thus, these *in vitro* results are promising steps towards using hAM as a natural biomaterial for bone tissue engineering.

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andrea.lindenmair@o.roteskreuz.at

P08 THE USE OF ENZYMES DURING ADIPOSE DERIVED STEM CELL ISOLATION AFFECTS CELL IDENTITY AND FUNCTIONALITY

Eleni Oberbauer[1,2], Christoph Wurzer[1,2], Carolin Steffenhagen[1,2], Susanne Wolbank[1,2], Christian Gabriel[1,2,3], Heinz Redl[1,2]

1 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria
2 Austrian Cluster for Tissue Regeneration, Vienna, Austria
3 Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria

Subcutaneous human adipose tissue is an attractive and abundant source for therapeutic cells. The isolated stromal vascular fraction (SVF) is a heterogeneous cell population including the adipose-derived stromal/stem cells (ASC), which have been shown to possess regenerative potential in many preclinical models and several clinical trials. Current cell isolation methods for cells from adipose tissue are dependent on enzymes such as collagenase. However, the use of enzymes may have an impact on safety, potentially on efficacy, complicates regulatory authorization and is expensive which is reflected in the cost of cell products. Therefore we compared classical enzymatic cell isolation methods to reduced enzyme methods regarding cell yield, identity and functionality. Our results demonstrate that enzyme treatment is necessary for applications which require large cell numbers, since reduced enzyme to 30% and 10 % result in inferior cell yields. In contrast, the metabolic activity of the isolated cells as determined via intracellular ATP decreased with increasing collagenase concentration. Reduced enzyme treatment during isolation resulted in a higher number of endothelial progenitor cells (CD45-/CD31+/CD34+), pericytes (CD45-/CD31-/CD146+), and supra-adventitial cells (CD45-/CD31-/CD146-/CD34+). Furthermore, reduction of collagenase concentration showed significant higher adipogenic, osteogenic and chondrogenic differentiation potential. In this work we could show that the use of recombinant enzymes such as collagenase has an influence on cell properties and these findings should be considered in future clinical applications.

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Eleni.Oberbauer@trauma.lbg.ac.at

P09 INFLUENCE OF SODIUM HYDROXIDE AND HYDROCHLORIC ACID ON ARTICULAR CARTILAGE DECELLULARIZATION

C. Schneider[1], S. Wolbank[1], C. Gabriel[2], H. Redl[1], G. van Osch[3], S. Nürnberger[1,4], F. Hildner[2]

1. Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Vienna, Austria
2. Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria
3. Depts of Otorhinolaryngology & Orthopaedics, Erasmus MC University Medical Center, Rotterdam, The Netherlands
4. Department of Trauma Surgery, Medical University of Vienna, Vienna, Austria

Current cartilage regeneration strategies often show poor integration of the implant, lack early mechanical stability and require a two-staged surgical procedure. In this project we pursue a new concept combining decellularized donor cartilage with primary mesenchymal stem cells. Since cartilage tissue is tightly packed with glycosaminoglycans (GAGs), depletion of GAGs is necessary to allow repopulation. The aim of this study was to decellularize articular cartilage as well as to reduce the density of the tissue by GAG depletion, while the collagen structure should be retained.
Cartilage biopsies were obtained from human femoral heads of three donors and subjected to decellularization procedures adapted from Kheir et al., using sodium hydroxide or hydrochloric acid as main decellularizing agents. Remaining DNA and GAG content were quantified and alcian blue staining and collagen type II immunostaining were performed using an antibody binding to the collagen type II triple helix.
Sodium hydroxide significantly reduced the DNA content and was most effective in the removal of GAGs, however the collagen triple helix structure was disrupted in the outer layer of the samples. Hydrochloric acid reduced the DNA content to <25 ng/mg while GAGs and collagen in the outer layers were only slightly affected. H&E staining showed full cell removal in the superficial zone but presence of some cell residues in the deeper zones, though there were fewer residues when hydrochloric acid was used.
By subsequently combining sodium hydroxide and hydrochloric acid we will fine-tune DNA and GAG content for optimal de- and recellularization.

cornelia.schneider@o.roteskreuz.at

P10 GLYCINE-BASED POLYPHOSPHAZENE SCAFFOLDS SUPPORT CHONDROGENIC DIFFERENTIATION OF ADIPOSE DERIVED STEM CELLS

C. Schneider[1], F. Hildner[2], M. Rigau[2], I. Teasdale[3], E. Oberbauer[1], S. Nürnberger[1,4], H. Redl[1], C. Gabriel[2]

1. Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Vienna, Austria

2. Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria
3. Institute of Polymer Chemistry, Johannes Kepler University, Linz, Austria
4. Department of Trauma Surgery, Medical University of Vienna, Vienna, Austria

Polyphosphazenes provide interesting inherent features for tissue engineering applications due to their highly diverse properties, depending on the side group substituents. The aim of this study was to evaluate the applicability of glycine-based polyphosphazenes as scaffolds for cartilage regeneration.
Photo-polymerizable scaffolds with controlled hydrolytic degradability were designed and prepared via short chain poly(organo)phosphazene building blocks. Porous matrices with or without glutathione were fabricated by thiol-ene photo-polymerization and subsequently seeded with adipose derived stem cells (ASC). Cell adhesion and proliferation were quantified and cells were then differentiated for up to 5 weeks under chondrogenic conditions. Micromass pellet cultures and TissuFleeceE®, a clinically used collagen sponge, served as controls.
While cell adhesion and proliferation were slightly higher on TissuFleeceE®, qRT-PCR showed significantly higher collagen type II (COL2A1) and aggrecan (AGC1) expression for the glutathione containing polyphosphazene compared to the controls. The expression of these key chondrogenic markers was slightly inferior for the polymer without glutathione, demonstrating expression in the range of TissuFleeceE®. Collagen type II immunohistochemistry and alcian blue staining corroborated the gene expression results. Quantification of glycosaminoglycans using dimethyl-methylene-blue demonstrated significantly enhanced GAG expression in glutathione containing polyphosphazenes compared to pellet cultures.
In conclusion, glycine based polyphosphazenes demonstrated excellent properties for supporting synthesis of cartilage matrix proteins. ASC showed significantly higher chondrogenic potential when seeded to polyphosphazene polymers, especially in the presence of glutathione.

cornelia.schneider@o.roteskreuz.at

P11 EXTRACORPOREAL SHOCKWAVE TREATMENT HAS BENEFICIAL EFFECTS ON MOTOR, SENSOR AND MIXED SCHWANN CELL CULTURE

Christina M.A.P Schuh[1,2]*, David Hercher[1,2], Susanne Wolbank[1,2], Dominik Rünzler[2,3], Heinz Redl[1,2], Thomas Hausner[1,2]

[1] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology
[2] Austrian Cluster for Tissue Regeneration- Donaueschingenstrasse 13, A-1200 Vienna – Austria
[3] University of Applied Sciences Technikum Wien–Department of Biochemical Engineering, Vienna, Austria

Schwann cells are a crucial factor in peripheral nerve regeneration. As new treatment approaches are sought, there is an increasing demand for native Schwann cells for in vitro testing and/or reimplantation. In this study we evaluated differences between Schwann cells from a motor nerve (N. femoralis, motor branch), a sensor nerve (N. femoralis, sensor branch), as well as from a mixed nerve (sciaticus). We present a method to increase initial cell yield, proliferation rate and to prolong culture of Schwann cells with proliferative phenotype.

Sciatic nerves, as well as both N. femorali of adult Sprague Dawley rats were explanted and treated with extracorporeal shockwaves (ESWT). Subsequently Schwann cells were isolated and maintained for 8 weeks (passage every 6 days). Cells were counted at first passage and proliferation was analyzed with BrdU assay. Evaluation of proliferative phenotype was done with flow cytometry (P75, S100, P0).
Schwann cells isolated from nerves treated with extracorporeal shockwaves showed an up to 50% higher initial cell number (normalized on nerve wet weight). Evaluation with flow cytometry revealed a significantly lower amount of myelin component P0 in the ESWT group compared to untreated control. Cultured for up to 8 weeks (P6) Schwann cells treated with ESWT showed no change in marker expression (P75, S100) or proliferation (BrdU), while marker expression and proliferation of control group steadily decreased.
Extracorporeal shockwave treatment of donor nerves shows beneficial effects on rat Schwann cell isolation and culture.

