Program

von-Behring-Röntgen-Symposium 2015

Bone Disorders and
New Treatment Concepts

October 9th – 10th, 2015
Giessen, Germany

BFS (Biomedizinisches Forschungszentrum Seltersberg)
Justus-Liebig-University Giessen

von behring|röntgen|
stiftung

www.von-behring-roentgen-symposium.com

JUSTUS-LIEBIG-
UNIVERSITÄT
GIESSEN

Philipps Universität Marburg

Synovasure® PJI

Alpha Defensin Test for
Periprosthetic Joint Infection

Demand More From Your Diagnosis

High accuracy
with 97 % sensitivity and 96 % specificity[1]

Reproducible
results not affected by antibiotics treatment[1]

Quick
results in 10 minutes

Easy to use
no laboratory needed

synovasure.zimmer.com/eu

1 Deirmengian et al – Combined Measurement of Synovial Fluid a-def and CRP level – J Bone Joint Surg Am. 2014;96:1439-45

©2015 Zimmer Biomet

ZIMMER BIOMET
Your progress. Our promise.™

Lit.No. 06.02708.012

INDEX

Universities of Giessen and Marburg
Univ.-Prof. Dr. Christian Heiss (Giessen)
Univ.-Prof. Dr. Steffen Ruchholtz (Marburg)

WELCOME ADDRESS

© Justus-Liebig-University; Pressestelle Franz Möller

Dear distinguished conference participants,

I would like to warmly welcome you in Giessen to the von-Behring-Röntgen Symposium on "Bone Disorders and New Treatment Concepts". It is a pleasure that the international and interdisciplinary group of researchers will be meeting at Justus-Liebig-University (JLU) this year.

The topic of the symposium is of great relevance as diseases such as Osteoporosis and other musculoskeletal disorders affect millions of individuals. They can lead into drastic changes in bone physiology and structural properties, which has effects on the implementation feasibility of bone substitute materials. That is why it is of high importance to develop novel solutions to such bone defects and investigate them. I am sure that this symposium will bring about new ideas and contribute to the advancement of the scientific community in this field. I am glad to see that the tradition of our eponym Justus Liebig, namely using interdisciplinary synergies, promoting excellence in research training and broad international networking figure very prominently also within the scope of this symposium. Not only since the foundation of the Universities of Marburg and Giessen Research Alliance has the brilliance of Mittelhessen as a medical location been recognized both nationally as well as around the globe: The participation in several German centers for health research as well as large collaborative projects in the Excellence Initiative of the federal and state governments, special research areas of the DFG and the state excellence program LOEWE leave no doubt about the large potential of the region in medicine: Giessen and Marburg represent the third largest university medical center in Germany. At this excellent location, the international symposium shall provide the basis for a network for bone disorders, promoting scientific communication and exchange. At JLU Giessen, we strive to support young researchers and to provide networks for their academic careers. Therefore, it shall not remain unmentioned that I am very thankful to the von-Behring-Röntgen-foundation for its commitment in supporting students and early career researchers.

I wish all participants an interesting and rewarding symposium with lots of inspiring academic insights and opportunities to generate new ideas for successful and creative science in a multidisciplinary environment.

Prof. Dr. Joybrato Mukherjee
President of Justus-Liebig-University Giessen

Sehr geehrte Damen und Herren,

zum siebten Mal kommen herausragende Wissenschaftlerinnen und Wissenschaftler zum von-Behring-Röntgen-Symposium zusammen, um aktuelle medizinische Forschungsfragen zu diskutieren. Ich freue mich, dass sich diese internationale und interdisziplinäre Konferenz in Mittelhessen so gut etabliert hat.
Gute Lösungen für die Heilung von Knochendefekten zu finden, bleibt eine wichtige Aufgabe für die Forschung und in der Patientenversorgung. Das Konferenzprogramm zeigt, wie vielfältig diese Aufgabe ist. Trotz vieler Fortschritte etwa bei der Behandlung der weit verbreiteten Osteoporose, in der Unfallchirurgie oder bei der schwierigen Diagnose und Behandlung der Multiplen Myeloma geht die Suche nach Verbesserungen weiter. Dazu gehört auch die für die Lebensqualität von Patienten wichtige Forschung zu Implantaten.
Mit dem diesjährigen Gastgeber der Konferenz, der Justus-Liebig-Universität Gießen, verbindet die Philipps-Universität Marburg eine gute Tradition der Zusammenarbeit in der Medizin. An den beiden Standorten wirken die diesjährigen Konferenzleiter Prof. Dr. Steffen Ruchholtz aus Marburg und Prof. Dr. Christian Heiss aus Gießen als renommierte Wissenschaftler und Kliniker, die die ganze Bandbreite von der Behandlung erblich bedingter Knochendefekte bis hin zu Schwerstverletzungen beherrschen. Die Stärke mittelhessischer Forschung auf diesem Gebiet zeigt sich auch in dem in Gießen angesiedelten DFG-geförderten Sonderforschungsbereich zu neuen Knochenersatzmaterialien und Implantatwerkstoffen.

Ich wünsche den Teilnehmerinnen und Teilnehmern eine erfolgreiche Konferenz mit wertvollen Einblicken, neuen Erkenntnissen und Anregungen für die weitere Zusammenarbeit in der internationalen Wissenschaftsgemeinschaft.

Prof. Dr. Katharina Krause
Präsidentin der Philipps-Universität Marburg

WELCOME ADDRESS

Dear distinguished conference participants,

we welcome you on behalf of our foundation to the 6th von-Behring-Röntgen-Symposium entitled “Bone disorders and new treatment concepts”. We are very pleased that the von-Behring-Röntgen-Symposium, which is first held in 2009, this year is home at Justus-Liebig-University of Giessen.

The von-Behring-Röntgen-Stiftung was founded by the federal State of Hessen in 2006.

With its capital of 100 million euros our foundation is one of the largest in the field of medical foundations in Germany. She supports and promotes the medical faculties of the Justus-Liebig-University of Giessen and the Philipps-University of Marburg in their network of life sciences and other academic fields: National and international research cooperation, development projects for new methods in research and education, young scientists, projects related to applied research, joint Projects combining the medical faculties of the universities of Giessen and Marburg and scientific communication through conferences and symposiums.

