9th Student Symposium on Molecular Medicine

Perspectives in Trauma Research

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April 16, 2016
Symposium Programme

MOLECULAR MEDICINE ULM
CONNECTING TWO WORLDS

Morning Session

08:30	**Introduction**	
08:30 - 09:15	**Prof. Markus Huber-Lang, MD** *Ulm University Medical Centre*	Molecular Danger Management after Polytrauma
	Rebecca Wiegner, MSc *Ulm University Medical Centre*	
09:15 - 10:00	**PD Marcin Osuchowski, DVM, PhD** *Ludwig-Boltzmann-Institute, Vienna*	Age and gender – small steps in modeling of trauma and infection
	Susanne Drechsler, DVM *Ludwig-Boltzmann-Institute, Vienna*	
10:00 - 10:30	**Coffee Break**	
10:30 - 11:15	**Prof. Georg Duda, PhD** *Julius-Wolff-Institute, Charité Berlin*	Research topic: Biomechanics and Musculoskeletal Regeneration
	Taimoor Qazi, MSc *Julius-Wolff-Institute, Charité Berlin*	Modulating regenerative potential of MSCs for muscle regeneration
11:15 - 12:00	**Prof. Martijn van Griensven, MD, PhD** *Klinikum rechts der Isar, TU Munich*	Trauma research – from shock and sepsis to bone regeneration
	Sònia Font Tellado, MSc *Klinikum rechts der Isar, TU Munich*	Fabrication, characterization and osteogenic properties of 3D printed PCL and PCL-Bioactive glass composites
12:00 - 13:00	**Lunch Break**	

Afternoon Session

13:00 - 13:45	**Poster Session**	
13:45 -14:30	**Prof. Armin Blesch, PhD** *Indiana University School of Medicine, USA*	Axonal Regeneration and Neuronal Relays after Spinal Cord Injury
	Ioana Goganau, MSc *University Hospital Heidelberg*	Electrical Stimulation to Enhance Sensory Axon Regeneration after Spinal Cord Injury
14:30 - 15:00	**Student Representatives** *Ulm University*	Master Programme & International Graduate School in Molecular Medicine
15:00 - 15:30	**Coffee Break**	
15:30 - 15:45	**Poster Prize Award Ceremony**	
15:45 - 16:30	**Sebastian Konzok, MSc** *Fraunhofer ITEM, Hannover*	Use of ex vivo Organotypic Lung Tissue in Translational Research of Respiratory Injury and Inflammation Diseases
16:30 - 17:15	**Inga Schalinski, PhD** *Department of Psychology, Konstanz University* **Susanne Breinlinger, MSc** *Department of Psychology, Konstanz University*	Type and Timing of Childhood Adversities on Trauma-Related Symptoms in Adult Inpatients
17:15	**Get-together with Cake and Drinks**	

Abstracts

Molecular danger management after Polytrauma

Prof. Markus Huber-Lang, MD
& Rebecca Wiegner, MSc
Clinical and Experimental Trauma Immunology, Department of Orthopaedic Trauma, Hand, Plastic, and Reconstruction Surgery, Ulm University Medical Centre

Severe tissue injury exposes the body to various PAMPs and DAMPs, resulting in an almost synchronic release of pro- and anti-inflammatory mediators. The “first line of defence” formed
by organ-blood barriers, the serine protease systems, and leukocytes is extensively challenged after trauma by PAMPs/DAMPs-exposure, -sensing, and translation into an effective danger response.
In polytraumatised humans, we have recently described an early excessive activation of the serine protease system, mainly consisting of an intensively cross-talking coagulation and complement system, associated with dysfunctional features and imbalanced regulatory systems. In particular, the complement activation product C5a acts as a strong chemoattractant, recruiting and activating neutrophils within the first 24 h after trauma. Excessive exposure of neutrophils lead to molecular-, cellular- and organ dysfunction, clinically manifested as multiorgan failure and death. Blockade of the C5a-C5aR interaction significantly improved cellular function and overall outcome. Besides complement modulation, novel therapeutic avenues are provided by mesenchymal stem cell application, which might improve regeneration processes and the fatal course of severely injured patients.