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christina.schuh@trauma.lbg.ac.at

P12 REGENERATIVE DENTISTRY: HYPOXIA-MIMETIC AGENTS AND GROWTH FACTORS FOR ORAL TISSUE REGENERATION

Hermann Agis (1, 2), Barbara Cvikl (1, 2, 3), Michael B. Fischer (4, 5), William V. Giannobile (6,7), Reinhard Gruber (8,9,2), Ulrike Kuchler (8,2), Andreas Moritz (1, 2)

(1) Department of Conservative Dentistry and Periodontology, Medical University of Vienna, Vienna, Austria
(2) Austrian Cluster for Tissue Regeneration, Vienna, Austria
(3) Department of Preventive, Restorative and Pediatric Dentistry, School of Dental Medicine, University of Bern, Bern, Switzerland
(4) Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Vienna, Austria
(5) Center for Biomedical Technology, Danube University Krems, Krems, Austria
(6) Periodontics and Oral Medicine, School of Dentistry, University of Michigan, Ann Arbor, Michigan, United States of America
(7) Department of Biomedical Engineering, College of Engineering, University of Michigan, Ann Arbor, Michigan, United States of America
(8) Department of Oral Surgery, Medical University of Vienna, Vienna, Austria
(9) Laboratory of Oral Cell Biology, School of Dental Medicine, University of Bern, Bern, Switzerland

Strategies for oral tissue regeneration involve collagen matrices and bone substitutes. In regenerative endodontology, revascularisation strategies are applied. When healing is compromised personalized approaches to stimulate regeneration are required. We assessed regenerative strategies with hypoxia-mimetic agents (HMA) and growth factors from platelets.
We proposed an innovative approach for oral surgery and periodontology with HMA loaded bone substitutes and collagen matrices. HMA were released from bone substitutes and collagen matrices and maintained their pro-angiogeneic capacity in vitro. HMA decreased the proteolytic activity and osteoclastogenesis. In diabetic rats HMA loaded bone substitutes stimulated angiogenesis in calvaria defects but not bone regeneration. For regenerative endodontology we assessed the response of dental pulps to HMA. HMA increased pro-angiogenetic capacity in pulp-derived cells and tooth slices also under simulated diabetes and influence of capping material.
Platelet-derived growth factor-BB and platelet-rich plasma can stimulate oral tissue regeneration. Here we tested collagen matrices and bone substitutes as carriers for platelet supernatants. Proliferation of periodontal cells and pulp-derived cells was increased by the supernatants while the effect on osteoclastogenesis was dependent on the preparation of supernatants. The growth factors were released from the biomaterials in a burst-like kinetic. In a novel ex vivo wound repair model we revealed the effect on cell population and gene expression kinetics in collagen matrices.
Overall, HMA and supernatants of activated platelets released from bone substitute materials and collagen matrices maintained their biological activity. These results are a primer for research on HMA and platelet supernatants to overcome compromised oral tissue regeneration.

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All authors in alphabetic order

hermann.agis@meduniwien.ac.at

P13 TRANSCRIPTOME CHANGES IN DYSTROPHIC EPIDERMOLYSIS BULLOSA RESEMBLE SKIN AGING

Jenny S. Breitenbach[1], Mark Rinnerthaler[2], Michael Breitenbach[2], Andrea Trost[3] and Johann W. Bauer[1]

[1]Laboratory for Molecular Therapy, EB House Austria, Division of Experimental Dermatology and EB House Austria, University Hospital of Dermatology, Paracelsus Medical University Salzburg, Muellner Hauptstrasse 48, 5020 Salzburg, Austria
[2] Faculty of Science, Department of Cell Biology, Division of Genetics, University of Salzburg, Austria.
[3]Department of Ophthalmology and Optometry, Paracelsus Medical University, Muellner Hauptstrasse 48, 5020 Salzburg, Austria

Recessive dystrophic Epidermolysis bullosa (RDEB) is caused by mutations in the gene coding for collagen VII, *COL7A1*, a component of the dermo-epidermal basement membrane zone. The patients present with skin blistering after minor trauma, defects in wound healing, a general pro-inflammatory state, fewer epidermal layers, and impaired skin barrier function. The skin of RDEB patients and aged skin share common characteristics: structural changes in the epidermis and dermis, defects in wound healing and increased inflammation. We compared the skin transcriptome of young adults with RDEB with that of sex- and age-matched healthy probands. We also compared the skin transcriptome of healthy young adults and elderly healthy donors. Next, we generated an overlap list of genes that were significantly regulated in both comparisons. We found a large and highly significant overlap of genes concerning a limited number of functional protein families. Most prominent among these families are proteins of the cornified envelope or mechanistically involved in cornification, structural skin proteins, and proteins with a role in inflammation or immunity (e.g. pro-inflammatory cytokines and chemokines and their receptors, proteins of the innate immune response and transcription factors). To summarize, our data support our working hypothesis that common biochemical mechanisms are involved in skin aging and the pathomechanisms of RDEB.