Both universities steeped in tradition. Philipps-University of Marburg it is the oldest university in the world that was founded as a Protestant institution in 1527. It has been a place for research and teaching for almost 5 centuries. In the course of more than 400 years since its founding in 1607, the Justus-Liebig-University of Giessen has also grown to a full-scale university with a various fields of excellence.

With their namesakes, Emil von Behring and Wilhelm Conrad Röntgen, the von-Behring-Röntgen-Stiftung recalls two outstanding individuals. Emil von Behring (1854 - 1917) was a bacteriologist and serologist in Berlin and later in Marburg, invented sera against diphtheria and tetanus.

Wilhelm Conrad Röntgen (1845 – 1923) was from 1879 to 1888 professor of physics in Giessen and discovered later in Würzburg the “x-rays”, before he changed to Munich.

Both earned the Nobel Prize when it was ever awarded for the first time in 1901: Emil von Behring for Medicine and Wilhelm Conrad Röntgen for Physics.

Since 2008 our foundation had already supported about 70 projects with about 13 million euros and among other conferences this one with its important and highly topical subject.

One principal part of research is sharing and discussing theories. The von-Behring-Röntgen-Symposium will give the chance to learn about achievements in the treatment of bone disorders and fractures especially osteoporosis and multiple myeloma.

We are very proud that this year´s von-Behring-Röntgen-Symposium is devoted to these important diseases and that such a prestigious group of renowned investigators followed our invitation to come to Mittelhessen to present and discuss the latest research and discoveries in this field.
We wish you all an inspiring conference and a pleasant stay in Giessen!

Friedrich Bohl
President, von-Behring-Röntgen-Stiftung

WELCOME ADDRESS

Dear distinguished conference participants,

On behalf of the Faculty of Medicine of the Justus-Liebig-University, I kindly wish to welcome you to this year's von-Behring-Roentgen-Symposium on "Bone Disorders and New Treatment Concepts". The von-Behring-Roentgen Foundation was founded in 2006 with the primary purpose of supporting and promoting both basic and clinical research and teaching at the medical faculties of the Universities of Giessen and Marburg. As one of its primary activities, it hosts annual international symposia on topics related to profile areas of research at both medical faculties. I am extremely grateful to the members of the Executive Board of the Foundation for this ongoing support. Alterations in bone physiology and structure properties, resulting from bone disorders, are significantly linked to research activities in bone substitute material integration and function. Osteoporosis and multiple myeloma are especially important challenges in this context, requiring a comprehensive approach in the improvement of bone diseases and consecutive fractures. Tissue regeneration is one of the profile research areas of our faculty and the SFB Transregio 79, which addresses "Materials for tissue regeneration within systematically altered bone," has been recently reevaluated and granted until 2018 by the DFG under the direction of Univ.-Prof. Dr. Christian Heiss. This transregional research group is compiled of a network of activities from different universities including Giessen, Heidelberg, and Dresden. Our medical faculty participates in several German centers for health research, collaborative projects in the Excellence Initiative of the federal and state governments, special research areas of the DFG, and the state excellence program LOEWE. In cooperation with the UKGM GmbH, our medical faculty represents the third largest university medical center in Germany. The faculty of medicine is the largest faculty of the JLU, hosting approximately 2.850 students, and benefiting from an innovative training concept, a graduate school, and an international Ph.D. program. On behalf of all members of our faculty, I wish you a successful and exciting symposium, filled with new ideas and fruitful discussions with colleagues and friends. Finally, I wish to take this opportunity to thank the speakers and the participants for their contributions toward this meeting, as well as the hosts for putting together an excellent program and for working hard to make it a highlight of the joint activities of the Faculties of Medicine in Giessen and Marburg.

Wolfgang Weidner
Dean, Faculty of Medicine, Justus-Liebig-University Giessen

Dear Colleagues and Guests,

The Medical Faculty is happy to welcome you to the von-Behring-Röntgen-Symposium.

'Mobility is life´ is a famous slogan in modern societies. This counts especially for elderly patients. Mobility is directly related to a longer and healthier survival. We have to be aware that mobility and activity highly depend on a loadable musculoskeletal system.

Particularly the aging bone is at a high risk for the development of relevant chronic systemic or local disorders. Bone diseases like osteoporosis and multiple myeloma can lead to important alteration of bone physiology and structure.

These are the topics of this symposium. The intention is to highlight and discuss actual results and concepts of basic research all around the musculoskeletal system. The presentations will cover the development of osseous diseases, local and systemic treatment strategies as well as the implementation of bone substitute materials.

I wish you all a pleasant stay in Giessen and a fruitful scientific exchange and inspirations for your personal research and clinical work.

Prof. Dr. Helmut Schäfer
Dean, Faculty of Medicine,
Philipps-University Marburg

**Dear Colleagues, Dear Fellow Scientists,
Dear Guests,**

We are pleased to personally welcome you to the von-Behring-Röntgen-Symposium at the BFS (Biomedizinisches Forschungszentrum Seltersberg) of the Justus-Liebig University of Giessen.
A core principle of the von behring|röntgen|foundation and the SFB/Transregio 79 is that successful and creative science depends upon innovative and multidisciplinary teamwork; we hope this meeting will promote and facilitate this goal. Musculoskeletal disorders can lead into drastic alteration in bone physiology and structural properties, which reflects on the implementation feasibility of bone substitute materials. When we contemplate the advancements in bone disorders, especially osteoporosis and multiple myeloma, our thoughts go to the contributions of German research institutions as an integral part of the European and International scientific community. For the next days we hope to instigate an opportunity for debate, discussion and the exchange of ideas in the rapidly developing field of musculoskeletal disorders. We have tried to incorporate greater opportunity for participants to present their work in an interactive environment. We have an impressive list of oral and poster presentations from all over the world which we trust will stimulate further discussions and research ideas.

