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Abstract Book

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Michael Comer - PACT Chairman

For a number of years now the “cell” has been used as a vehicle or “factory” to manufacture so called “biopharmaceuticals”. Apart from the proven medical benefit these compounds also share a multi-billion euro annual turnover. Products like Erythropoietin (EPO), Enbrel, Betaseron, Herceptin and many other proteins or antibodies are now firmly embedded in the repertoire of clinicians and are used on a daily basis to combat serious conditions or diseases. Since the cell was recognized a few centuries ago, as the basic biological unit of all living matter, the “fascination” and the intriguing possibility to enlist the cell itself as a therapeutic agent was made much more a reality by the relatively recent realization of pluri-potency of so-called “stem” and progenitor cells. These cells can be cultivated in-vitro and “persuaded” to use their capability to differentiate into the cell types of various tissues and organs of the body. So a new potential for “regenerative” medicine has also become a potentially powerful instrument in the available arsenal to the physician and surgeon to combat diseases or conditions that are often rare in nature but nonetheless just as overwhelming.

In Austria and Vienna the knowledge, expertise and experience in the use of the cell as a “factory” is well established also, there is a long and profound traditional capability and presence in research and developmental medicine. It would seem obvious therefore, to harness and combine these attributes. This concept was the driving force in establishing an alliance between the major Universities and Institutes in Austria in order to take advantage and harvest the benefits of the potential synergies anticipated from creating an infrastructure (platform) that will accommodate a larger critical mass of interest, expertise and endeavours in the area of cell based therapies.

The Platform for Advanced Cellular Therapies, “PACT” is an alliance of several key players in research, medicine, veterinary medicine and the life sciences. The vision of PACT is summarized in the “Memorandum of Understanding” signed in December 1012: Initially the Foundation Partners will utilize their combined effort in obtaining funding with the purpose to support fundamental and pre-clinical research in the life sciences and medicine. Furthermore, in a second phase, they plan to build a ‘Centre’ focused on personalized cell based therapies and advanced medical treatments. The proposed “Cell based Therapies Centre” will provide “state of the art” facilities for treatments for patients, accessible to the Foundation Partners, their collaborators, agencies or other relevant organizations and/or charitable institutions”.

This “Foundation Symposium” is dedicated to establishing a venue for key players in medical applications, eminent scientists and regulatory professionals in dealing with the status quo and for the possible technological prospects for conceivably new therapeutic approaches. The following Abstracts will document where we are and what is feasible in the foreseeable future.

ADULT MESENCHYMAL STEM CELLS: THE NEW MEDICINE

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Marrow derived adult Mesenchymal Stem Cells (MSCs) can be isolated and culture expanded. Although these cells are capable of differentiating into lineages that result in the fabrication of bone, cartilage, muscle, marrow stroma, tendon/ligament, fat and other connective tissues, MSCs have recently been shown to be intrinsically therapeutic. Such culture expanded adult/MSCs are immuno-modulatory especially in muting T-cells and, thus, both autologous and allogeneic MSCs have been used to mute or cure graft-versus-host-disease, Crohn's disease, AMI, MS, diabetes, and are now being tested in over 384 clinical trials for a huge spectrum of diseases (clinicaltrials.gov). Furthermore, these MSCs set-up a regenerative micro-environment which is anti-apoptotic, anti-scarring, mitotic for tissue intrinsic progenitors and angiogenic. These immuno and trophic activities result from the secretion of powerful bioactive molecules that, in combination, support localized host controlled regenerative events.
The MSCs reside in every tissue of the body and function as perivascular cells (pericytes) until a focal injury occurs. At sites of injury, the pericyte is released becomes an MSC, becomes activated and functions to provide molecular assistance in activities leading to tissue regeneration. Although it is proposed that all MSCs are pericytes and have common capacities, it is expected that MSCs from different tissues location or anatomical sites of injury will not be equivalent. Thus, adipose-derived and marrow-derived MSCs naturally reside as pericytes and have different functional capacities. The fact that uncultured, freshly isolated autologous "stromal vascular fraction (SVF)" from fat has been shown to be therapeutically effective strongly argues that the MSCs in the SVF are a potent multi-drug and site-specific delivery vehicle. This full thesis that adult MSCs are potent therapeutic agents is the theme of this lecture.

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INNOVATING PRECLINICAL DRUG DISCOVERY AND HUMAN CELL THERAPY

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There has been significant interest in the therapeutic and scientific potential of stem cells since reconstitution of the haematopoietic system was first realized by bone marrow transplantation in the 1960s. The isolation of tissue-specific, multipotent stem cells from adult organs and the derivation of pluripotent human embryonic stem cells offer the potential for regeneration of a number of different tissues and organs susceptible to age-related degenerative conditions and traumatic injury. In the not-too-distant future, it will be possible to repair heart tissue damaged by myocardial infarction, to replace neuronal cells

lost in Parkinson's and Alzheimer's diseases, to transplant new insulin producing cells for diabetics and myelinating cells for individuals afflicted with multiple sclerosis, and to replace bone and cartilage lost through aging and inflammatory disease. In addition, the generation of specific populations of defined subtypes of human cells has tremendous potential to revolutionize the fields of drug discovery and investigation into the cellular bases of human disease. The newly emerging field of Regenerative Medicine will fundamentally alter clinical medicine and significantly influence our perceptions of aging, health and disease, with a myriad of consequences for society at large.

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DEVELOPMENT AND VALIDATION OF AN IMMUNE GENE EXPRESSION-BASED BIOMARKER THAT PREDICTS RESPONSE TO DNA-DAMAGING CHEMOTHERAPY

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It is estimated that 25% of breast cancers are defective for the Fanconi anemia/BRCA (FA/BRCA) DNA-damage response pathway resulting in sensitivity to DNA-damaging agents. Microarray- based transcriptional analysis of 107 breast tumors enriched for BRCA1/2 mutations identified a molecular subgroup characterized by loss of the FA/BRCA pathway. This molecular group was defined by activation of a specific type of interferon signaling. A 44-transcript DNA damage response deficient (DDRD) assay was developed to identify this subgroup in formalin- fixed paraffin-embedded (FFPE) samples. In a cohort of 203 patients, the assay predicted response to neoadjuvant anthra-cycline/ cyclophosphamide-based chemotherapy (odds ratio 3.96 (95% CI:1.67-9.41)). In a cohort of 114 node-negative breast cancer patients treated with adjuvant 5-fluorouracil, epirubicin and cyclophosphamide, the assay predicted 5-year relapse free survival (hazard ratio 0.37 (95% CI:0.15-0.88)). The assay was not prognostic in 664 patients who did not receive DNA-damaging chemotherapy. These data indicate that the DDRD assay is an independent predictor of outcome in early breast cancer following DNA-damaging chemotherapy. Importantly, DDRD assay positive tumours display a specific type of lymphocytic infiltration that may allow the use of immune based therapies in the future.

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ENGINEERING OF VASCULARIZED HUMAN TISSUES

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Tissue engineering is an emerging multidisciplinary field involving biology, medicine and engineering. Depending to the biomedical application, our group uses cell lines or primary (stem) cells combined with biological-based matrices that are specifically adapted to mimic the In-vivo microenvironment of selected tissues. Thus, we have previously established a process for manufacturing collagen matrices with a persisting blood circulation system (BioVaSc® technology). The matrices are based on decellularized organs such as porcine intestine, with intact blood vessel structures allowing us to generate and maintain 3D tissue structures. Based on the BioVaSc® technology, In-vitro models of various organ systems such as the human gastrointestinal, respiratory tracts or skin have been established and functionally tested. Further, it has been shown that mechanical parameters such as media flow, rotation, tension, extension or pulsation stress are critical for the development of 3D bioartificial tissues.
Our 3D tissue equivalents allow us to study different steps of tumor development, drug absorption or infections without the need for testings in animals.

Supported by the program »Bayern FIT« of the Bavarian State Government.

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A BIOREACTOR-BASED 3D CULTURE SYSTEM FOR SKELETAL MUSCLE ENGINEERING IN FIBRIN SCAFFOLDS

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Fibrin is a versatile biomaterial that has been used extensively in a variety of tissue engineering applications. We have developed a 3D *in vitro* culture system using a bioreactor (MagneTissue) which allows mechanical stimulation of myoblasts embedded in a ring shaped fibrin scaffold by application of strain. Using this system we sought to analyze the effects of mechanical strain on cell alignment and distribution, viability and expression of myogenic markers.

We demonstrate that over a culture period of at least 9 days the cells remain viable and partially differentiate into myotubes. Additionally, application of mechanical strain leads to parallel cell alignment and may also facilitate nutrient diffusion within the scaffold, demonstrated by I mproved cell distribution. Furthermore, myogenic differentiation is confirmed by transcriptional up-regulation of myogenic markers and by histological analysis. Our findings demonstrate the feasibility of fibrin in 3D skeletal muscle engineering applications. In addition, the use of this 3D culture system may provide a powerful tool to study myogenic differentiation, muscle physiology and disease. In this respect, further optimization of different strain application patterns might increase the degree of myogenic differentiation and functionality, with the long-term goal of providing patients with fully functional engineered skeletal muscle transplants.