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jenny.breitenbach@salk.at

P14 SHIFT OF ANG1/ANG2 BALANCE CAUSES REDUCED MIGRATION IN AGING ENDOTHELIAL CELLS

Ebenbauer B[1,2], Hohensinner PJ[1], Kaun C[1], Maurer G[1], Huber K[2,3], Wojta J[1,2]

[1]Department of Cardiology, Medical University of Vienna,
[2] Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna, Austria
[3]3rd Medical Department, Wilhelminenspital, Vienna, Austria

Introduction: Aging is associated with a variety of cell type specific changes. Endothelial cells have been reported to have reduced angiogenetic capability upon aging. An important pathway for endothelial cell activation is the Angiopoietin (Ang) 1/2 pathway with its receptors Tie1 and Tie2. Whereas Ang1 is critical for vessel maturation and quiescence, Ang2 is an activating factor for endothelial cells promoting neovascularization.
Aim: The aim of our study was to identify a possible regulation of the Ang1/2 system upon endothelial cell aging.
Results: Aging endothelial cells (EC) were generated by passaging them at least 8 times. Aging endothelial cells were characterized by increased senescent associated cytokine expression (e.g. PAI-1, MCP1 and GMCSF) and reduced telomere length. Upon aging, endothelial cells showed increased levels of Ang1, both in mRNA and secreted protein levels . In addition, Ang2 levels droped significantly in both mRNA (as well as protein levels resulting in a shifted ratio of Ang1/2 in aging endothelial cells. The Tie1/2 receptor system was not affected by aging and its expression remained stable. This change in Ang1/Ang2 relation was represented in a change in migration speed in an in vitro scratch wound assay. Aging EC were significantly slower in migration compared to the young counterpart. Adding soluble Ang2 to aging cells in the scratch assay restored endothelial cell mobility.
Conclusion: Aging induces a shift towards increased Ang1 expression in endothelial cells resulting in a reduced migration speed.

benjamin.ebenbauer@hotmail.com

P15 LINKING GLIOBLASTOMA MULTIFORME MICRORNA PROFILES WITH A POTENTIAL SUSCEPTIBILITY TO DENDRITIC CELL-BASED CANCER IMMUNOTHERAPY

Friedrich Erhart[1], Alexander Dohnal[1], Thomas Felzmann[2], Matthias Hackl[3], Rene Reitermeier[2], Carmen Visus[2]

1 Laboratory for Tumor Immunology, St. Anna Children's Cancer Research Institute, Vienna, Austria
2 Activartis Biotech GmbH, Vienna, Austria
3 TAmiRNA GmbH, Vienna, Austria

Glioblastoma Multiforme (GBM) is the most frequent and most aggressive brain cancer in adults. In children it accounts for 7-9% of all central nervous system tumors and can thus be regarded as an orphan disease. Currently, a phase II clinical trial of a novel, Dendritic Cell (DC)-based immunotherapy against GBM, developed by the CCRI, is being conducted on more than 80 adult patients. Our treatment approach makes use of autologous, tumor antigen-pulsed DCs. While final results of the trial are still pending, preliminary data already allows grouping patients into long-term and short-term survivors. Using miRNA sequencing of tumor tissue from 16 study patients, we recently identified miRNAs with a differential regulation in the treatment group long-term survivors as opposed to the control group long-

term survivors. Among them are miRNAs with a known association with brain cancer. MiR-216 (up-regulated) is the one with the highest difference in treatment versus control. In general, miR-216 has so far only been briefly mentioned in supplementary tables of two publications about miRNAs found in GBM samples. However, no publications regarding its regulatory targets in GBM cells could be identified. Its function in GBM is completely unknown. Our sequencing data is the first hint at its connection with susceptibility to immunotherapy in GBM. Further experiments will clarify its regulatory targets in GBM tissue and its specific biological role.

friedrich.erhart@ccri.at

P16 RGS16, A PUTATIVE EXTRACELLULAR IMMUNE CHECKPOINT

Bastien A. Huber[1], Angela Halfmann[1], Klara Soukup[1], Thomas Felzmann[2], Alexander M. Dohnal[1]