We would like to encourage all researchers and clinical fellows to start and maintain provocative links and communication. We remind them of their obligation to bring the developed concepts of future project to the attention of interested communities and raise awareness of the general public. Also to extend the experience to their personal and institutional networks as well as funding bodies.

The coordination office of the Transregio 79 hosted by the University of Giessen will help collect ideas and initiate a smooth follow-up to create new joint-project incubators.

We wish you all an inspiring conference!

Univ.-Professor Dr. Christian Heiss
Chairperson of Scientific Committee, JLU-University of Giessen

aap

Quality & Innovation in Fracture Treatment
Made in Germany

LOQTEQ®

Locking Compression Technology by aap

One Hole One Screw One Step

Made in aap Germany

aap Implantate AG
Lorenzweg 5 • 12099 Berlin
www.aap.de

CONGRESS VENUE

BFS (Biomedizinisches Forschungszentrum Seltersberg)
Justus-Liebig-University Giessen
Schubertstr. 81
35392 Giessen, Germany

CONGRESS HOURS

Friday, October 9th, 2015	08.30 am – 06.00 pm
(Registration: 07:45 am)	
Saturday, October 10th, 2015	07.45 am – 01.00 pm

POSTER SESSIONS

Session I:	
Friday, October 9th, 2015	01.00 pm – 02.00 pm during lunch break
Session II:	
Saturday, October 10th, 2015	07.45 am – 08.45 am

SOCIAL PROGRAM

Get-together with dinner and live music (underCover)
Friday, October 9th, 2015 06.00 pm

CONGRESS SECRETARIAT

Department of Trauma, Hand and Reconstructive Surgery
Laboratory of Experimental Trauma Research
Rudolf-Buchheim-Str. 7
35392 Giessen, Germany
Phone: +49 641 985-44613
Fax: +49 641 985-44609
E-mail: martina.hofacker@chiru.med.uni-giessen.de

CONGRESS ORGANIZATION / REGISTRATION OF DELEGATES

wikonect GmbH
Hagenauer Str. 53
65203 Wiesbaden, Germany
Phone: +49 611 20 48 09-22
Fax: +49 611 20 48 09-10
E-mail: info@wikonect.de
Web: www.wikonect.de

ACCREDITATION | CME

The von-Behring-Röntgen-Symposium is certified with 13 CME credits by the 'Landesärztekammer Hessen'. Please do not forget to bring your Barcode-stickers (EFN-No.) for each day.

SUPPORTED BY

von behring|röntgen| stiftung

SFB/TRR79

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Philipps Universität Marburg

DFG Deutsche Forschungsgemeinschaft

LOEWE

Bundesministerium für Bildung und Forschung

GGL International Giessen Graduate Centre for the Life Sciences

Univ.-Prof. Dr. Christian Heiss (Giessen)

Univ.-Prof. Dr. Steffen Ruchholtz (Marburg)

Prof. Dr. Dr. Volker Alt (Giessen)

Prof. Dr. Michael Frink (Marburg)

Prof. Dr. Katrin S. Lips (Giessen)

Dr. Phillip Lechler (Marburg)

FRIDAY, OCTOBER 9th, 2015

BFS (Biomedizinisches Forschungszentrum Seltersberg), B, Großer Hörsaal

8:30 am — **Opening Ceremony**
J. Mukherjee, President
Justus-Liebig-University, Giessen
F. Bohl, President von-Behring-Foundation, Marburg
W. Weidner, Dean Faculty of Medicine,
Justus-Liebig-University, Giessen
H. Schäfer, Dean Faculty of Medicine,
Philipps-University, Marburg
S. Ruchholtz, scientific board,
Philipps-University, Marburg
C. Heiss, scientific board, Spokesman SFB TRR 79
Justus-Liebig-University, Giessen

9:15 – 10:45 am — **Session 1: Bone – A fascinating organ**
Chairs:
E. Baumgart-Vogt, Giessen (Germany),
T. Pohlemann, Homburg/Saar (Germany)
V. Ziller, Marburg (Germany)

9:15 am
Keynote — **Ossification and bone remodeling**
E. Baumgart-Vogt, Giessen (Germany)

9:30 am
Keynote — **Mouse models: Valid approach for exploring bone healing and metabolism?**
T. Pohlemann, Homburg/Saar (Germany)

9:45 am
Keynote — **Sarcopenia, obesity and bone health in community-dwelling older adults**
D. Scott, Monash (Australia)

10:00 am
Keynote — **Bone extracellular matrix proteins – true regulators**
T. ElKhassawna, Giessen (Germany)

10:15 am
Free paper — **Neovascularisation of the critical size defect in osteoporotic bone: a morphometric micro-CT study**
M. Kampschulte, Giessen (Germany)

10:25 am
Free paper
Optimization of the electrospinning process for production of mesenchymale stem cell (MSC) seeded, nanofiber scaffolds for bone regeneration
K.- F. Schüttler, Marburg (Germany)

10:35 am
Free paper
Effect of M3 muscarinic acetylcholine receptor deficiency on collagen antibody-induced arthritis
K. S. Lips, Giessen (Germany)

10:45 am
Coffee break

11:15 – 1:00 pm
Session 2: Multiple myeloma and associated bone lesions
Chairs:
W. Seeger, Giessen (Germany)
D. Hose, Heidelberg (Germany)
A. Neubauer, Marburg (Germany)

11:15 am
Keynote
How do I diagnose myeloma?
T. Möhler, Heidelberg (Germany)

11:30 am
Keynote
How and when do I treat multiple myeloma?
H. Salwender, Hamburg (Germany)

11:45 am
Keynote
What makes up myeloma molecularly – dissecting the clonal architecture?
D. Hose, Heidelberg (Germany)

12:00 am
Keynote
How does asymptomatic myeloma evolve and progress?
A. Seckinger, Heidelberg (Germany)

12:15 am
Keynote
When does myeloma start? Timing of initial events in myeloma genesis?
S. Sahota, Southampton (United Kingdom)