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CHALLENGES OF MANUFACTURING OF ATMP'S FOR CLINICAL TRIALS

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Fraunhofer IZI operates, with more than 60 employees, two state-of-the-art clean room facilities for aseptic manufacturing of cell-based medicinal products for clinical trials. Due to the complexity of the manufacturing processes and quality controls for cell therapies there are many challenges and potential pitfalls on the way to bringing such a product into the clinic. This starts with the enormous and mostly underestimated costs, which are related to the - mainly manual - labor-intensive manufacturing processes and the necessity of a high grade clean room environment. Other underestimated issues are the long timelines and intensive efforts involved in obtaining product-specific GMP-manufacturing authorization and the regulatory approval for a clinical trial. During process transfer or process development various setbacks could occur regarding selection of appropriate raw materials and the subsequent supplier qualification. This is of particular importance for materials of human, animal or recombinant origin that must comply especially with viral and TSE safety regulations. The high process complexity, the variations of the cellular starting material (especially in autologous settings) as well as the variety of different cell therapy products is often related to difficulties in regard to a robust process validation. The same issue occurs for the validation of the highly sophisticated analytical methods, for which standard ICH guidelines or Pharm. Eur. are often not directly applicable. All these issues need special consideration and strategies that require frequent ongoing consultation and interaction with the regulators.

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UPDATE ON GLOBAL REGULATION OF CELL THERAPY: MEETING REGULATORY EXPECTATIONS FOR THE CMC ASPECTS OF CELL THERAPY PRODUCTS

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Presentation will review regulatory expectations from the EU (European Medicines Agency), US (Food and Drug Administration, Centre for Biologics Evaluation & Research) and Japan (Pharmaceuticals and Medical Devices Agency) on the CMC (Chemistry, Manufacturing and Control) aspects of cell therapy product development. These regulatory expectations will be illustrated with particular emphasis on virus and microbial risk mitigation strategies for raw materials, cell banks and expanded cell therapy products.

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REGULATORY UP-DATE AND CURRENT ISSUES ON ATMPS

Ilona Reischl, PhD

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The presentation will focus on current approaches to the regulation of ATMPs in Europe and touch upon the impact of the future clinical trials regulation on ATMPs. The latest activities and work plan of the EMA Committee for Advanced Therapies and the personal experience of the presenter in the assessment of ATMPs will be summarized.

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THE PHARMACEUTICAL DILEMMA OF CELL THERAPIES

Matthew Moran

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The Pharmaceutical industry has evolved from small molecule to large molecule as it strives to solve the many therapeutic challenges faced by man. Cellular biology presents the next phase to the industry challenged with addressing those diseases that have resisted research efforts so far. Can this research be converted into commercialization products that can help patients?

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MEETING REGULATORY REQUIREMENTS IN THE FIELD OF CELL AND TISSUE BANKING – A PRACTICAL VIEW

Simone Hennerbichler

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As advances in biomedical research led to human tissues and cells being used in an increasing variety of new indications and patients benefiting from these new therapies, quality and safety of these cell and tissue products need to be assured.
EU legislation consists of four directives (2004/23/EC, 2006/17/EC, 2006/86/EC, 2012/39/EC) which should be implemented in national legislation in all member states. In 2008 the Austrian Tissue Safety Act (Gewebesicherheitsgesetz BGBl. I Nr. 49/2008), based on these EC-directives, came into force.
This presentation will share the experiences as a tissue establishment focusing on the following main topics:

- procurement and transport
- processing and storage
- premises and equipment
- documentation and quality management
- quality control, release and distribution

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NASAL CHONDROCYTES FOR CARTILAGE REPAIR: SCIENTIFIC BASIS AND CLINICAL TRANSLATION

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In embryonic models and stem-cell systems, mesenchymal cells derived from the neuroectoderm can be distinguished from mesoderm-derived cells by their 'Hox-negative' profile, a phenotype associated with enhanced tissue regeneration capacity. We investigated whether developmental origin and Hox-negativity are related with self-renewal and environmental plasticity also in differentiated cells from adult individuals. Using hyaline cartilage as a model we show that adult human neuroectoderm-derived nasal chondrocytes (NC) can be constitutively distinguished from mesoderm-derived articular chondrocytes (AC) by the lack of expression of specific HOX genes. In contrast to AC, NC can be extensively cultured and serially cloned while conserving the ability to form cartilage tissue. NC can also stably adopt a Hox-positive profile typical of AC upon implantation into articular cartilage defects and directly contribute to their repair. Hence, HOX-negative differentiated neuroectoderm cells in adult individuals retain a previously unrecognized self-renewal capacity and degree of plasticity, typical of embryonic- or stem-cell systems. In the field of cartilage tissue engineering, the results reinforce previous findings on the more reproducible re-differentiation and cartilage forming capacity of human NC, not exposed to degenerative processes, as compared to AC from age-matched donors or in an isogenic setting. Based on the here presented findings, combined with the previous demonstration that NC favorably respond to mechanical forces typical of joint loading and can recover from inflammatory processes, the Basel University Hospital has started a first-in-man phase I clinical study to treat traumatic knee joint defects with cartilaginous constructs generated from autologous NC (http://clinicaltrials.gov: NCT01605201; 7th patient treated).

Supported by Swiss National Science Foundation, Deutsche Arthrose Hilfe

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DIFFERENTIAL GENE EXPRESSION PROFILING OF THE INTERMEDIATE AND OUTER INTERZONE LAYERS: INVOLVEMENT OF INFLAMMATORY PATHWAYS IN JOINT DEVELOPMENT

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Formation of stable articular cartilage is the ultimate goal and main challenge of cartilage tissue engineering. Since permanent articular cartilage is only generated during embryonic development, a better understanding of embryonic joint formation may provide essential clues for cartilage tissue engineering. Embryonic joint formation first becomes morphologically evident when the interzone forms at each prospective joint site. The interzone, consisting of 2 outer and an intermediate layer, constitutes a distinct cohort of progenitor cells responsible for the formation of the majority of joint tissues including articular cartilage. To date layer selective analysis of interzone cells has not been reported and therefore significant controversy exists regarding the exact role of the different interzone layers in joint development.
In this study, microarray-based differential gene expression analysis of laser microdissected murine intermediate and outer interzone layers was performed. Pathway analysis revealed a high representation of inflammatory pathways and functions in the differential gene expression profile specifically in the intermediate layer. The 25 genes most highly differentially up-regulated in the intermediate interzone also have an emphasis on inflammatory pathways, while in the outer interzone they play roles in cartilage matrix formation, chondrocyte differentiation (hypertrophy) and endochondral ossification. Further studies are needed to look into the specific role of the inflammatory pathways in the interzone and to examine whether the differentially up-regulated genes relevant to chondrocyte hypertrophy and endochondral ossification in the outer interzone reflect its cell fate.

Supported by Science Foundation Ireland

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STRATEGIES FOR WHOLE TOOTH TISSUE ENGINEERING.

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Tooth development is an exceptionally complex process, during which an initial soft tissue tooth organ eventually gives rise to erupted, functional teeth composed of highly mineralized dentin, enamel and cementum tissues. Our published report in 2002 demonstrated, for the first time, the possibility of using Tissue Engineering approaches to bioengineer functional teeth. Since that time, we have been working to devise methods for optimized dental tissue engineering with the goal of created biological based, fully functional, human replacement teeth. Here we review the advantages and disadvantages of a variety of biodegradable scaffold materials and designs for dental tissue and whole tooth tissue engineering applications. We describe novel three dimensional in vitro tooth

models, and in vivo rat and porcine implantation models, to facilitate our goal to generate functional biological tooth substitutes in humans.

This research is supported by NIH/NIDCR/NIBIB R01 DE016132 (PCY), and NIH/NIDCR R01 DE018043 (PCY) and R01 DE16962 (PCY).

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VETERINARY REGENERATIVE MEDICINE – CLINICAL PATIENTS AS POTENTIAL MODELS FOR NATURALLY OCCURRING DISEASE

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When it comes to translating research from bench to bedside many of the pioneering innovations are achieved by cooperating teams of natural scientists, human medical and veterinary medical scientists. The symbiosis is particularly apparent in the rapidly growing field of regenerative medicine. The veterinary profession has an important role to play in the translational process offering the missing link between basic science and human clinical applications. Many naturally occurring diseases encountered in humans also pose a problem in veterinary patients. These diseases certainly raise the interest in regenerative medical treatments on the veterinary side but at the same time offer the perfect model for human patients, better than artificially created diseases in lab animals which do not properly reflect the clinical situation in humans. Tight cooperation between basic sciences, human medicine and veterinary medicine would therefore not only be beneficial for veterinary patients but would drive the field of regenerative medicine forward for the benefit of both human and veterinary patients. The purpose of this presentation is to give an overview on the current clinical state of the art in veterinary regenerative medicine and potential animal models for diseases with similar pathology and aetiopathogenesis as in humans.