[1]Children's Cancer Research Institute Vienna, St. Anna Kinderkrebsforschung Wien
[2]Activartis Biotech, Wien

Dendritic Cells (DCs) are important in immune regulation to prevent excessive effector T cell functions after lipopolysaccharide (LPS) encounter. Based on human data from monocyte-derived DCs we detected a potential target to drive DCs into their regulatory phenotype, the regulator of G-protein signaling protein 16 (RGS16). Except its function as a transduction inhibitor in G-Protein coupled receptor signaling, not much is known about RGS16 in DCs. DCs silenced for RGS16 expression were able to induce higher proliferation in $CD8^+$ T-cell populations compared to control DCs. RGS16 protein is not only present in DCs, we also found it in the supernatant and furthermore in small vesicle isolations of LPS-stimulated bone-marrow-derived DCs from mice. As we are interested in immune-regulatory mechanisms in the tumor microenvironment we inoculated B16F10 melanoma into wild-type mice. In tumor-resident DCs mRNA expression was 2-fold lower than in the respective splenic DCs. However in the tumor cells, RGS16 expression was almost 10-fold higher. Further investigation will determine the role of RGS16 in immune regulation and its impact in the tumor microenvironment.

bastien.huber@gmx.at

P17 DIFFERENTIATION OF URINE-DERIVED MESENCHYMAL STEM CELLS FOR SKIN REGENERATION

Viktoria Kantner[1], Markus Schosserer[1,5], Rita Reynoso[1], Verena Wally[2], Bogdan Jug[1], Sylvia Weilner[3,5], Ivana Buric[1], Johann W. Bauer[2], Regina Grillari-Voglauer[1,3,5], Johannes Grillari[1,3,4,5]

[1] Department of Biotechnology, BOKU - University of Natural Resources and Life Sciences Vienna, A-1190 Vienna, Austria
[2] Division of Experimental Dermatology and EB House Austria, Department of Dermatology, Paracelsus Medical University Salzburg, A-5020 Salzburg, Austria
[3] Evercyte GmbH, Muthgasse 18, A-1190 Vienna, Austria
[4] Christian Doppler-Laboratory on Biotechnology of Skin Aging, A-1190 Vienna, Austria

[5] Austrian Cluster for Tissue Regeneration, Vienna, Austria

The currently available methods for skin regeneration are for many reasons not always satisfying. Especially for the rare genetic skin disease Epidermolysis bullosa (EB), there is much need for novel therapies. Due to a mutation in anchoring proteins of skin cells in these patients, even mild mechanical friction causes the skin to blister. Novel therapeutical methods, like regenerative medicine and gene therapy, depend on a supply of epidermal stem cells. Therefore, Urine is an easily accessible, non-invasive and unlimited source for mesenchymal stem cells. Since autologous cultures containing genetically modified epidermal stem cells showed promising results for the reconstruction of skin of EB patients, autologous, urine-derived mesenchymal stem cells (USC), expanded and differentiated in vitro, could even improve this therapeutic approach. We could already show that these USCs differentiate readily into the osteogenic and chondrogenic lineage. The next step will be to (trans-) differentiate these cells into dermal and epidermal cells and subsequently generate artificial skin from these USCs, in order to regenerate healthy skin.

viktoria.kantner@boku.ac.at

P18 LAB ON A CHIP: AUTOMATION AND MINIATURIZATION OF DENDRITIC CELL CULTURES

Olga Kuten[†], Mario Rothbauer[†], Sneha Schneider[*], Caterina Barresi[*], Thomas Felzmann[*] and Peter Ertl[†]

[†]BioSensor Technologies, AIT Austrian Institute of Technology GmbH, Vienna Austria
*Activartis Biotech GmbH, Vienna Austria