12:30 am
Free paper
Dendritic glycopolymers and their polyelectrolyte complexes as efficient drug delivery systems for retarded release of bortezomib from calcium phosphate cements
B. Mamitzsch, Dresden (Germany)

12:40 am
Free paper
Biomaterials for myeloma bone lesions – In vitro release of bortezomib from bioactive calcium phosphate-containing silica/collagen xerogels and in vivo effect on bone remodeling
S. Rössler, Dresden (Germany)

12:50 am
Free paper
Unravelling the biocompatibility and new bone formation capabilities of bortezomib-loaded biomaterials
F. Alagboso, Giessen (Germany)

1:00 pm
Lunch & Poster Session I

2:00 – 3:30 pm
Session 3: Osteoporosis – Basics and medical management
Chairs:
A. Schaeffler, Giessen (Germany)
P. Aspenberg, Linköping (Sweden)
P. H. Kann, Marburg (Germany)

2:00 pm
Keynote
Bisphosphonates in orthopedics
P. Aspenberg, Linköping (Sweden)

2:15 pm
Keynote
Antibodies for treatment of osteoporosis
M. Schieker, Munich (Germany)

2:30 pm
Keynote
New clinical and pharmaceutical aspects of osteoporosis treatment
P. Drees, Mainz (Germany)

2:45 pm
Keynote
Osteoporosis – Cellular basics and medical management
P. Ebeling, Monash (Australia)

3:00 pm
Free paper
Preservation of bone ultrastructure using high pressure freezing and microwave assisted chemical fixation
D. E. S. Daghma, Giessen (Germany)

3:10 pm
Free paper
ToF-SIMS analysis of lipids in hMSCs harvested from osteoporotic and control bone – comparative study of cells from adipogenic, osteogenic and basal culture conditions
K. Schaepe, Giessen (Germany)

3:20 pm Free paper	**Evaluation and establishment of a sheep model of osteoporosis – an insight in the T-Value standard** D. Weisweiler, Giessen (Germany)
3:30 pm	Coffee break

4:00 – 6:00 pm **Session 4: Disturbance of fracture healing – Mechanical or biological problem or both?**
Chairs:
A. Ignatius, Ulm (Germany)
L. Cheung, Hong Kong (China)
S. Ruchholtz, Marburg (Germany)

4:00 pm Keynote	**Inflammation and fracture healing: A matter of balance** A. Ignatius, Ulm (Germany)
4:15 pm Keynote	**Beyond mechanical stability: Fracture healing under healthy and compromising conditions** S. Geissler, Berlin (Germany)
4:30 pm Keynote	**Problems of osteoporotic fracture healing – Mechanobiology and mechanosensitivity** L. Cheung, Hong Kong (China)
4:45 pm Keynote	**Expression of the cholinergic system in bone and bone defect healing** K. S. Lips, Giessen (Germany)
5:00 pm Free paper	**Influence of mast cells on fracture healing in mice** J. Kroner, Ulm (Germany)
5:10 pm Free paper	**Regulation of biological processes in fracture repair** D. Malhan, Giessen (Germany)
5:20 pm Free paper	**Influence of neutrophil granulocyte depletion on fracture healing in mice** A. Kovtun, Ulm (Germany)
5:30 pm Free paper	**Comparison of metaphyseal fracture defect healing between osteoporotic and non-osteoporotic bone of the rat** U. Thormann, Giessen (Germany)
6:00 pm	**Get-together**

SATURDAY, OCTOBER 10th, 2015

BFS (Biomedizinisches Forschungszentrum Seltersberg), B, Großer Hörsaal

7:45 – 8:45 am **Poster Session II**

9:00 – 10:30 am **Session 5: Bone and implant infections – What is the evidence?**
Chairs:
V. Alt, Giessen (Germany)
G. Schmidmaier, Heidelberg (Germany)
M. Lohoff, Marburg (Germany)

9:00 am Keynote	**The clinical problem of implant-infections in bone surgery** V. Alt, Giessen (Germany)
9:15 am Keynote	**Biofilms on medical devices: composition, complications and consequences** A. Moter, Berlin (Germany)
9:30 am Keynote	**Microbiomic analysis of removed internal fixation devices** E. Domann, Giessen (Germany)
9:45 am Keynote	**Antibiotica-coated implants for prevention of implant related infection** G. Schmidmaier, Heidelberg (Germany)
10:00 am Free paper	**Impact of CLP-induced sepsis on bone in a murine animal model** W. Floel, Giessen (Germany)

10:10 am
Free paper
Intracellular Staphylococcus aureus in osteoblasts can be killed via TLR9-mediated induction of oxidative stress
W. Mohamed, Giessen (Germany)

10:20 am
Free paper
Microbiological analysis of antibacterial effectivity of silver-nanoparticle (Agnp)-dopted bone substitute materials in an experimental rat model
A. Lampe, Giessen (Germany)

10:30 am
Coffee break

11.00 – 12.45 am **Session 6: Smart and new implants – How smart and new are they really?**
Chairs:
C. Heiss, Giessen (Germany)
P. Roschger, Vienna (Austria)
M. Frink, Marburg (Germany)

11:00 am
Keynote
Bone material quality in health, disease and treatment
P. Roschger, Vienna (Austria)

11:15 am
Keynote
Fracture monitoring – Endeavors and obstacles
M. Ernst, Davos (Switzerland)

11:30 am
Keynote
New implants in acetabular surgery
W. Lehmann, Hamburg (Germany)

11:45 am
Keynote
Bridging the gap between biochemically and biomechanically smart materials: Anorganic/organic composites for bone replacement
T. Hanke, Dresden (Germany)

12:00 am
Free paper
Development of new beta-Ti alloys for hard tissue replacement
R. Schmidt, Dresden (Germany)

12:10 am
Free paper
Role of osteocyte in implant degradation and bone – Implant anchorage
S. Ray, Giessen (Germany)

12:20 am
Free paper
Bone Morphogenetic Protein 7 (BMP-7) influences tendon-bone integration in vitro
T. Schwarting, Marburg (Germany)