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LIGAMENT REGENERATION USING SILK SCAFFOLDS AND ADIPOSE DERIVED STROMA CELLS

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The regeneration of tendon/ligament tissue relies on scaffold devices that provide mechanical stability, while providing the optimal environment for the tissue to grow. We have developed a novel, silk-fiber based degradable scaffold device that is used for anterior cruciate ligament (ACL) regeneration and present the first results of an in-vivo large animal study. Raw *bombyx mori* silk was manufactured in a wire rope design, braided in a certain pattern that should allow cell seeding and tissue ingrowth. Biomechanical testing was performed to compare the scaffold with the native ACL. Using a bioreactor system, we tested the regenerative capability, in terms of cell adhesion and tissue growth, under mimicking the natural biochemical/mechanical conditions. An in-vivo, large animal (sheep) study was conducted to test the structure under true biological conditions. In this study, plain scaffolds where compared to scaffolds loaded with Adipose-derived stromal cells (ASCs). Micro-CT, MRI and histological work-up were performed 6 and 12 months after implantation. We were able to manufacture a scaffold matching the mechanical properties

of the native ACL. The in-vivo experiments revealed the formation of new ligament tissue in parallel with the degradation of the silk-based scaffold device independently of added ASCs. With the novel scaffold design, we were able to mimick the mechanical properties of the ACL, providing stable joint conditions during the regeneration process. Histologically, we saw a considerable reduction of scaffold fibers, already after six months. Long-term follow up is needed to see if the scaffold will be completely replaced by the regenerated ACL.

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ULTRASOUND-MEDIATED GENE TRANSFER IN FIBRIN BASED MATRICES: POTENTIAL USE IN BONE TISSUE REGENERATION.

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Tissue regenerative gene therapies rely on controlled transgene delivery *in situ* and should be compatible with stem cells and biomaterial application. Sonoporation enables minimally invasive, spatially controlled non-viral gene delivery *in vivo*. Since this method relies on proximity of therapeutic DNA, microbubble contrast agents and target cells for efficient delivery, it was the aim of the study to develop a novel 3D sonoporation approach using all components embedded within a matrix.

A protocol for Matrix-assisted Sonoporation (MAS™) was developed and gene transfer efficacy was monitored using luciferase in C2C12 cells *in vitro*, followed by osteoinduction using a BMP2/7 co-expression plasmid. Subsequently, MAS was applied *in vivo* in an ectopic nude mouse model for luciferase monitoring of gene delivery and BMP2/7 induced bone formation.
In vitro data indicated successful gene delivery within matrices and expression for up to one week. Alkaline phosphatase assays demonstrated osteoinduction by MAS-based delivery of BMP2/7. *In vivo* application showed effective gene delivery within matrices and expression for 14 days. Activation with ultrasound displayed increased gene expression when compared to matrix implantation-mediated passive gene delivery, demonstrating the feasibility of the ultrasound trigger. Evaluation of MAS-mediated ectopic bone formation demonstrated efficient bone formation at ectopic sites, with higher bone volumes and frequency compared to standard sonoporative gene transfer.
We conclude from these studies, that this approach enables spatially controlled, minimally invasive non-viral gene delivery to target cells in 3D within matrices leading to improved bone induction and can be harnessed for tissue engineering applications in the future.

Supported by the EUROSTARS Project UGen.

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LEVELS OF CIRCULATING VESICULAR MICRORNA-31 INCREASE WITH AGE AS WELL AS IN THE CASE OF OSTEOPOROSIS AND INHIBIT OSTEOGENIC DIFFERENTIATION CAPACITY OF MESENCHYMAL STEM CELLS

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Aging is a complex process resulting in the decline of physiologic functions due to accumulation of damage in cells and tissues. Mesenchymal stem cells (MSCs) counteract this decline but their regenerative power decreases with age. In particular osteogenic differentiation potential of MSCs has been shown to decrease with age thereby contributing to slowed down bone formation and osteoporosis. While much is known about cellular aging of MSCs, little is known about factors of the aged systemic environment influencing their functionality. While searching for extrinsic factors that influence osteogenesis of MSCs extracellular vesicles (EVs) were found. Exposition of MSCs to EVs secreted by senescent endothelial cells (senECs), which were shown to accumulate with age in vivo, or isolated

from plasma of human elderly donors failed to induce osteogenesis compared to MSCs incubated with secreted EVs of young endothelial cells or plasma derived EVs of young donors. We attributed the age-dependent impairment of osteogenesis by EVs to vesicular miR-31 which was shown to be enriched within EVs of senECs and within plasma derived EVs of elderly donors but also in EVs of patients suffering from osteoporosis. Overexpression of miR-31 in MSCs reduced while inhibiting miR-31 enhanced osteogenesis *in vitro*. MiR-31s underlying molecular inhibitory effect was illuminated by demonstrating that miRNA-31 targets FZD3, a factor which was previously unknown to be necessary for osteogenesis. Finally we were able to rescue MSCs from the inhibitory effect of EVs isolated from senECs or from plasma of elderly donors by transfecting them with a miR-31 inhibitor.

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CELL BASED THERAPY IN NEPHROLOGY: BETWEEN HOPE AND REALITY!

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Currently numerous experimental evidence exists that cell therapy is beneficial in models of acute renal failure, inflammatory kidney disease as well as kidney transplantation. In contrast, clinical studies in patients with kidney disease are lacking.
So far, it has been proven that Tregs are decreased in patients with active Goodpasture's disease whereas effector T cells are increased. In contrast, patients in the convalescent phase have increased numbers of Tregs. The importance of Tregs in inflammatory kidney disease is supported by experimental data providing evidence that Tregs are of crucial importance to suppress nephrotoxic serum nephritis (NTS). Tregs also play a key role in kidney transplantation. Patients with a stable graft function have increased numbers of antigen-specific Tregs, which decrease in patients with chronic allograft failure. Furthermore, renal grafts with acute rejection have a better outcome in case of increased Treg infiltration. Even though the experimental and descriptive clinical data are very promising there exist currently no clinical studies evaluating the therapeutic potential of Tregs in patients with inflammatory kidney diseases or transplantation. This will be changed in the near future since the University Regensburg is currently executing "The ONE study", which is funded by the European Union (more than 10Mio Euro) and evaluates the potential of different Treg populations in kidney transplantation.
In summary, cellular therapy, especially using Tregs, might be an interesting therapeutic approach in kidney diseases and transplantation. So far only experimental data exist, but interventional clinical studies are lacking.

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KEY LESSONS LEARNED FROM CELL THERAPY CLINICAL TRIALS

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SURVIVAL OF STEM CELLS IN CARDIAC CELL-BASED THERAPY

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Several factors influences stem cell survival, such as 1) mechanic factors: around 90% of the survival status of the cells are lost during injection and after transplantation, 2) little to no extracellular matrix of the injured host tissue, supporting survival of the cells; 3) lack of oxygen or nutritive substrates, but massive excess of free-radicals, neutrophils, and scavenger cells. Last 15 years, several new approaches have been introduced to increase stem cell survival: 1) Pre-treatment of cells with homing factors (SDF-1, GRO-1, MCP-3, HGF, IGF), chemical transmitters, engraftment factors (proteases, PAI-1, eNOS, MMP-9), survival coctails including integrins, eNOS transcription enhancer, HMG-CoA reductase inhibitors, thymosin beta-4; 2) ischemic conditioning of the cells; 3) genetic modifications of the cells for overexpression of tenascin-C, relaxin, periostin, CD18, phosphorylated-Akt, Bcl-2, SDF-1, 5-Azacytidine, HSP, protein kinase G1-alpha, or 4) increasing telomeric length with Pim-1 kinase, 5) engineering of the cells to increase cell-to-cell interaction. Another approach is to change the host milieau via enrichment with angiogenic factors (VEGF, HGF, FGF-2, IGF-1), or activation of SDF-1 – CXCR4 axis. All of these pretreatment led to improved cell survivals, increased LV EF and decrease in infarct size under experimental conditions. However, until now, no one of these procedures resulted in a robust regeneration of the human myocardium. Based on the fact, that apoptotic cells produces several regenerative factors in larger amount than do the viable cells (“Dying cell hypothesis”), it seems that the survival of the cells might not be absolutely necessary to achieve tissue regeneration.

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STEM CELLS FOR CARDIAC REGENERATION IN MICE AND MEN

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Cardiovascular disease remains the leading cause of death in industrialized countries. Advancements in medical, interventional and surgical treatment have improved the

prognosis of patients with ischemic conditions. However, none of these approaches results in restoration of viable myocardium after a heart attack.
20 years ago stem-/ progenitor cells were tested for the first time for their potential to reduce scar size after myocardial infarction. Application of stem cells via various routes led to significant and reliable improvement of myocardial function after ischemic heart damage by both angiogenesis and myogenesis. The initial hype caused by almost miraculous reports from experimental studies performed mostly in mice was followed by some degree of disillusion after only modest results could be achieved in clinical trials.
20 years on stem cell therapy has still not made the transition from bench to bedside. There is a plethora on experimental and clinical studies in the scientific literature examining the the most critical questions in stem cell therapy: number of cells needed, most effective route of administration, mechanism(s), stem cell type. Conclusive answers are still lacking.
In cardiac surgery there is a strong need for stem cell therapy and tissue engineering with the most important fields of investigation: 1. myocardial repair after infarction, 2. use of stem cells as biological pace maker, 3. tissue engineering (TE) of off the shelf vessels for coronary artery bypass grafting, 4. TE of heart valves and 5. building of human hearts.