Dendritic cell (DC)-based cancer vaccines represent a fully individualized somatic cell therapy. Activartis' phase II clinical study was testing a DC-based cancer immune therapy vaccine (AV0113) in individuals with glioblastoma multiforme. For each patient, independent manufacturing and quality control (QC) of AV0113 was required since patient-derived tumor tissue and white blood cells were used for the manufacturing of AV0113. Automation is the most straightforward strategy for assuring a maximum of reproducibility but also addresses the need for minimizing labor costs. Although QC compared to manufacturing is the more labor intensive procedure, so far no concepts exist that allow the automation of QC. A lab-on-a-chip (LOC) micro device integrates several laboratory functions on a single chip of only millimeters to a few square centimeters in size. Here we use a computer controlled and automated microanalysis platform to culture DCs on a cell-chip system consisting of embedded optical and electrical biosensors as well as integrated fluid handling systems. After 48 hours culture the DCs were still viable (78%) and we could also detect the expression of functionally important DC membrane molecules such as CD86, CD80, CD83, MHC-I and –II by flow cytometry. On a chip cultured DCs also secrete IL-12, a key cytokine that supports cytolytic immunity. Future plans include further research on the creation of advanced QC measurements for cellular cancer immune therapy products. These preliminary results reveal that the LOC technology has the potential to provide the next generation of cell analysis tools under controlled and reproducible measurement conditions.

olga.qte@gmail.com

P19 GEN EXPRESSION CHANGES IN ISCHEMIC PRECONDITIONING OF THE PORCINE HEART

Dominika Macejovska, Katrin Zlabinger, Abelina Zimba, David Lorant, Georg Goliasch, Dietmar Pils, Hendrik Jan Ankersmit, Maximilian Y. Emmert, Simon P. Hoerstrup, Gerald Maurer, Peter Ferdinandy, Mariann Gyöngyösi, Noemi Pavo

Department of Cardiology and Surgery, Medical University of Vienna, Austria, University and University Hospital of Zürich, Switzerland, Department of Pharmacology, Semmelweis University of Budapest, Hungary

Pre-infarction angina mimicking ischaemic preconditioning (IPC) operates as cardioprotection (CP) reducing infarct size. We analyzed the effects of brief repetitive episodes of myocardial ischemia/reperfusion (I/R) without subsequent infarction in a porcine model using next generation sequencing (NGS) and visualized IPC gene expression in time lapse as a 3D model. Domestic pigs (n=12) underwent repetitive cycles (3x) of 10min I/R by percutaneous intracoronary balloon inflation/deflation of the mid left anterior descending (LAD) coronary artery. Animals were randomized and hearts were explanted at 5h (n=6) and 24h (n=6). Animals with sham intervention (n=8) served as controls. Gene expression profiling using NGS of the basal, mid and apical segments of the LAD-region was performed. Expression maps of target genes were assessed by real-time PCR and visualized as 2D and 3D model of the entire heart. Signaling pathway impact analyses (SPIA) and ingenuity analysis (IPA) revealed significantly over-or down-regulated genes in a time- and spatial-dependency. Activated Ca-signaling pathway dominated the 5h gene deregulation, while genes in immunmodulatory, complement and coagulation cascade as well as extracellular matrix interaction pathways were involved in 24h post IPC stimulus. Predicted protein-protein interaction discovered large cluster of multiple strongly upregulated genes contributing to survival/apoptosis signaling both at 5h and at 24h post IPC. The temporal and spatial genetic mapping of CP in a translational model of IPC enables the differentiation of the genetic networks of the early and second window of protection, and identifies memory elements of genetic networks and pathways for targeted drug research against ischemic myocardial injury.

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dominika.macejovska@meduniwien.ac.at

P20 BIOMIMETIC VASCULAR SYSTEM AS NOVEL TOOL FOR NANOPARTICLE UPTAKE RESEARCH

Isabel Olmos Calvo[1,2], Verena Chawart[3], Christian Jungreuthmayer[3,4], Mario Rothbauer[1], Carina Huber-Gries[5], Peter Ertl[1]

[1] Austrian Institute of Technology, Vienna, Austria
[2] Medical University, Department of Internal Medicine III, Division of Rheumatology, Vienna, Austria
[3] University of Life Sciences Vienna, Department of Biotechnology, Vienna, Austria
[4] Austrian Center of Industrial Biotechnology, Metabolic Modeling Group, Vienna, Austria
[5] University of Applied Sciences Technikum Wien, Vienna, Austria

The vascular system is a complex network composed of a variety or arteries, capillaries and veins that transports nutrient, gases and waste products throughout the body. The size

distribution (dia. 8 µm to 25 mm) and composition of blood vessels varies significantly according to their function and location, which results in different blood flow velocities and pressure profiles throughout the body. To investigate the influence of flow velocity on nanoparticle-endothelium interactions, we have developed a biomimetic vascular system to assess active uptake of 250 nm polystyrene particles in the presence of increasing shear force conditions. Active uptake of nanoparticles was confirmed by blocking Clathrin-pathway of HUVECs and nanoparticle behavior under flow conditions was also modelled using computational fluid dynamic (CFD) simulations. Results of our nanoparticle uptake research revealed a distinct dependence between active uptake and flow rate, since the ability to incorporate nanoparticle by HUVEC cells decreased dramatically at elevated flow rates (above 5 dyne/cm^2).This means that uptake, biodistribution and accumulation of nanoparticles within the vascular system is highly dependent on location, type of blood vessel and applied blood flow conditions.