12:45 am
Award Ceremony

1:00 pm
End of Symposium

FRIDAY, October 9th, 2015
1.00 pm – 2.00 pm, during lunch break

1F
Deficiency of M3 muscarinic receptor aggravates joint destructive effects in murine collagen-antibody-induced arthritis

Nicole Dittmann[1], Janet Beckmann[1], Iris Schütz[1], Jochen Klein[2], Katrin Susanne Lips[1],

[1] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University, Giessen, Germany
[2] Department of Pharmacology, School of Pharmacy, Goethe-University Frankfurt, Frankfurt am Main, Germany

There is increasing evidence that the cholinergic system might be important for rheumatoid arthritis pathology, but the role of M3 muscarinic receptor (M3R) was not investigated, yet. Thus, we sought to analyze if M3R-deficiency might be protective for experimental arthritis. Collagen-antibody-induced arthritis (CAIA) was induced in M3R-/- and WT mice and severity of arthritis was assessed by scoring of paw swelling. The joints were analyzed for histopathological changes in synovial tissue, cartilage and bone by means of histological scoring, immunohistochemistry, and transmission electron microscopy (TEM). Synovial tissue of arthritic M3R-/- mice showed a tendency towards more severe pathological changes. A strong thickening and pannus formation was observed. Ultra-structural analysis of arthritic synoviocytes showed hypertrophy and an increase in number of vacuoles and endoplasmatic reticulum in snovial macrophages and fibroblasts, respectively. Using α-smooth muscle actin immunohistochemistry an enhanced number of positive blood vessels was found in the thickened walls of M3R-/- CAIA mice compared to LPS control mice. Articular cartilage of arthritic animals showed a rough surface that was destroyed at many places while cartilage the control animals had a smooth surface. Using TEM chondrocytes of arthritic M3R-/- mice looked less vital, with dense chromatin and cytoplasmic vacuoles. Toluidine blue stained sections of the knee joints revealed a significant enhancement of bone erosion in M3R-/-CAIA mice compared to WT with CAIA. Taken together, we showed that M3R-/- mice were not protected from CAIA. Unexpectedly, arthritic M3R-/- mice showed a significant stronger joint destruction, indicating that stimulation of M3R might be protective for arthritis.

Supported by LOEWE Hessen Germany (NNCS, project B2).

2F
Histological and enzymehistochemical investigations of osteoporotic bone of the rat

Asmaa Eldaey[1], Sabine Wenisch[1]

[1] Clinic of Small Animals, c/o Institute of Veterinary Anatomy, Histology and Embryology, Justus-Liebig-University of Giessen

Bone is a mineralized tissue and is composed of osteoblasts and osteoclasts which are responsible for bone formation and resorption. Imbalances between osteoblasts and osteoclasts result in a systemic disorder called osteoporosis. Osteoporosis is caused by hormonal alterations or glucocorticoid medication and leads to reduced bone strength followed by high fracture risk. Regarding the usefulness of animal models in osteoporosis research it was the aim of the present investigation to examine the distribution pattern of osteoblasts and osteoclasts as well as the morphological features of bone in normal and osteoporotic treated rat. Female rats were assigned to the following groups: controls, Sham, ovariectomized with diet deficiency (OD) and ovariectomized with steroid (OS). Rats were euthanized after 3 and 12 months. Lumber vertebrae were dissected and processed for histological and histochemical analyses. In controls intact bone and single layers of osteoblasts covering the bone surfaces were found. In the OD group, an increase of osteoblasts and thin widely separated trabeculae could be detected. In the OS group, cortical bone showed no changes whereas cancellous bone revealed severe loss. Two way-ANOVA performed after alkaline phosphatase staining revealed significant increases of stained areas in OD and OS groups compared to controls ($p=0.05$ and $p=0.001$). Interestingly, bone of the OS showed a distinct increase of osteoblasts ($p=0.09$) compared to the OD. This might be caused by greater remodeling activities via osteoblasts proliferation in order to compensate the severe bone loss caused by steroid application in combination with ovariectomy. Next, osteoclast and connexin 43 distribution pattern will be examined enzyme- and immunohistochemically.

3F

A functional examination of human bone material from the upper extremety of a Complex Regional Pain Syndrom (CRPS) patient

Stina Maria Langer[1], Janet Beckmann[1], Gabor Szalay[2], Christian Heiss[2], Heidrun H. Krämer[3], Katrin Susanne Lips[1]

[1] Laboratory for Experimental Trauma Surgery, Justus-Liebig-University, Giessen, Germany
[2] Department of Trauma Surgery Giessen, University Hospital of Giessen-Marburg, Campus Giessen, Giessen, Germany
[3] Department of Neurology, University Hospital of Giessen-Marburg, Campus Giessen, Giessen, Germany

Complex regional pain syndrome (CRPS) is a painful disorder, occuring at the distal parts of the limbs after trauma. The acute phase of CRPS is characterized by inflammatory symptoms like swelling, reddening and warming of the affected skin, as well as by pain. The chronic stage of CRPS is dominated by atrophic changes. Progress has been made to unravel the pathophysiology of CRPS, showing that neurogenic inflammation plays an important role at least in the early stages of CRPS. However, changes deriving from deep structures, e.g. bone, like osteoporosis and decreased range of motion are still matter of investigation. Recently, alterations in bone metabolism have been linked to CRPS pathogenesis. In the present investigation we aimed to further elucidate the processes taking place during chronification of CRPS. For this reason we examined the osseous parts of an amputated arm from a chronic CRPS patient, regarding the functionality of bone and joint.

Different sections of the CRPS affected arm were examined histologically and a histomorphometrical investigation was performed. Furthermore cell- and molecularbiological methods, including functional assay for cell viability (MTT), ELISA and realtime RT-PCR, contributed to a widespread analysis, regarding differences from proximal to distal parts of the upper limb.

Histologically, a clear reduction of bone mass by CRPS was observed, particularly the cortical bone was discontinuous and seemed instable. Synovial fibroblasts from the CRPS joint revealed a significant reduction in vitality and an up-regulation of the pro-inflammatory cytokine interleukin 6 compared to cells from healthy donors.