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A RANDOMIZED CLINICAL TRIAL FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME WITH THE INDIVIDUALIZED DENDRITIC CELL-BASED CANCER IMMUNOTHERAPY AV0113

Thomas Felzmann for the GBM-Vax study consortium

Activartis Biotech GmbH

Background: We recruited 78 patients aged 18-70 years suffering from newly diagnosed glioblastoma multiforme (GBM) into a randomized clinical trial designed for demonstrating the efficacy of the individualized dendritic cell-based cancer immunotherapy concept AV0113 (GBM-Vax, EudraCT 2009-015979-27). DC are charged with autologous tumor antigens and contacted with lipopolysaccharide (LPS) in the presence of Interferon (IFN) -γ enabling IL-12 secretion for 1 day thus priming cytolytic anti-tumor immune responses. Exposure to LPS/IFN-γ is limited to 6 hours in order to permit DC/T-lymphocyte interaction in the presence of IL-12.
Methods: Patients underwent first line GBM therapy (surgery, radiotherapy, chemotherapy) according to the standard of care; patients randomized into the treatment group received AV0113 as add-on therapy. Primary and secondary objectives were PFS and OS. After progression, patients were treated with Bevacizumab, which was not part of the protocol. The study still collects follow up information.
Results: AV0113 DC-CIT was well tolerated. Reactions at the injection site were mild and included redness and swelling; some patients developed fever. There are some imbalances of severe adverse events between control and treatment groups, which, however, didn't appear to be linked to CIT. No signs of autoimmunity were observed. A trend analysis of one-year survival of all patients receiving Bevacizumab as second line therapy revealed that 45% of control group patients died during the first year; in the AV0113 treatment group only 16% died during the first year of their disease. A similar trend is observed in overall survival. DC-CIT didn't improve progression free survival. Accompanying immunological assays demonstrated a correlation of longer survival with signs of polarization of the immune system towards cytotoxicity.
Conclusions: Randomization for the GBM-Vax study was completed in May 2013; hence we expect a complete data set for the one-year survival in May 2014 and will present this outcome. If the current trend is confirmed, we expect a first demonstration of efficacy for IL-12 secreting DC in the treatment of GBM.

Supported by AOP Orphan Pharmaceuticals AG

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MESENCHYMAL STEM/PROGENITOR CELL FUNCTION: BEYOND REGENERATION

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Mesenchymal stem/progenitor cells (MSPCs) comprise a heterogeneous cell population which is thought to contribute to stromal homeostasis and regeneration in almost every mammalian organ. Established criteria identify MSPCs based on ≥95% CD73/90/105 expression with the lack of CD45/34 and CD14/19 (or 11b/79a, respectively) as well as lack of major histocompatibility complex (MHC) class II antigens (all ≤2%) when recovered after adherent cell culture. A three-lineage differentiation potential represents the in vitro potency of MSPCs to propagate osteoblasts, chondroblasts and adipocyted. So far neither clonogenicity/stemness nor definitive multipotentiality (derivation of multiple [>3] cell lineages from single stem/progenitor cells) has been definitively proven for the majority of MSPC populations. A variable functionality in several models of local or systemic transplantation despite a clear lack of cell recovery after transplantation precipitated the current view that MSPC execute dominant trophic (anti-apoptotic, anti-scaring, angiogenic, mitotic) and various immune modulatory effects without the need for sustained engraftment. Examples for definitive tissue and organ regenerative potential of human MSPCs and a novel humanized bone and marrow niche model will be presented. Trophic as well as immune modulatory aspects of MSPC function will be discussed as well as additional facets of their functionality including anti-septic activities.

KEYWORDS: Stem cell research, stem/progenitor cells, transplantation, engraftment, multipotentiality

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CENTRE FOR GENE AND CELLULAR THERAPIES IN THE TREATMENT OF CANCER (ONCOGEN) TIMISOARA - A STEP TOWARDS THE MULTIDISCIPLINARY RESEARCH IN EASTERN EUROPE

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ONCOGEN Centre Timisoara is a result of structural funding granted through EU policy in the field of research and technological development intended to establish the European Union as a leading knowledge-based economy. The Centre is aiming to sustain and facilitate the translation of research results to advanced therapies. It will enable interdisciplinary integration of knowledge in immunology, molecular biology, biochemistry, imaging techniques, bioinformatics and genetic engineering; application of worldwide progresses by using advanced therapies in the field of oncology; setting up of the integrated-interventional clinical research in view of developing standard clinical protocols; validation of innovative cancer therapies developed by the Centre or its partner centres; development of research funding programmes to train highly skilled researchers in the field. The clinical-use Clean Room will approach: regenerative medicine, based on previous experience within FP7 projects (CASCADE, REBORNE); selection of antigen specific CTLs for autologous transplantation in cancer patients and innovative therapies in immune diseases. The main goal is to integrate our centre in the European networks on advanced therapies, therefore contributing to reduce the gap between Romanian and EU research systems.

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STEM CELL THERAPIES IN DERMATOLOGICAL DISEASES

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The skin is uniquely suited to accommodate cellular therapies. This is exemplified in the genetic blistering skin disease Epidermolysis bullosa (EB). Here besides gene therapy, gene editing/engineering and protein replacement therapy, cell therapies have gained interest, recently. These include allogeneic fibroblasts, mesenchymal stromal cells (MSCs), bone marrow stem cell transplantation, culturing/grafting revertant mosaic keratinocytes, and clinical application of inducible pluripotent stem cells. Although a cure for EB still remains elusive, these data have raised the expectations of patients and professionals. The lessons learned in treating EB will have significant implications for improving the management of other genetic diseases.

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URINE AS A NON-INVASIVE SOURCE OF KIDNEY EPITHELIAL CELLS THAT CAN BE REPROGRAMMED TO INDUCED PLURIPOTENT STEM CELLS

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The transformation of somatic cells into induced pluripotent stem cells (iPSCs) using exogenous factors, also termed reprogramming, may be used for personalized medicine in the future and can produce valuable in vitro models of human diseases. So far, human iPSCs have been generated from skin, amniotic fluid, human umbilical vein endothelial cells, extra-embryonic tissues, cord blood, periosteal membrane, dental tissue, adipose tissue, neural stem cells, hepatocytes, and peripheral blood cells. The reprogramming from these tissues has been achieved with varied frequencies, indicating that the cells of origin are an important determining factor. In addition, there is intense debate regarding whether human embryonic stem cells (ESCs) and iPSCs are equivalent, and donor cell

heterogeneity may further complicate this due to difficulties in setting standards for performing such comparisons. The ideal cell source for reprogramming should be easily accessible, easily reprogrammed, and universal (any age, sex, ethnic group, and body condition). Here, we report the generation of human iPSCs from cells obtained non-invasively from human urine. Urine-derived cells from 12 donors yielded iPSCs with excellent differentiation ability. Therefore, we propose urine to become the preferred source for generating iPSCs in many instances. The ease of this method may facilitate the standardization of iPSC technology, will boost the generation of cell based disease model systems and is also an advance in the direction of clinical use of iPSCs.

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SCHWANN CELL LIKE CELLS FOR PERIPHERAL NERVE REGENERATION APPROACHES

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The gold standard in peripheral nerve regeneration is the autologous nerve transplant. Due to limiting factors like length and diameter of the graft and the disadvantage of a second incision site, alternatives are sought. New approaches include novel nerve conduits and nanoscaled guiding structures, but also Schwann cells as they are a crucial factor in nerve regeneration. After injury, they start proliferating to form bands of Büngner enhancing and guiding axonal regeneration. To avoid incisions on nerves, as it could result in painful neuromas, Schwann Cell like Cells (SCLs) differentiated from Mesenchymal Stem Cells have become of great interest as an alternative to native Schwann cells. In this study we evaluated SCLs concerning their differentiation and activation status (flow cytometry: S100b, P75, GFAP, MAG), finding an expression of P75, GFAP and S100 in presence, as well as increased expression of MAG and P0 in absence of forskolin, indicating a change of activated status into beginning myelination. The SCLs were applied to treat injuries to the sciatic nerve in the rat in two different systems – preseeded on electrospun matrices and encapsulated in collagen, both resulting in improved regeneration compared to the control groups. Concluding, SCLs are a promising tool to improve peripheral nerve regeneration.

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USE OF PBMC-SECRETOMES IN CUTANEOUS WOUND HEALING

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Wound healing is a complex process involving a vast number of mechanisms. In this study we investigated the effects of cell-free supernatants derived from PBMC cultures ($PBMC^{Sec}$) in animal models of skin regeneration after full-thickness defects. Six mm punch biopsy wounds were set on C57BL/6J-mice and full thickness burn injuries were created in a porcine model. In addition, in the porcine model skin grafting with subsequent topical application of $PBMC^{Sec}$ was performed. Morphology and neo-angiogenesis were analyzed by H&E-staining and CD31 immuno-staining, respectively. In vitro effects on diverse skin cells were investigated by migration assays, cell cycle analysis and tube formation assay. Signaling pathways were analyzed by Western blot analysis.
Histological analysis revealed a better regeneration of epidermal and dermal structures after application of $PBMC^{Sec}$ in both skin models. Moreover, inflammation was markedly reduced. In the mouse model, reduction of wound size after topical application was observed. Staining revealed more advanced healing and angiogenesis compared to control wounds. In vitro treatment of primary skin cells showed increased proliferation and migration. In endothelial cells $PBMC^{Sec}$ induced proliferation and tube-formation in a matrigel-assay. In addition, $PBMC^{Sec}$ treatment of skin cells led to the induction of multiple signaling-pathways involved in cell migration, proliferation and survival.
We could show that the secreted paracrine factors of human PBMC have regenerative potential in vitro and in vivo. These effects are based on improved wound healing, increased angiogenesis and a better quality of the dermal/epidermal structures after full-thickness cutaneous injuries.