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is.olmos.ca@gmail.com

P21 ADIPOSE-DERIVED STEM CELL SECRETOME AMELIORATES INFLAMMATION AND ORGAN FAILURE AFTER HEMORRHAGE & TRAUMA IN RATS

Carina Penzenstadler, Mostafa Ashmwe, Arian Bahrami, Anton Klotz, Mohammad Jafarmadar, Asmita Banerjee, Susanne Wolbank, Heinz Redl and Soheyl Bahrami

Ludwig Boltzmann Institute for Experimental and Clinical Traumatology & AUVA Research Center, Vienna, Austria

It has been speculated that the repeatedly reported beneficial effects of stem cells (SCs) may be attributed to the mediators released by the SCs. We tested the therapeutic effect of a rat adipose-derived SCs secretome (AdSC-Secretome) preparation in a rat hemorrhagic traumatic shock (HTS) model followed by reperfusion.
Rats were subjected to HTS (mean arterial pressure 30-35 mmHg till decompensation) and a resuscitation protocol that mimicks prehospital restrictive reperfusion (30 ml/kg/h, MAP maintained at 50-55 mmHg for 40 min) followed by an adequate reperfusion phase (75ml/kg/h over 60 min, MAP to baseline). 20 minutes after the onset of reperfusion, animals (n=7/group) received an intravenous bolus of either the secretome produced by 2x10^6 AdSC over 24 hrs or vehicle. Blood samples were obtained at baseline, end of resuscitation (EOR), 24 and 48 hrs after shock.
Cell injury markers creatine kinase and lactate dehydrogenase peaked at 24 hrs with no difference between AdSC-Secretome and control groups (27223±9306 vs 17375±7577 U/L and 11657±2057 vs 25216±8039 U/L, respectively) returning to baseline at 48 hrs. HTS-induced inflammatory response represented by IL-6 was markedly inhibited in the AdSC-Secretome treated group at 24 (p=0.042) and 48 hrs (p=0.061). HTS-induced liver dysfunction determined by plasma alanine aminotransferases was significantly ameliorated by AdSC-Secretome at EOR (1247±286.6 vs 2774±463 U/L, p=0.026) and at 24 hrs (2417±436 vs 5527±1068 U/L, p=0.020).
Systemic administration of AdSC-Secretome during reperfusion may ameliorate the HTS-induced inflammation and/or organ failure in rats.

carina.penzenstadler@trauma.lbg.ac.at

P22 ASSESSMENT OF PROGNOSTIC VALUE OF TCR Vβ REPERTOIRE IN PERIPHERAL BLOOD OF PATIENTS ENROLLED IN CANCER-IMMUNOTHERAPY PHASE II CLINICAL TRIAL IN GLIOBLASTOMA.

René Reitermaier[1], Katrin Fischuber[1], Simone Klingenbrunner[1], Friedrich Erhart[2], Thomas Felzmann[1], and Carmen Visus[1]

[1]Activartis Biotech GmbH, [2]St. Anna Children's Cancer Research Institute; Vienna

Glioblastoma multiforme (GBM) is the most common and aggressive primary malignant astrocytic brain tumor. Standard treatment includes surgery, radiation therapy and chemotherapy with temozolomide. In this study we assessed the T cell receptor β chain variable region (TCR Vβ) repertoire by flow cytometry before and after cancer immune therapy (CIT). All patients were in a randomized, open- label, 2- arm, phase II clinical study receiving dendritic cells (DCs) cancer vaccine (AV01139) developed at Activartis Biotech GmbH. The analysis was performed on peripheral blood T cells obtained from 21 GBM patients and 6 healthy donors, using antibodies against 24 TCR Vβ families. Changes on the TCRVβ from different T cell subsets like CD3+/CD8+/CD45RO/CD4+ as well as regulatory T cells before and after CIT could be observed. Interestingly the overall survival (OS) correlates with a high expansion of specific TCR Vβ families.
Further, we used next generation deep sequencing to measure clonality of individual rearranged T cell receptor beta genes from tumor samples of 12 GBM patients. It seems that the value of clonality (accumulation of certain T cell clones at the tumor site) distinguishes between long and short term survivors.
These data suggest that TCR Vβ families' expansion in the peripheral blood and the clonality at the tumor site could be considered as prognostic factors in GBM patients vaccinated with DC-CIT.