In the chronic stage, inflammatory processes persist in the osseous parts of the affected limb. These changes might cause an imbalance in bone homeostasis and an alteration in bone metabolism, leading to a dysfunction of the limb. Thus, suppression of osseous reorganisation, triggered by inflammation might be a promising therapeutic strategy to avoid the decomposition of bone during CRPS.

4F

Influence of high-fat diet on murine bone architecture

Hinrich Fehrendt[1], Sonja Hartmann[1], Christian Heiss[1,3], Thomas Linn[2], Reinhard Schnettler[1], Katrin Susanne Lips[1]

[1] Laboratory for Experimental Trauma Surgery, Justus-Liebig-University Giessen, Kerkraderstrasse 9, 35394 Giessen, Germany
[2] Clinical Research Unit, Medical Clinic and Polyclinic 3, Justus Liebig University Giessen, Klinikstrasse 33, 35392 Giessen, Germany
[3] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Rudolf-Buchheim-Strasse 7, 35392 Giessen, Germany

Many health-problems like diabetes or cardiovascular diseases are negatively associated with obesity, whereas the effect of high-fat diet on bone metabolism is still discussed controversially. The purpose of this study was to examine changes in bone structure after high-fat diet.

The study was conducted with thirteen 4 week old C57BL/6J male mice, who were fed either a high-fat diet (HFD) (60% fat, n=7) or regular laboratory chow (10% fat, n=6) for 23 weeks. To explore the effects of a high-fat diet for changes in bone structure we used dual-energy X-ray absorptiometry (DEXA), enzyme- and immunohistology, histomorphometry, RT-PCR and transmission electron microscopy (TEM). The results were statistically evaluated with the Mann-Whitney-Test considered to be significant at $p<0.05$.

Histomorphometric results showed a significant reduction ($p=0.026$) of trabecular surface in distal femora of HFD mice. The cancellous bone of HFD mice showed gaps between trabecular lamellae. Differences in number or differentiation grade of osteoblasts and osteoclast were not observed. Ultrastructurally, a loosening of cell-cell contacts between osteoblasts was found as well as a smaller rim of osteoid.

The mRNA expression of collagen 1 was significantly reduced in humera (P=0.002) and vertebra L3 (P=0.004) of HFD mice.

The study demonstrates negative effects of a high-fat diet on bone status of mice. Clinical trials have determined that overweight adolescents have a higher fracture risk because of poorer posture control. But changes in microstructure or bone metabolism might also contribute to the increased fracture incidence of obese patients.

6F
Bone of Acetylcholinesterase and Butyrylcholinesterase Knockout Mice

Vivien Kauschke[1], Malte Haupt[1], Mathias Kneffel[1], Wolfgang Floel[1], Sonja Hartmann[1], Marian Kampschulte[2], Jonas Sender[2], Lutz Dürselen[3], Anna Kovtun[3], Christian Heiss[1,4], Katrin Susanne Lips[1]

[1] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University Giessen, Giessen, Germany
[2] Department of Radiology, University Hospital of Giessen-Marburg, Giessen, Germany
[3] Institute of Orthopedic Research and Biomechanics, University of Ulm, Ulm, Germany
[4] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Campus Giessen, Giessen, Germany

Acetylcholine (ACh) is degraded by acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE). Knockout (KO) of these enzymes inhibits this process and ACh accumulates. In turn, ACh enhances osteoblasts proliferation and differentiation in vitro. Therefore, we investigated effects of AChE- and BChE-KO on bone in vivo.

Bone of 16-week-old female heterozygous AChE- or homozygous BChE-KO mice and their corresponding wildtypes (WT) were analyzed using biomechanical testing, histology, micro-computed tomography (micro-CT), dual energy X-ray absorptiometry (DEXA) and real-time RT-PCR.

Biomechanical testing showed that bending stiffness and maximum bending force did not differ significantly between AChE-KO and WT mice. However, histology revealed significantly less osteoclasts in AChE-KO mice, whereas their number was increased in BChE-KO mice. Micro-CT analysis showed significantly decreased cortical area fraction in the mid-diaphysis of femur in AChE-KO mice. In BChE-KO mice cortical area fraction, trabecular thickness and trabecular separation of femurs were increased. DEXA measurement revealed similar bone mineral densities between AChE-KO and WT mice. No statistically significant differences were detected in mRNA expression of alkaline phosphatase, cathepsin K and connexin 43 in AChE-KO or BChE-KO mice.
Considering that AChE is essential for survival, it is interesting that a heterozygous AChE-KO does not affect development, including bone-tissue. Similar results between KO and WT mice might be reasoned by mutual compensating effects of AChE and BChE. Possible non-proliferative effects of BChE might explain increased osteoclasts numbers in BChE-KO mice. A bone-specific double-KO of both enzymes or inhibitor administration might clarify consequential effects on bone.

Funded by SFB/TRR 79 and LOEWE NNCS

7F
A histological processing technique that preserves the integrity of calcified tissues and minimize numbers of sacrificed animal models

Thaqif El Khassawna[1], Diaa Eldin S. Daghma[1], Christian Heiss[1,2]

[1] Laboratory for experimental trauma Surgery, Justus-Liebig University of Giessen, Germany
[2] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Giessen, Germany

Hypothesis:
Histological analysis of bone tissue must be decided as either decalcified or undecalcified before sample processing. This study utilized sequential and alternating undecalcified and decalcified sections from the same bone sample to enhance statement deduction, minimize the number of experimental animals and increase the benefit of patient's biopsies.

Methodology:
Intact Rat spine, fractured rat femur and a human tooth were embedded in Polymethylmethacrylat (PMMA). Controls were embedded in paraffin after decalcification in EDTA. PMMA samples were sectioned and grinded at 5 and

10-15 µm thick respectively. Sections from PMMA embedding were then decalcfied in EDTA solution for 2 weeks at 4°. Experimental and control samples were both tested by a hematoxylin eosin stain, an Enzymohistochemichal stain (TRAP) and an immunehistochemical stain (ERK).