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ENGINEERING OF CHIMERIC ANTIGEN RECEPTOR (CAR)-MODIFIED T CELLS FOR ADOPTIVE IMMUNOTHERAPY OF CANCER – EMERGING OPPORTUNITIES AND CHALLENGES

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Adoptive immunotherapy with T cells that were modified by gene-transfer to express a tumor-targeting chimeric antigen receptor (CAR) is being investigated as a novel and transformative way for treating cancer. CARs are synthetic receptors with an extracellular antigen-binding domain derived from the VH/VL chains of an antibody, an intracellular signaling domain – most commonly CD3zeta *in cis* with a co-stimulatory domain such as CD28 or 4-1BB, and recognize surface molecules independent from HLA. The CAR-transgene can be inserted into autologous T cells to provide a personalized tumor-reactive T-cell product for an individual patient. Pilot clinical trials at centers in the US have demonstrated the curative potential of this approach with dramatic and durable complete anti-tumor responses in a subset of patients with chemo-radiotherapy refractory $CD19^+$ B-cell acute and chronic leukemia (ALL/CLL) that received T cells modified with a CAR specific for the B-lineage marker CD19. Importantly, clinical responses correlated with engraftment and persistence of CAR T cells following adoptive transfer. An ongoing effort in the field is to identify and validate alternative tumor antigens to extend applications of CAR T-cell therapy. Our group has developed a CAR specific for the ROR1 molecule that is expressed on several hematologic malignancies and epithelial cancers, including triple-negative breast cancer and demonstrated the ability of ROR1-specific CAR T cells to confer anti-tumor reactivity in pre-clinical models. We are in the process of establishing the GMP manufacturing process for CAR T cells and preparing clinical trials to implement this powerful new therapeutic modality at our institution.

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TREG INDUCED CHIMERISM FOR TRANSPLANTATION TOLERANCE

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Long-term outcome after organ transplantation remains unsatisfactory as many grafts are lost due to chronic rejection and as patients suffer from substantial morbidity associated with chronic immunosuppression. Induction of donor-specific immunological tolerance would be a potential solution for this unmet medical need. The establishment of hematopoietic chimerism through the transplantation of donor bone marrow is a promising strategy in this respect leading to robust tolerance in numerous experimental models. However, the toxicity of the myelosuppressive and cytotoxic recipient conditioning usually

required to allow bone marrow engraftment precludes widespread clinical translation. Recently we developed a murine chimerism protocol devoid of myelosuppressive and cytotoxic elements. To this end, we combined the transplantation of conventional doses of fully allogeneic donor bone marrow with cell therapy with polyclonal recipient T regulatory cells (Tregs). In addition, recipients received a short course of costimulation blockade (CTLA4Ig, anti-CD40L) and rapamycin. With this non-cytotoxic protocol lasting multi-lineage chimerism and durable donor-specific tolerance (as assessed by grafting donor and 3rd party skin and hearts) were achieved. Hence, combining chimerism induction with Treg therapy provides a novel approach to achieve transplantation tolerance with clinically acceptable toxicity.

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IMMUNOTHERAPY OF THE PROSTATE CANCER WITH DENDRITIC CELLS LOADED WITH IMMUNOGENIC TUMOR CELLS

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Immunotherapy has emerged as another treatment modality in cancer. The goal of immunotherapy in advanced cancer patients does not have to be the complete eradication of tumor cells but rather the restoration of a dynamic balance between tumor cells and the immune response. Appropriate combination of tumor mass reduction (by surgery and/or chemotherapy) and neutralization of tumor-induced immunosuppression might set the right conditions for the induction of anti-tumor immune response by active immunotherapy. We initiated two Phase I/II clinical trials using mature dendritic cells (DCs) pulsed with killed LNCap prostate cancer cell line in patients at two distinct stages of the prostate cancer. In the first trial, patients with biochemical relapse, defined as three consecutively rising levels of 3rd generation PSA, are treated with continuous subcutaneous administration of DCs. Second trial is designed for patients with hormone refractory prostate cancer and patients receive alternate treatment with DC-based vaccine and palliative chemotherapy with docetaxel to reduce the tumor cell burden. After one year of follow up, we observe a significant prolongation of the PSA doubling time in the cohort of biochemical relapse patients when compared to the historical controls. In patients with hormone refractory prostate cancer, the alternate administration of DC-based cancer immunotherapy and docetaxel results in the stabilization of the disease progression and longer than expected survival rather than to the reduction of the tumor cells burden. We conclude that the continuous DC-based cancer immunotherapy can represent an efficient adjuvant treatment modality for prostate cancer patients.

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

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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 target joint or to the connective tissue of the target joint. Since 2011, 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, 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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P1 DETERMINATION OF MECHANICAL AND HISTOLOGICAL PROPERTIES OF EQUINE MENISCI – TARGET VALUES FOR THE DEVELOPMENT OF A MENISCUS MODEL

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The healing potential of the meniscus is limited and no optimal treatment strategy is currently available. Therefore 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. To define the target properties of the meniscus model, biomechanical and histological attributes were examined.
A histological study with special emphasis on vascularisation patterns, collagen types and alignment was performed. To evaluate the stiffness and energy loss during dynamic compressive loading and the impedance of the menisci during compressive loading, a Walter+Bai AG materials testing system was used. The specimens (n=36, age 0.5-27 years), were fixed in a custom designed jig for examination. To determine the shore hardness, a PCE-DD-A Shore A durometer was used.
A shore hardness of 37.0–85.0 Shore A (mean: 59.4±9.5 Shore A) was determined. Stiffness yielded 520±140 N/mm for the center portion of the meniscus, respectively 501±180 N/mm for the outer portion of the meniscus (overall: 576±263 N/mm). Energy loss overall, represented by the area of hysteresis of each loading measurement, was 0.55±0.18 Nm. The angled jig for the biomechanical tests allows for distribution of force in an equine physiologic manner and good reproducibility independent of different meniscal sizes and shapes.

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P2 HUMAN AMNIOTIC MEMBRANE: A PROMISING SOURCE FOR TISSUE ENGINEERING

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Human amniotic membrane (hAM) represents an abundantly available biomaterial already being applied in many surgical procedures for years. Furthermore, it is consistently gaining

access to new areas in tissue engineering and regenerative medicine. For tissue engineering applications, cells are usually combined with suitable carrier substrates or as cell sheets. The hAM, a pre-formed sheet containing stem cells with pluripotent properties, is known to have low immunogenicity, anti-inflammatory, anti-fibrotic, and non-tumorigenic properties. Furthermore, it has been shown to engraft in allogeneic as well as xenogeneic settings without eliciting adverse effects. Our aim was a new approach for bone, cartilage and nerve tissue engineering via differentiation of the hAM *in toto*. We found that hAM can be differentiated towards the osteogenic, chondrogenic and Schwann cell-like lineage. Hence, hAM in its viable form may be a suitable general tool for tissue regeneration.

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P3 HIGHLY PURIFIED HUMAN COLLAGEN ISOLATED FROM THE HUMAN PLACENTA IS A POTENTIAL MATERIAL FOR PRIMARY HEPATOCYTE CULTIVATION

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Collagens are routinely used as substrate for the expansion of specific human cell types *in-vitro*. They are mainly provided as xenogeneic products, associated with the risks for transmission of zoonosis. Collagens can be alternatively produced as recombinant human proteins from cell culture procedures, resulting however in low yields at high expenses. Primary hepatocyte cell culture is crucial for new *in-vitro* test systems. However, these cells need an appropriate surrounding for attachment and long-term viability.
Human collagens type I, III. IV and V were isolated by pepsin digestion followed by subsequent sequences of salt precipitations and buffer exchanges by dialysis, resulting in high yields (550± 71mg collagen/100g wet tissue). Isolation of primary rat hepatocytes was established by a two-step collagenase perfusion which consists of a non-recirculating collagenase perfusion through the portal vein. The isolated cells were filtered through a 100µm pore size cell strainer, and cultured on 24 well plates, coated with placental collagen I&III, and compared to gelatin coated plates. 3 different coating concentrations (1mg/ml, 0.1mg/ml, 0.01mg/ml) were compared.
CK-18 immunostaining revealed hepatocyte specificity of the cell isolate. MTT tests showed a significantly higher cell viability for hepatocytes seeded on placental collagens compared to gelatin after one day of culture for all coating concentrations ($n=8$, $p<0.05$). The cells were kept in culture up to 7 days.
We conclude that placental collagens are an appropriate substrate for the cultivation of hepatocytes compared to gelatin coated cultures. The isolated human placental collagens support the attachment and viability of primary rat hepatocytes.