Age and gender – small steps in modeling of trauma and infection

PD Marcin Osuchowski, DVM, PhD
& Susanne Drechsler, DVM
Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna

Severe injury induces a systemic inflammatory response associated with widespread activation, but also subsequent impairment of immune responses. Therefore, polytrauma patients are at risk of developing secondary sepsis and/or multiple organ dysfunction syndrome (MODS). Evidence shows that elderly and/or male polytraumatized patients have worse outcome compared to their young and/or female counterparts. Preclinical rodent studies have identified age and gender as two key players in morbidity and mortality of polytrauma and posttraumatic sepsis. However, the specific pathways/mechanisms responsible for this disparity are unclear. Moreover, the inherent resistance of rodents to injury/infection aggravates animal-to-human translation.
While in our mouse 2-hit model of posttraumatic sepsis age/gender affected survival as expected, activation of the humoral inflammatory compartment was surprisingly similar across all groups. We developed mouse models of increasing severity to establish whether more severe polytrauma (e.g. fracture, splenectomy and hemorrhage; TSH) better recapitulates the impaired immune responses observed in human trauma patients. Interestingly, within 48h, TSH induced a systemic increase of CD4+, CD8+ T-cells, MHC-2 expression, C5a, phagocytosis and in-vitro cytokine release capacity. Furthermore, TSH was protective in the subsequent polymicrobial abdominal sepsis. TSH failed to induce immunosuppression in mice but was a strong activator of the immune system. Supported by FWF.

Abstracts

Biomechanics and Musculoskeletal Regeneration

Prof. Georg Duda, PhD
Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration &
Berlin-Brandenburg Center for Regenerative Therapies, Charité Berlin

Bone is a unique and highly regenerative tissue in vertebrates. Unlike to most injuries that lead to fibrotic scar formation and incomplete restoration of the tissue structure and function, bone healing restores pre-fracture properties under optimal conditions. Thus, a scarless repair of structures such as after fracture is possible leading the path to unravel mechanism of true regeneration. Consequently, the investigation of bone regeneration has significant impact on our understanding of how such processes of regeneration are driven and how it is affected by risk factors such as aging. An understanding of the underlying mechanisms and processes might serve as blue-print to other organ systems where regeneration appears even more challenging.
The formation of callus tissue - as intermediate material to reconstitute the body's own structure and function - proved to be mechano-responsive in both, the type and the amount of tissue formed. Demanding mechanical conditions such as in instable fracture fixations lead to a delay of bone bridging, a prolonged cartilaginous phase of endochondral ossification, a reduced and delayed angiogenesis and a prolonged inflammatory phase. All of the relevant cascades of bone healing and formation are directly influenced by mechanical means. The way tissues are formed, the way they mature and aspects of their re-organization are directly influenced by mechanical constrains. Even though the general nature of mechano-sensitivity are widely known, details of their interplay and specially how the mechano-sensitivity at the various length scales from macroscopic mechanics to sub-cellular signaling are yet not fully understood.
Further, the process of bone healing seems to recapitulate aspects of the embryonic skeletal tissue formation and development. It is yet unclear if the processes of formation and repair are indeed similar. To what degree the key-regulator, the mechano-sensitivity, remains constant with time and across processes such as development, maintenance and regeneration is also relatively unknown. Using mesenchymal stromal (or stem) cells (MSCs) as a key element of regenerative capacity, studies from our and other groups in humans and animals have demonstrated an age dependent regeneration potential that seems to decline with increasing age. The reduced mechano-sensitivity of one of the key-elements of regeneration – mesenchymal stroma cells - combined with a shift in material characteristics and change in tissue straining in aged species compared to their younger counterparts illustrates the importance to characterize mechano-senstivity of biological systems not as static and somehow stable systems but as adaptive systems with changing capacities in all stages of aging.
Mechano-biology seems to be apparently a central aspect of the phases of bone healing and regeneration; it plays a key role in maintenance and seems to be also important in early developmental phases. A further understanding of the underlying mechanism of the link between biology and mechanics and their direct interactions at the various lengths scales and across aging seems to be essential to understand healing cascades, their interaction and limitations in healing in clinically demanding situations. This understanding is mandatory to allow effective stimulation of regenerative cascades even under compromised healing conditions.