Results:
Decalcified PMMA sections preserved their integrity were not different from paraffin sections. The differentiation steps in all staining protocols were maintained according to the paraffin sections.

Conclusion:
In experimental trauma surgery, histological evaluation is crucial. Pre-clinical studies of bone cement require undecalcified embedding to preserve biomaterials. However, most antibodies for IHC or probes for in situ hybridization are established on paraffin-embedded decalcified specimens. In most cases, extra animals are planned to obtain both information. Here we propose a new protocol that allows the work on successive sections and alternately decalcify every second one to obtain all possible information minimizing animal numbers and individual differences between the animals in the differently processed groups.

9F
Influence of ovariectomy, diet and glucocorticosteroids on bone mineral density and morphology of the ovine spongious bone: a micro-CT study

Marian Kampschulte[1,2], Jonas Sender[1,2], Georg Erdmann[2], Wolfgang Böcker[3,4], Thaqif El Khassawna[3], Christian Heiss[3,4], Gabriele A. Krombach[1,2], Alexander C. Langheinrich[2,5]

[1] Department of Diagnostic and Interventional Radiology, University Hospital Giessen and Marburg GmbH, Giessen
[2] Laboratory of Experimental Radiology, Justus-Liebig-University Giessen
[3] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University Giessen
[4] Department of Trauma Surgery, University Hospital Giessen and Marburg GmbH, Giessen
[5] Department of Diagnostic and Interventional Radiology, BG Trauma Hospital Frankfurt/Main

To describe the influence of ovariectomy, diet, and dexamethasone on bone mineral density (BMD) and morphology in a sheep model of osteoporosis.

Adult female merino sheep (n=32) were randomly subjected to control (n=8), ovariectomy (OVX, n=8), ovariectomy plus multideficiency diet (OD, n=8), and ovariectomy plus multideficiency diet plus bi-weekly dexamathasone injections (ODS, n=8). Animals were euthanized 8 months after study initiation. Deep frozen vertebral specimens (L2) were micro-CT scanned with a spatial resolution of 33 µm isotropic voxel side length. Bone mineral density (BMD), bone volume/ tissue volume (BV/TV), trabecular thickness (Tb.Th.), trabecular number (Tb.Nr.) and trabecular separation (Tb.Sp.) were analyzed.
BMD was significantly reduced ($p < 0.001$) in ODS (mean: 0.26 g/cm3, SD: 0.047) compared to control (mean: 0.51 g/cm3, SD: 0.055), OVX (mean: 0.49 g/cm3 SD: 0.047), and OD (mean: 0.46 g/cm3 SD: 0.047). BV/TV and Tb.Th. were significantly reduced ($p < 0.001$) in ODS compared to control, OVX, and OD. Analysis of Tb.Nr. and Tb.Sp. did not show significant differences. We did not find statistical differences between control- and OVX-group resp. control- and OD-group.

Micro-CT imaging revealed a significant loss of BMD with a change of morphometric parameters in the ODS treatment combination. This specific animal model can be considered as a candidate for preclinical research in osteoporosis as it is necessary for the development of bone augmenting biomaterials.

10F
Enzyme-histochemical analysis of cellular bone metabolism in an osteoporotic sheep model

Felix Merboth[1], Wolfgang Böcker[1,2], David Weisweiler[1,2], Katrin Lips[1], Thaqif El Khassawna[1], Christian Heiß[1,2]

[1] Laboratory for experimental trauma Surgery, Justus-Liebig University of Giessen, Germany
[2] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Giessen, Germany

Osteoporosis is an ongoing challenge in trauma surgery and orthopedics. This study aims to investigate the activity of osteoblasts and osteoclasts as key players of bone metabolism.

Four groups of female merino sheep served as control, ovariectomized (OVX), OVX and a calcium-deficient diet (OVXD), and OVXD with corticosteroids (OVXDS). 8 months post treatment femora and lumbar vertebra were

explanted, processed into decalcified sections and then stained with tartrate resistant acid phosphatase (TRAP) for osteoclasts and alkaline phosphatase (ALP) for osteoblasts. Histomorphometry was performed in ImageJ software and statistical analysis was ran in IBM SPSS software.

Osteoclasts number and their Howship`s lacuna length were highest in OVX femur compared with all groups. In contrast, these values were lowest in OVXDS vertebra compared with all other groups. However, ALP area and surface length were higher in OVXDS in the femora, where as in the vertebra the increase between control and OVXDS was non/significant but with a trend. Interestingly, triple treatment OVXDS shows a non/significant change in osteoclasts parameters with high osteoblasts parameters compared to the control-group. This finding could result from the lack of vitamin D and the steroid administration resulting in secondary hyperparathyroidism, which affects osteoblasts activity.

On the other hand, differences between femur and vertebra might result from discrepant biomechanical loading on either region. Therefore, the femur shows a deviant response to the treatments compared to the vertebra. Further analysis of bone metabolism by fluorochrome labeling is currently under investigation.

11F

Histological analysis of non-mineralized portion at fracture risk regions in an osteoporotic sheep model

Matthias Mülke[1], Wolfgang Böcker[1,2], David Weisweiler[1,2], Katrin Lips[1], Thaqif El Khassawna[1], Christian Heiss[1,2]

[1] Laboratory for experimental trauma Surgery, Justus-Liebig University of Giessen
[2] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Giessen, Germany

Osteoporosis is an ongoing challenge in industrialized societies. Relevant animal models for preclinical testing of drugs and biomaterials are crucial to characterize. Therefore, the understanding bone metabolism especially the mineralized to non-mineralized tissue balance is of great importance. This study investigated the imapact of various treatment of osteoporosis induction in a sheep model.

31 skeletally mature female Merino sheep were ovariectomized (OVX) and treated with calcium- and vitamin-D2/3-deficient diet (OVXD) and with the steroid in addition to the diet (OVXDS) compared with OVX alone and Sham control. Iliac Crest (IC) biopsies were taken at (M=month) 0M, 3M and 8M. Also femora and spine samples were taken post mortem. Sections were stained with Kossa van Gieson then histomorphometrical analysis was performed in ImageJ and statistical analysis ran in SPSS v 21.0.