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P4 TRANSIENT *IN VIVO* BDNF/GDNF GENE OVEREXPRESSION PROMOTES MOTO-NEURON SURVIVAL AND FUNCTIONAL REINNERVATION IN A VENTRAL ROOT AVULSION MODEL IN THE RAT

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Axonal injury implied close to cell bodies of motoneurons, such as ventral root avulsion induces the death of the vast majority of affected motoneurons. Brain derived neurotrophic factor (BDNF) and glial cell-line derived neurotrophic factor (GDNF) are potent survival factors for damaged motoneurons, but if produced *in situ* in a non-regulated manner they induce axonal sprouting without functional reinnervation, a consequence known as the "candy store effect".
In a lumbar ventral root avulsion-reimplantation rat model, we applied a plasmid-based vector system to induce transient expression of BDNF and/or GDNF in the close vicinity of the damaged motoneurons. Rat adipose tissue-derived stem cells (rASCs) were transfected *in vitro* with these constructs and applied *in vivo* around the reimplanted ventral root, embedded in collagen gel.
Evaluation of expression kinetics *in vitro* showed high expression levels at early timepoints after transfection and a desired decline after 2 weeks. Spatiotemporally limited neurotrophic factor therapy induced not only the survival of the injured motoneurons, but promoted the regeneration of their axons into the vacant ventral roots, too. Morphological reinnervation was accompanied by considerable functional improvement of hind limb locomotor activity. BDNF, GDNF and a combination of both factors induced a similar extent of reinnervation.
These findings provide evidence that damaged motoneurons require spatiotemporally restricted expression of BDNF and/or GDNF to support their survival and regeneration

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P5 HUMAN ADIPOSE DERIVED STEM CELLS RETAIN THEIR CHONDROGENIC POTENTIAL DURING EXPANSION WITH HUMAN PLATELET LYSATE

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Fetal calf serum (FCS) bears a potential risk for carrying diseases and eliciting immune reactions. Nevertheless, it still represents the gold standard as medium supplement in cell culture.
In the present study human platelet lysate (hPL) has been tested as an alternative to FCS for the expansion and subsequent chondrogenic differentiation of human adipose derived stem cells (ASC). ASC were isolated from liposuction material of 8 donors and expanded up to passage 3 with 10% FCS (group 1) or 5% hPL (group 2). Subsequently, three dimensional micromass pellets were created and cultured for 5 weeks in chondrogenic differentiation medium without hPL or FCS but supplemented with 10 ng/mL bFGF and 10 ng/mL TGF-β3. In order to evaluate the effect of hPL on chondrogenesis during cell condensation, micromass pellets of group 2 were additionally treated with 5% hPL within the initial 3 days of micromass pellet culture (group 3).
Growth curves revealed that medium supplementation with hPL strongly increases cell proliferation. Chondrogenic differentiation has been evaluated by qRT-PCR, glycosaminoglycan (GAG) quantification and histological staining. Ten cartilage related markers (COL2A1, COL1A1, SOX9, COL9A2, COL10A1, AGC1, CSPG2, MIA, COMP, CRTL1) were evaluated with qRT-PCR and demonstrated chondrogenic differentiation of both, hPL and FCS expanded ASC. GAG quantification did not reveal significant differences between the three groups, although hPL expanded cells tended to express higher levels of GAG. Histologically, collagen type II and GAGs could also be detected in all groups.
The present study demonstrates that hPL strongly induces proliferation of ASC while retaining the chondrogenic differentiation potential, suggesting that hPL is equal or superior to FCS as supplement for the expansion of ASC particularly with regard to chondrogenic differentiation.

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P6 COMPARISON OF HUMAN PLATELET LYSATE AND FETAL CALF SERUM AS MEDIUM SUPPLEMENTS FOR ENDOTHELIAL CELLS

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Vascularization of tissue-engineered constructs is still a challenging aspect in tissue engineering and regenerative medicine. To successfully culture endothelial cells (EC) for prevascularization strategies fetal calf serum (FCS) is widely used. However, some critical aspects like possible xenogeneic protein or virus transmissions have to be considered. Therefore, the aim of this project was to determine if human platelet lysate (PL) is a reasonable candidate to replace FCS as a medium supplement in the culture of human endothelial cells.

The usability of PL for human endothelial cell culture was tested by analyzing the morphology, endothelial marker expression, metabolic activity and vasculogenic potential of outgrowth endothelial cells (OEC), lymphatic endothelial cells (LEC) and human umbilical vein endothelial cells (HUVEC).

The results show a similar morphology of the EC types cultured in both FCS and PL. In addition, EC marker expression did not differ significantly. Moreover, OECs, LECs and HUVECs formed tube-like structures in Matrigel when cultured in PL. Employing MTT assays we found that the metabolic activity of OEC and LEC was decreased when cells were cultured with PL compared to FCS. HUVEC did not show a significant decrease in metabolic activity.
In conclusion, the use of PL on different EC types did not reveal any substantial negative effects on EC behavior. Thus, PL appears to be a favorable candidate to replace FCS as a medium supplement in the culture of endothelial cells.

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P7 UROKINASE PLASMINOGEN ACTIVATOR PROTECTS CARDIAC MYOCYTES FROM OXIDATIVE DAMAGE AND APOPTOSIS

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The uPA-uPAR system has been established as an important contributor in tissue reorganization and wound healing. In addition, uPA has been reported to stabilize p53, a key cell cycle control, DNA repair and apoptosis initiation protein. We investigated the role and function of uPA and its control of p53 and its downstream targets in the human heart and in isolated human cardiac myocytes. To evaluate, if the uPA-uPAR-p53 system is activated in failing hearts we determined expression levels of uPA, uPAR, p53, PAI and p53 targets BAX (proapoptotic) and OGG (DNA repair) in explanted failing hearts (n=15). uPA correlated strongly with uPAR (0.997; $p<0.001$), PAI (0.992; $p<0.001$), p53 (0.962; $p<0.001$) as well as with BAX (0.972; $p<0.001$) and OGG (0.958; $p<0.001$). To determine, if uPA is pushing the cell towards a certain fate, we analyzed the role of uPA in H_2O_2 induced DNA damage and apoptosis. Preconditioning of cardiac myocytes with uPA reduced H_2O_2 induced apoptosis by 13% ($p=0.05$) determined by TUNEL staining. In addition uPA treatment reduced oxoguanine foci at the DNA by 46% ($p<0.001$) indicating increased DNA repair capacity. This protection is probably due to an upregulation of OGG by uPA (1.4 fold after 24h). We conclude that uPA might have a tissue protective role in human hearts besides its role in tissue remodeling.

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P8 3D DIFFERENTIATION OF ADIPOSE-TISSUE DERIVED STROMAL CELLS IN A THERMOSENSITIVE POLY-CAPROLACTONE SCAFFOLD

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In tissue engineering (TE) strategies, the natural process of regeneration is imitated by using bioresorbable scaffolds that support cellular attachment, migration, proliferation and differentiation. Based on the idea of combining a fully degradable polymer (Poly(ε-caprolactone) with a thermoresponsive polymer (polyethylene glycol methacrylate) a scaffold was developed [1], which liquefies at 4°C and solidifies at 37°C. In this study, it was combined with either C2C12 cells or human adipose-tissue derived stromal cells (ASCs) to generate an expandable 3D construct for soft or bone TE.
As a first step, biomaterial seeding was optimized for C2C12 cells as well as ASCs and their attachment, survival, distribution and persistence within the 3D material was characterized. C2C12 cells were differentiated towards the osteogenic as well as myogenic lineage, while ASCs were differentiated with adipogenic or osteogenic media. Differentiation was examined using qRT-PCR for the expression of osteogenic, myogenic and adipogenic markers. C2C12 cells differentiated towards the myogenic lineage and ASCs treated with adipogenic differentiation medium showed increased expression of specific markers in 3D compared to 2D, suggesting that the thermoresponsive scaffold qualifies for 3D in vitro differentiation towards soft tissue. Next, we evaluated whether the scaffold was able to support vascularization in an in vivo angiogenesis model. An increase in newly formed vessels was detected during the first two weeks followed by a decline in total vascularization in week four. No signs of inflammation were visible. This suggests that the scaffold supports angiogenesis and is therefore a promising candidate for further TE approaches.

[1] R. Cheikh Al Ghanami, B. R. Saunders, C. Bosquillon, K. M. Shakesheff, und C. Alexander, „Responsive particulate dispersions for reversible building and deconstruction of 3D cell environments“, *Soft Matter*, Bd. 6, S. 5037, 2010.

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P9 LASER CAPTURE MICRODISSECTION OF MURINE INTERZONE CELLS: LAYER SELECTION AND PREDICTION OF RNA YIELD

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Articular chondrocytes originate from a cohort of progenitor cells in the so-called interzone in embryonic developing joints. We conducted this study to determine the possibility to identify the intermediate and outer interzone layers histologically without in situ hybridization or immunohistochemistry; to establish whether sufficient amounts of RNA can be harvested from each interzone layer of individual embryos for gene expression analysis; to estimate RNA yield prior to costly amplification steps.
Cells from the outer (OI) and intermediate (II) interzone of the femorotibial joint and the epiphyseal cartilage (EC) of femur and tibia of murine embryos (13.5 and 15.5 days gestation) were harvested using laser capture microdissection (LCM). Microarray analysis was performed to confirm appropriate layer selection. The surface area and the grey value (gv) of photomicrographs was measured and the relative optical density (ROD) was calculated and degree and significance of the correlation with RNA yield determined.
Cells from the OI, II and EC were successfully harvested with LCM and yielded sufficient amounts of RNA for linear amplification and microarray analysis. The RNA yield correlated significantly with the tissue surface area harvested, the mean gv and the corresponding ROD.
This study provides a technique for selective laser capture microdissection, microarray analysis of murine interzone cells and a method to estimate RNA yield. We recommend to harvest a minimum of 1 x $10^6 \mu m^2$ of 13.5 and 3 x $10^6 \mu m^2$ of 15.5 days murine embryos to obtain approximately 10 ng total RNA for linear T7-based amplification and subsequent microarray analysis.