Modulating regenerative potential of MSCs for muscle regeneration

Taimoor Qazi, MSc
Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration, Charité Berlin

While muscles adapt after minor injuries, major trauma causes permanent functional and structural deterioration. Intramuscular bolus MSC administration has demonstrated effectiveness in restoring muscle function, but the underlying interaction between stromal cells and tissue progenitor cells remains unclear. We demonstrate that stimulation of MSCs with recombinant IGF-1 and VEGF165 enhances their paracrine factor secretion, and that the resulting bioactive factors profoundly influence myoblast survival and function in vitro. Importantly, in a clinically relevant model of severe muscle trauma, we show that the local control of paracrine signaling via engineered niches that condition MSCs significantly improves muscle function by remodeling scar tissue and promoting new fiber formation. The outcomes of this work imply that enhancement of paracrine signaling via local growth factor stimulation of MSCs represents a potent strategy to regenerate compromised muscle tissues, one that outperforms both local MSC and growth factor delivery alone.

Trauma research – from shock and sepsis to bone regeneration

Prof. Martijn van Griensven, MD, PhD
Experimental Trauma Surgery, Klinikum Rechts der Isar, Technical University of Munich

In the acute phase of trauma, many intrinsic systems are activated and try to stabilize the organism and restore homeostasis. Among them, the immune system plays a pivotal role. Gender differences and differences in Bone Mass Index influence the outcome of such patients. Thus, hormones seem to be involved. Indeed, the endocrine system is also a major player influencing the immune system as was shown by measurements in polytraumatized patients. These are on the one hand sex-hormones and on the other hand adipose-derived hormones. This could be confirmed in an in vivo model of combined trauma (lung contusion, liver rupture, femur fracture and hemorrhagic shock). Here, it was also shown that miRNAs play a pivotal role in bone regeneration during trauma. This also influences bone healing in traumatized patients. A set of miRNA could be identified and modulating them modifies the healing response. Furthermore, in these patients, large bone defects may be present that need even more extensive therapy. For those cases, bone tissue engineering using drug delivery systems for growth factors, 3D-printed scaffolds and mesenchymal stem cells are used.

Fabrication, characterization and osteogenic properties of 3D printed PCL and PCL-Bioactive glass composites

Sònia Font Tellado, MSc
Experimental Trauma Surgery, Klinikum Rechts der Isar, Technical University of Munich