rene.reitermaier@activartis.com

P23 IN VIVO LONG-TERM SERIAL TRACKING OF LIVING MESENCHYMAL STEM CELLS SEEDED ON BIOENGINEERED ARTIFICIAL PULMONARY VALVE SCAFFOLD IN SHEEPS

Katrin Zlabinger, Dominika Macejovska, Imre J Pavo, Hendrik Spriersterbach, Laszlo Balkay, Maximilian Y. Emmert, Rita Garamvölgyi, Joseph C. Wu, Simon P. Hoerstrup, Mariann Gyöngyösi

Department of Cardiology, Medical University of Vienna, Austria, Deutsches Herzzentrum Berlin, Germany, University of Debrecen, Hungary, University and University Hospital of Zürich, Switzerland, University of Kaposvar, Hungary, Stanford University School of Medcine, USA

Tissue engineered heart valve (TEHV) with adaptive self-growth attracts increasing attention in paediatric cardiology. We have tracked the survival of the mesenchymal stem cells (MSC) seeded on the TEHV via serial in-vivo non-invasive PET-CT imaging. Decellularized TEHV scaffolds were seeded with MSCs transfected with PET-reporter gene (PET-MSCs). Static cultivation of scaffold with the PET-MSCs led to successful ingrowth of the PET-MSCs into the scaffolds, with an average cell number of 2x10^6/TEHV. The TEHVs were implanted percutaneously into the pulmonary position of sheeps. PET images were acquired with a 10-min scan after injection of 10 MBq/kg [^{18}F]-FHBG 3h, 6h, 24h and 3 weeks after valve

implantations. For quantitation of survived cells in the TEHV, vials containing 5x10^4, 2x10^5 and 4x10^5 PET-MSCs were mixed with PET tracer for 1h. After wash-out of the non-bound tracer, the vials were in vitro scanned with PET-CT, which showed accumulation of the seeded cells at the base of the leaflets. In vivo PET-CT images of sheeps 3h after implantation of the TEHV demonstrated a clear signal of PET-MSCs, with a mean estimated number of living cells of 1.2x10^6 with no meaningful decrease of cell number at 6h or 24h. At 3 weeks PET-CT image showed living PET-MSCs in the TEHV (estimated cell number 0.6x10^6). Immunofluorescense at 1-month showed alpha-smooth muscle actin positivity on valve surface of TEHV. This is the first report on serial non-invasive in vivo tracking of long-term survival of MSCs seeded onto TEHVs and percutaneously implanted into the pulmonary position of sheeps.

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katrin.zlabinger@meduniwien.ac.at

CELLTOOL GMBH:

LABEL-FREE AND NON-DESTRUCTIVE: A NOVEL TECHNOLOGY TO ANALYZE CELL BEHAVIOR

Karin Schütze[1], Tanja Buchacher[2], Viktoria Weber[2], Eleni Oberbauer[3], Susanne Wolbank[3]

[1] CellTool GmbH, Bernried, Germany
[2] Christian Doppler Laboratory for Innovative Therapy Approaches in Sepsis, Danube University Krems, Krems, Austria
[3] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Linz, Austria

We present Raman spectroscopy as a new tool for gentle and non-invasive analysis in two independent examples focusing on stem cell differentiation and identification of pathogen infected cells.
In the first application Raman was used to investigate the successful differentiation of fibroblasts to chondrocytes. After a cultivation period of 7 days the cells were analyzed by Raman and we found that 60% of the cells were differentiated to chondrocytes showing a higher level of collagen.
In the second application Raman was used to study the infection of human blood monocytes with the intracellular pathogen *C. pneumoniae*. It revealed different molecular fingerprints for infected and non-infected monocytes, which were mainly due to changes in lipid and fatty acid content.
These two examples show that Raman spectroscopy is a fast and non-invasive technology to identify, characterize and monitor cell behavior also providing highly specific molecular information. The method could even be applied in-line during ongoing cell culture as cells remain vital and unaffected.

k.schuetze@celltool.de