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P10 DEVELOPMENT OF A PERSONALIZED CELLULAR EX-VIVO CBL-B SILENCING CANCER IMMUNE THERAPY

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The E3 ubiquitin ligase cbl-b has been identified as an important gatekeeper limiting T cell activation. Concordantly, the immune system of cbl-b deficient mice can effectively fight tumors. We have recently shown in proof-of-concept experiments that transfer of transiently cbl-b silenced murine T cells had efficacy to enhance the anti-tumor immune response in mouse models, thereby validating cbl-b as an excellent target to enhance anti-tumor immune activity.

An efficient siRNA transfection protocol that allows simultaneous transfection of T, B, NK cells and monocytes with minimal cell damage was established, resulting in inhibition of cbl-b expression for more than 7 days in stimulated human T cells. Consequently, cbl-b silenced human T cells displayed strongly increased cytokine production and proliferation. Moreover, simultaneous silencing of cbl-b in all immune cells of the PBMCs yielded additional advantages, most notably enhancing NK cell reactivity against tumor cell and IL-2 stimulation. Silencing of cancer patient PBMCs yielded similar results ex vivo, and intranodal transfer of autologous cbl-b silenced cells together with activated DCs to patients with advanced cancers was feasible and well tolerated.
To enable the clinical implementation of a cbl-b ex vivo silencing treatment, we have established and tested a protocol that can be easily performed on any clinical unit that applies adoptive cell therapies to patients. Based on these results, a Phase I trial for the systemic administration of cbl-b silenced PBMCs to patients with advanced cancers has been initiated.

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P11 STEM CELL CHARACTERISTICS OF HUMAN AMNIOTIC EPITHELIAL CELLS: IMPACT OF CULTURE

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Human amnion represents an abundantly available and uncontroversial source of cells with stem cell properties. Cell-therapy, applying stem cells with characteristics such as differentiation and immunomodulatory capacity, has become an important strategy in regenerative medicine. For this reason, we have expanded and thoroughly characterized amniotic cells cultured in different media. To optimize in vitro expansion, three specific media for amniotic cells were compared to our standard media. Proliferation was assessed by calculating cumulative population doublings. Surface antigen expression profiles were determined by flow cytometry. Osteogenic and adipogenic differentiation were evaluated by von Kossa and Oil Red O staining, respectively. Differentiation efficiency was estimated by quantitative real-time polymerase chain reaction. We found that the expression of surface antigens clearly depends on the culture medium. Irrespective of a relatively high donor dependency, the best proliferation in vitro was achieved with the special media AmniochromPRO and Quantum 3-21. Interestingly, although proliferation was reduced in MesenPRO, cells cultured under these conditions showed a great potency for differentiation towards the osteogenic lineage. Regardless of the medium used for expansion, no adipogenic differentiation could be induced. Thus, amnion is a promising source of stem cells for cell therapy and regenerative medicine.

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P12 TWO PHASE CULTIVATION FOR THE EXTRACTION OF CELLS WITH MELANOCYTIC POTENTIAL FROM THE EQUINE HAIR FOLLICLE

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For more than thirty years, the hair follicle has been attentively studied by stem cell biologists. There is a lack of literature about culturing equine cells extracted from horse hair follicles.
For this reason, the study presented here focuses on the isolation of hair follicle cells with melanocytic potential by a method of Savkovic et al (2012). The aim of this work is the successful adaptation of the aforementioned protocol to equine skin.
Skin samples from five horses were taken from the frontal region. Firstly, the two phase cultivation depended on a cultivation of micropreparated hair follicles on transwells to accomplish an air liquid interface. Cells grew under hypoxic conditions and were fed every three to four days with Derma Life Melanocyte Medium. After being 50% confluent, wells

were flooded with medium and further outgrowth was provided. Subsequently, the second phase of cultivation as an adherent culture was performed. This facilitated the cell monitoring in hindsight of morphology as well as being the prerequisite for characterization by immunofluorescence and RT-PCR.
The weekly photodocumentation showed the proliferation of cells around the displayed hair follicles on transwells. Furthermore, they onwardly proliferated in the adherent culture with a melanocytic growth pattern presenting typical dendritic processes.
Results obtained by using immunofluorescence and RT-PCR supported the morphological findings.
The initial findings presented here indicate that equine hair follicles can serve as a source for melanocytic cells and are promising for further research to reveal their potential for autologous regenerative therapies.

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P13 PURINERGIC SIGNALING IS INVOLVED IN ENDOTHELIAL CELL SPROUTING.

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Endothelial cells utilize purinergic signaling in order to respond to various stimuli such as hypoxia or inflammation. Yet, the role of purinergic signaling in angiogenesis and sprouting is poorly understood. Here, we studied the role of purinergic signaling in endothelial cell

sprouting using the HUVEC spheroid assays and luminometry to assess ATP release from HUVEC monolayers. In response to stimulation with VEGF (25 ng/ml), HUVEC release ATP in a dose- and time-dependent fashion. Pretreatment of HUVEC spheroids with the gap junction inhibitor carbenoxolone (50 µM) significantly reduced the number VEGF-induced sprouts by 50% when compared to controls. Supplementing growth media with 10 µM ATP also significantly reduced VEGF-induced sprouting while no effect was observed in the presence of 1 or 100 µM ATP. Neither the non-hydrolysable ATP analog ATPγS, nor ADP or adenosine affected sprouting. Inhibition of the ectonucleotidase CD39 followed by the addition of ADP also did not affect sprouting of HUVEC. In the absence of VEGF, HUVEC sprouting was not affected by ATP or its hydrolytic products. These data suggest that specific sets of purinergic receptors are involved in sprouting. Our results suggest that ATP release is involved in the response of HUVEC to VEGF, that localized purinergic signaling regulates sprouting, and that exogenous addition of ATP can interfere with these signaling processes. We conclude that purinergic signaling mechanisms may be potential therapeutic targets to alter sprouting and angiogenesis in wound healing and in diseases such as cancer.

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P14 APPLICATION OF L-MIMOSINE IN DENTAL PULP TISSUE REGENERATION: IN VITRO STUDIES IN 2D CELL CULTURES AND TOOTH SLICE ORGAN CULTURES UNDER BASAL AND DIABETIC CONDITIONS

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Healing of the dental pulp after traumata or carious lesions is difficult to predict. Moreover, diabetes mellitus can reduce the healing capacity of dental pulp tissue. Novel regenerative strategies are based on the pro-angiogenic effect of prolyl hydroxylase inhibitors. Among these inhibitors is L-mimosine, a non-proteinogenic amino acid. However, the response of the dental pulp to L-mimosine is unknown.
In this study we investigated the in vitro response of the dental pulp to L-mimosine in single layer cell cultures based on viability, proliferation and vascular endothelial growth factor production utilizing MTT-tests, ^{3}[H]thymindine, and immunoassays, respectively. Moreover, viability and VEGF production of tooth slice organ cultures was assessed after stimulation with L-mimosine. Diabetic milieu was mimicked with advanced glycolysed end-products.
Our results show that at non-toxic concentration L-MIM enhances under basal conditions the production of vascular endothelial growth factor. L-minosine also elevated the vascular endothelial growth factor levels in single layer cell cultures and tooth slice organ cultures performed in the presence of advanced glycolysed end-products.
In summary these results indicate that the dental pulp respond to L-mimosine under basal and under diabetic conditions. Further studies will show if this pro-angiogenetic response to L-mimosine found in vitro translates to enhanced pulp regeneration.

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P15 OPTICAL CONTROL OF RECEPTOR TYROSINE KINASES

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Cells respond to environmental stimuli through multifarious cell surface receptors. Receptor tyrosine kinases (RTKs) are a large receptor family that senses growth factors and hormones and is critically involved in normal and aberrant development and physiology. We developed engineered RTKs that are activated by light and termed optically-activated RTKs (Opto-RTKs). Optical control of RTKs was achieved by supplementing mammalian RTKs with light-sensing protein domains from phototropic organisms. Opto-RTKs enabled non-invasive activation of signaling pathways associated with survival and growth in multiple cell types.

Spatio-temporal control of cellular signaling with genetically-encoded and optogenetic RTKs will open new avenues in basic and applied research.

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P16 IN VITRO SHOCKWAVE TREATMENT INFLUENCES LYMPHATIC ENDOTHELIAL CELL MARKER EXPRESSION AND PROLIFERATION

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Shockwave treatment (SWT) is a promising therapy for treating orthopaedic diseases and chronic wounds. Moreover, it has been shown to increase blood and lymphatic vessel growth. In this study we analyzed the effects of shock waves in lymphatic endothelial cells (LECs) in vitro. LECs were stimulated in a waterbath with a MTS Dermagold 100 device. Twenty four hours later, flow cytometry analyses for endothelial marker expression were performed. The LEC proliferation and viability changes after SWT were determined by manual counting and MTT assays. Moreover, 2D as well as 3D migration assays were

employed and cells were stimulated on either fibronectin, collagen or uncoated surfaces to observe the influence of extracellular matrices during treatment. The proliferation rates of LECs varied when stimulation with different energy densities was applied, whereas the 2D and 3D migration was not altered by shockwave treatment. The endothelial markers CD31, VE-Cadherin, VEGFR2 as well as the LEC marker VEGFR3 expression did not change after SWT. However, we found an energy density-dependent increase in expression of podoplanin, another marker for LECs. Moreover, the extracellular matrix was shown to influence podoplanin expression upon SWT. Since lymphatic vessels play a key role in tissue haemostasis, regeneration of lymphatic vessels is of crucial interest for tissue engineering purposes or for lymphedema patients. Our results add new insights into SWT-induced changes of LEC behaviour and reveal podoplanin as one of the target molecules of SWT. SWT influences the proliferation of isolated lymphatic endothelial cells and mediates podoplanin upregulation in these cells.