INTRODUCTION: Composite scaffolds of polymers and glasses are promising for bone tissue engineering because of their tuneable properties and similarity to the native bone structure. This work describes the fabrication and characterization of scaffolds produced by additive manufacturing combining poly-ε-caprolactone (PCL) and a new class of bioactive glass (BG) particles. This new glass was self-developed using natural origin raw materials. It proved to have osteo- as well as angiogenic properties in 2D culture. Therefore, the main objective of this study was to produce a 3D scaffold composite containing the glass particles. We hypothesise that this composite material could be relevant for bone engineering and regeneration.
METHODS: PCL and PCL-BG scaffolds were prepared as previously described (Poh et al., 2013). Briefly, BG particles (53% SiO2, 24% Na2O and 23% CaO, ≤ 38 µm) were incorporated into 10% (w/v) PCL solution (CAPA 6500, Perstorp, United Kingdom) at 10%wt. Scaffolds were produced by melt extrusion-based additive manufacturing technology at 100 Cº using a 21G nozzle with a lay-down pattern of 0-90°. Scaffolds' porosity and morphology were analysed by µCT scan and SEM. Bioactivity was evaluated by incubation in Simulated Body Fluid (Koko et al., 1990) followed by surface characterisation by SEM-EDX. For mechanical testing, scaffolds were subjected to 10% compression at a rate of 1 mm min−1 in a zwicki1120 microtester fitted with a 2.5 kN load cell (n=8). Cytocompatibility and osteogenic properties were analysed in vitro by culturing human adipose mesenchymal stem cells (AMSCs) in osteogenic media in absence of dexamethasone. Cell viability was evaluated by MTT, LDH and live/dead staining. Osteogenic differentiation was analysed by osteogenic gene expression (qPCR) and mineral deposition (Kossa staining). Experiments were performed in triplicates of 3 independent donors (N=3, n=9). Statistical analysis was done with GraphPad Prism using two-way Anova.
RESULTS: µCT and SEM analysis showed that the PCL and PCL-BG scaffolds had a uniform morphology with open and highly interconnected pores (table 1). The distribution of the BG particles in the PCL matrix was homogeneous and lead to the deposition of a calcium phosphate layer on the surface of the scaffolds. In addition, the compressive modulus of PCL-BG scaffolds was increased in comparison to PCL at similar porosity. In vitro studies showed that incorporation of BG particles resulted in reduced toxicity and improved cell attachment to the scaffold's surface. In addition, enhanced mineral deposition was observed on the scaffolds containing BG particles. Finally, qPCR results showed that the expression of the osteogenic markers ALP and osteopontin and the vascularization marker VEGF was markedly upregulated in PCL-BG compared to PCL after 14 days of culture.
DISCUSSION & CONCLUSIONS: Additive manufacturing is a suitable technique for the fabrication of PCL-BG composites with an interconnected structure and homogenous mineral distribution. The BG particles used in this study promoted the expression of osteogenic markers in AMSCs and the deposition of a mineralized matrix. Importantly, the master vascularization regulator VEGF was highly upregulated suggesting that vascularization may be improved. In conclusion, the PCL-BG composite scaffolds described in this study show promise for bone tissue engineering applications.

Axonal Regeneration and Neuronal Relays after Spinal Cord Injury

Prof. Armin Blesch, PhD
Spinal Cord Injury Center, Heidelberg University Hospital &
Spinal Cord and Brain Injury Center, Indiana University School of Medicine, Indianapolis, USA

Over the last 2 decades, major advances have been made in understanding the mechanisms limiting axonal regeneration in the injured adult mammalian CNS. Besides inhibitory signals and a lack of stimulatory influences in the environment of injured axons, a means to activate the regenerative capacity of injured neurons might be needed to achieve long-distance growth of injured axons. Regeneration of dorsal column sensory axons has been one focus of our efforts to achieve functional target reinnervation across a spinal cord lesion site using a combination of cellular graft, neurotrophin gradients and stimulation of the intrinsic growth competence of injured neurons. As an alternative approach, neural stem cells grafted to a lesion site might be able to serve as a neuronal relay that receives supraspinal input and sends projections to distal target neurons. This approach has recently been shown to partially restore some sensorimotor and cardiovascular parameters in rats with complete spinal cord transections. Our talks will summarize some of these data highlighting the complexity of axonal regeneration for meaningful functional recovery.

Electrical Stimulation to Enhance Sensory Axon Regeneration after Spinal Cord Injury