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P17 DECELLULARIZATION PROCESS OF HUMAN ARTICULAR CARTILAGE FOR RECELLULARIZATION WITH AUTOLOGOUS CELLS

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The optimal cartilage regeneration procedure provides early mechanical stability and results in a long-term stable, functional cartilage tissue which is well integrated into the defect site. Since existing concepts do not fulfill all of these requirements, we are pursuing a novel strategy: decellularized cartilage matrix as biomimetic scaffold, seeded with adipose derived stem cells.
Cartilage biopsies were obtained from human femoral heads and subjected to decellularization procedures including freeze/thaw cycles in hypotonic buffer, sonication, treatment with 0.1% sodium dodecyl sulfate (SDS) with or without the presence of nucleases. Further we evaluated glycosaminoglycan (GAG) depletion aiming to partly remove GAGs in order to improve cell migration into the decellularized tissue. After decellularization, cell content and matrix components were quantified using biochemical assays and qualified by histology.
By combining freeze/thaw cycles in hypotonic buffer with SDS treatment the DNA content could be reduced to <50 ng/mg even without the use of nucleases. H&E staining demonstrated full cell removal in the superficial zone but presence of some cell residues in the deeper zones. Trypsin demonstrated most effective in removal of GAGs.
We have established a decellularization process for human articular cartilage which we aim to further improve using supercritical CO2 resulting in a 3D scaffold capable of supporting chondrogenic cell differentiation.

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P18 A DUAL LEVEL STRATEGY FOR TISSUE VASCULARIZATION

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We are following a new dual-level strategy to create prevascularized tissues, in vitro. By providing a larger vascular network upfront for the endothelial cells to integrate, we want to support their ability to form new microvascular structures from the decellularized vessel. The aim of this study was to demonstrate the biocompatibility of a vascular matrix after decellularization and to show that it is suitable to support vascular tube formation of endothelial cells within a 3D co-culture system (Holnthoner et al., 2012). Therefore we used

small pieces of the vascular matrix for pretesting our hypothesis. The decellularized vascular structure is used for recellularization with human endothelial cells. For sterilization a 0.18% peracetic acid solution, adjusted to pH 7 with NaOH, was used. Samples of decellularized blood vessels were opened in longitudinal direction and small pieces, 5x5 mm in size, were cut out. The luminal surfaces of the pieces were reseeded with GFP expressing HUVECs (1x10^5 cells/piece) and incubated for 7 days. After the recellularization the pieces were embedded into a fibrin matrix (5 mg fibrinogen/ml) mixed with ASCs to start our 3D co-culture system. As a control we used the same setup but without ASCs in the fibrin matrix. With our experiments we could demonstrate the biocompatibility of decellularized vascular tissue from the human placenta. The tissue was successfully recellularized with endothelial cells (ECs), which, in coculture with ASCs, displayed vascular tube formation from the scaffold. In future applications this dual-level approach should ensure oxygen and nutrient supply even in larger tissues and thus represents a novel vascularization strategy.

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P19 *IN VTRO* EXTRACORPOREAL SHOCKWAVE TREATMENT OF RAT- AND HUMAN ADIPOSE DERIVED STEM CELLS

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Adipose derived progenitor/stem cells (ASCs) are a promising tool for tissue engineering, addressing the problem of tissue and organ shortage. Limiting factors for the use of ASCs are donor variation and senescence, loss of differentiation capacity as a consequence to loss of multipotency. Extracorporeal shockwave treatment (ESWT) has been shown to have beneficial effects on regeneration of a variety of tissues in vivo.
In our study we show that human and rat ASCs respond strongly to repetitive shockwave treatment in vitro, resulting in maintenance and significant elevation of mesenchymal markers (flow cytometry: CD73, CD90, CD105), while cell viability and proliferation remain at a comparable level to control group. Another effect observed was a significant increase in differentiation capacity into osteogenic (von Kossa staining; PCR: osteocalcin, biglycan) and adipogenic lineage (Oilred O staining) as well as into Schwann like cells (flow cytometry: P75, S100, P0) in high passages.
Our results indicate that with ESWT multipotency of ASCs can be preserved in high passages after extensive expansion. Hence, ESWT might be a promising tool to improve ASCs for cell therapy in tissue engineering and regenerative medicine.

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P20 ISOLATION OF MESENCHYMAL STROMAL CELLS FORM OVINE PLACENTA COTYLEDONS

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Perinatal sources of MSCs, which are accessible using minimally invasive techniques, are attractive alternatives to bone marrow. In sheep however, collecting umbilical cord blood or Wharton´s jelly without performing a cesarean section is not possible due to animal distinctive characteristics.
In human placental chorionic villi, MSCs were shown to reside in a perivascular niche (Castrechini et al. 2010). However in contrast to the human discoid, hemochorial placentation, ruminants have a *cotyledonary, epitheliochorial placenta* with numerous small placentae built from Cotyledons (the fetal side of the placenta) and Caruncle (the maternal side of the placenta) together forming so called Placentomes. In the presented study ovine placenta cotyledons were identified to be a minimal invasively accessible source of MSC in sheep.

The ovine afterbirth was collected under sterile conditions and the Cotyledons were dissected. After collagenase digestion, erilysis and cell filtration the obtained mononuclear cell fraction was plated on adherent culture dish and cultured according to international standards (DMEM low glucose, 20% FCS, 1% L-Glutamine, Pen/Strep). The cells were maintained in culture at 37° and 5 % CO2 in a humidified atmosphere. Medium was changed twice weekly. Passaging was performed upon 80-90% confluence.
The obtained cells showed spindle shaped morphology, adherence to plastic, colony formation and in vitro differentiation into the mesenchymal lineages (bone, cartilage and adipose tissue).
Ovine placenta Cotyledons are suggested to be an abundantly available, minimal invasive source of MSC in sheep. Further investigation to characterize the obtained cells and to evaluate their regenerative effect *in vivo* will need to be carried out.

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P21 EFFECTS OF INTERLEUKIN-33 ON TISSUE FACTOR IN HUMAN ENDOTHELIAL CELLS IN VITRO

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Interleukin (IL)-33 is a member of the IL-1 cytokine family. IL-33 was previously shown to induce angiogenesis and the expression of inflammatory cytokines and adhesion molecules in endothelial cells. Tissue factor (TF) is a primary trigger of coagulation. Elevated levels of TF are found in atherosclerotic plaques. Here we investigated the impact of IL-33 on TF expression in human endothelial cells, as a new possible mechanism for IL-33 to regulate their thrombotic potential. Human umbilical vein endothelial cells (HUVEC) and human coronary artery endothelial cells (HCAEC) were treated with 1, 10 or 100 ng/ml recombinant human IL-33 for 3, 6, 9 and 24 hours (h). We found that IL-33 significantly ($p<0,001$) induced TF mRNA and protein expression in HUVEC an HCAEC in a time- and concentration-dependent manner. Stimulation with 100 ng/ml IL-33 for 3 and 6 h has also increased ($p<0,001$) cell surface TF activity level in HUVEC. ST2-siRNA-mediated gene knockdown inhibited IL-33-induced TF expression, suggesting that this effect of IL-33 is facilitated through its receptor ST2. Preincubation of HUVEC with 100µM dimethyl-fumarate, abrogated IL-33-induced TF protein synthesis. IL-1 receptor antagonist (IL-1RA) had no effect on IL-33-induced increase of TF expression. In human carotid atherosclerotic plaques (n=16), TF mRNA positively correlated with IL-33 mRNA expression ($r=0,86$, $p<0,001$). Our results

showed that IL-33 increases TF expression and activity in human endothelial cells, and that this effect is ST2/NFkB-dependent, but IL-1-independent. IL-33-induced changes in TF expression could affect the thrombotic potential of endothelial cells, as well as potentiate thrombotic events in the setting of ruptured human atherosclerotic plaque.

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P22 HIGH-THROUGHPUT MANUFACTURING OF HUMAN EPIDERMAL MODELS

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Human epidermal models are routinely used in an increasing number of skin irritation and toxicity testing. However, only a fraction of the current demand can be met by producing these models manually. The high-throughput manufacturing of such tissue models requires a standardized and automated production infrastructure, as implemented in the »Tissue Factory« prototype. The first model that was generated with the »Tissue Factory« was the Open Source Reconstructed Epidermis (OS-REp) model, originally developed by Henkel AG & Co. KGaA. For the transfer of this manual protocol into automated production individual process adaptions had to be implemented. The automation of this process was focused on a contactless and stress-free handling of the tissue models as well as on the scaled-up processing of biological materials under sterile conditions. The successful establishment of an automated production protocol for the OS-REp model was confirmed by performing quality control including histological analyses, viability testing and barrier function determination. Histologically, the tissue architecture of the OS-REp models revealed layers similar to those found in healthy human skin. The homogeneous production of epidermal models was demonstrated by analyzing the viability and histological cross sections of at least 12 models of a tissue culture plate. Additionally, a barrier function test was carried out in order to verify that the epidermal models were a suitable test system for skin irritation. In first experiments epidermal models with an ET50 value of at least 3 hours were produced. The »Tissue Factory« is designed for a maximum of 37,440 epidermal models a year.

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