Ioana Goganau, MSc
Spinal Cord Injury Center, Heidelberg University Hospital

Neuronal activity-dependent mechanisms shape the function of the nervous system during development and adulthood and influence axon sprouting, dendrite branching, pruning and synapse formation. This contributes to partial functional recovery after brain trauma, stroke and incomplete spinal cord injury. Whether neuronal activity can also modulate the intrinsic regenerative capacity of injured neurons has not been sufficiently investigated to date. Our work focuses on dorsal root ganglion (DRG) neurons that are easily accessible for electrical stimulation via their peripheral branch, or by paravertebral or epidural stimulation. Injuries to the peripheral branch of DRG neurons are also known to induce complex changes in neuronal gene expression that increase regeneration of the central branch ascending in the spinal cord (so called conditioning effect). This provides a positive control for potential effects of electrical stimulation. Using in vitro models of DRG neurite growth and KCl depolarization, and in vivo models of spinal cord injury with electrical stimulation with cuff electrodes around the sciatic nerve, our results indicate that the DRG growth capacity can be modulated by externally-induced activity, without adverse effects on pain behavior. Thus electrical stimulation might be useful in combination with other treatments to enhance axonal regeneration in the injured spinal cord.

Use of ex vivo Organotypic Lung Tissue in Translational Research of Respiratory Injury and Inflammation Diseases

Sebastian Konzok, MSc
Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover

Every day, the epithelial surface of our respiratory tract is exposed to 10.000 litres of air. This inhaled air contains a plethora of potential agents, from physical agents like cold air to chemical agents or biological ones such as bacteria. This exposure can lead to adverse health outcomes, ranging from minor dysfunctions to life-threatening diseases. These outcomes include inflammation, COPD, fibrosis, infections, cancer and lung injury such as acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), with the latter being the focus of our current research.
Usage of appropriate in vitro models with the highest impact on the real life situations is crucial in regards to airway biology and pathology. As an ex vivo organotypic model, Precision-Cut Lung Slices are comprised of epithelial cells, fibroblast, smooth muscle cells, nerve fibres, and immune cells. Cells are still viable and interact with each other, thus reflecting the highly specialized function of the lung. Lung tissue can be exposed to different substances and examined for phenotyping of cellular changes, respiratory toxicity and immune responses.
By this, different features of respiratory diseases can be investigated by using tissue of different species, including human. We found that the tissue response is highly comparable with the in vivo response and thus can be used for the prediction of toxicological endpoints and adverse health outcomes such as organ injury, respiratory sensitization and inflammation.

Type and Timing of Childhood Adversities on Trauma-Related Symptoms in Adult Inpatients

Inga Schalinski, PhD
& Susanne Breinlinger, MSc
Department of Psychology (Clinical Psychology), University of Konstanz

The initial portion of the talk provided by Inga Schalinski begins with the current literature regarding the relation of cumulative trauma on symptom severities: A dose-dependent effect of Adverse Childhood Experiences (ACE) on severity of symptoms has been frequently reported. Recent evidence indicates additional impact of type and timing of distinct ACE on symptom severity in support of stress-sensitive periods in (brain) development. Both models (dose-dependent and sensitive type and timing) have been supported by empirical data, however not yet sufficiently contrasted in their predictive power for symptom dimensions such as symptoms of posttraumatic stress disorder (PTSD), dissociation and depression. Susanne Breinlinger will focus on the methods and results of the study. Exposure to ten types of maltreatment up to age 18 were retrospectively assessed in N = 129 psychiatric inpatients using the Maltreatment and Abuse Chronology of Exposure (MACE). Symptoms of PTSD, shutdown dissociation, and depression were related to type and timing of ACE and their predictive power was compared to that of global MACE measures of duration, multiplicity and overall severity. Lastly, Inga Schalinski will discuss the findings how type and timing of ACE improves understanding of vulnerability, and informs diagnostics of psychopathology like PTSD, dissociation and depression.

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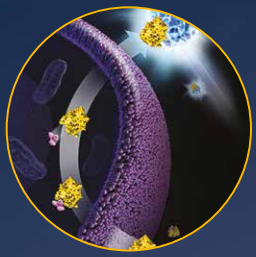

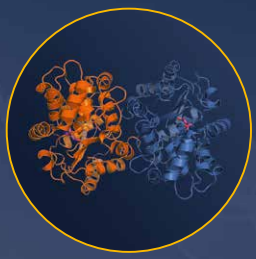

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