The ratio of osteoid/trabeculae bone was higher at 3M and 8M than 0M in the IC biopsies of OVX group. In the OVXDS the ratio showed progressive significant increase throughout the treatment. In OVXDS the ratio was higher than the control and OVX at 3M, then at 8M higher when compared to all groups. The osteoid portion was higher in the OVXDS spine compared to all groups. However, in the femur showed only OVXDS osteoid portion was higher than the control and OVX but not the OVXD.

The results suggest the induction of secondary osteoporotic status indicated by increased osteoid formation and mal mineralization. This might result from the increase of parathyroid hormone as secondary effect of the steroid administration and lack of vitamin D.

12F

Cartilage remnants and higher osteoid portion indicate bone healing delays in an osteoporotic sheep model

Sebastian Rosch[2], Annemarie Schäfer[2], David Weisweiler[1,2], Wolfgang Böcker[2], Christian Heiss[1,2], Thaqif El Khassawna[2]

[1] Department of Trauma Surgery, University Hospital of Giessen and Marburg GmbH, Giessen, Germany
[2] Laboratory for Experimental Trauma Surgery, Justus-Liebig University Giessen, Germany

Osteoporosis is one of the most common diseases in industrialized society, thus osteoporosis-associated fractures are a major clinical issue. Cellular and extracellular matrix (ECM) changes hallmark the multi-factorial nature of osteoporosis. The current study investigates ECM role and behavior during the healing of osteoporotic fracture.

31 female merino sheep with an average age of 5.5 years were divided in four groups. Group 1 (n=8, control-group), Group 2 (n=7) got a bilateral ovariectomy (OVX), group 3 (n=8) got OVX and a calcium-deficient diet (OVXD), group 4 (n=8) got OVX, diet and corticosteroids (OVXDS). At months (M) -5M and -8M from euthanasia drill-holes (7.5mm diameter) were created in the iliac crest signifying 5M and 8M of healing period, respectively. Histological evaluation with Toluidin blue and Movat pentachrom stains was performed and quantified using ImageJ V1.47 software. Statistical analysis ran in IBM SPSS V21.

Qualitatively, trabecular bone thickness was lower in OVXDS at both time points, however, cortical bone thickness was lower in control and OVXD with no significant change in OVX and OVXDS from 5M to 8M. Nonetheless, a higher total ossified tissue (TOT) and a lower total cartilage tissue (TCT) were seen in control group from -8M to -5M. In contrast, the highest TCT and the lowest TOT were seen at -8M in the OVXDS. Osteoid formation was lower after 8M than 5M in control and OVX group, in OVXD and OVXDS group osteoid was higher at 8M. Currently, osteoblast - osteoclast cross-talk is under investigation with specific RANKL, RANK and OPG staining.

13F
Effect of local and systemic application of bisphosphonates on bone defect healing in an osteoporotic rat femur diaphyseal bone defect model

Rene Aigner, Andre Wirries, Philipp Lechler, Justus Haferkemper, Mirko Schur, Christina Wack, Steffen Ruchholtz

Center for Orthopaedics and Trauma Surgery, University Hospital Giessen and Marburg GmbH, Location Marburg, Baldingerstraße, D-35043 Marburg

Introduction:
Antiresorptive drugs like bisphosphonates (BPH) are well established in osteoporosis therapy as they increase bone density and reduce the risk of osteoporotic fractures. But their effect on bone defect healing has not been investigated yet. In the past several authors reported that local application of BPH increases fixation of implants.
The aim of this study was therefore to analyze effects of local and systemic application of BPH on bone defect healing in a rat femur diaphyseal bone defect model.

Methods:
This study included 72 female Wistar rats. 36 rats were ovarectomized (OVX) at the age of 12 weeks. Afterwards animals were randomly assigned to one of the following three groups respectively (Control, BPH systemic, BPH local). At the age of 24 weeks diaphyseal femoral defect was performed and consecutively a fixateur externe was placed. Six weeks after surgery rats were euthanized. Analysis of bone defects and contralateral healthy bones included evaluation of bone density (pQCT) prior to surgery and post mortem, biomechanical properties (4 point bending), radiological (x- rays) and histological investigation.

Results:
Pre-operative evaluation of bone density showed significantly lower bone density in OVX rats. X-rays did not demonstrate any significant difference in bone healing between the control group and the group of systemic BPH application. Local application of BPH significantly decreased bone healing in the OVX as well as in the non-OVX group. Also biomechanical analysis showed a significantly negative effect after local BPH application. Histomorphometry revealed a delayed remodeling process in the local as well as in the systemic application group. But after local application the percentage of non osseous callus was higher compared to the other groups. Further histology proofed the lower bone healing ratio which was already shown by radiological analysis,

Conclusion:
Systemic BPH application led to delayed remodelling without having any negative effect on biomechanical properties and osseous bridging. Local application however does have a negative effect on bone defect healing in this osteoporotic bone defect model in the femur diaphysis in the rat.

14F
Cellular metabolism and bone repair in osteoporotic sheep model

Annemarie Schäfer[2], Sebastian Rosch[2], David Weisweiler[1,2], Wolfgang Böcker[1,2], Katrin S. Lips[2], Christian Heiss[1,2], Thaqif El Khassawna[1,2]
[1] Department of Trauma Surgery, University Hospital of Giessen and Marburg GmbH, Giessen, Germany
[2] Laboratory for Experimental Trauma Surgery, Justus-Liebig University Giessen, Germany

Osteoporosis often remains unnoticed until a fracture occurs. Since bone

mass and quality are reduced these pathologic fractures are difficult to treat. This project aims to provide further understanding of the altered fracture healing caused by osteoporosis.

31 female merino sheep with an average age of 5.5 years were divided in four groups: Group 1 (n=8, control-group), Group 2 (n=7) got a bilateral ovariectomy (OVX), group 3 (n=8) got OVX and a calcium-deficient diet (OVXD), group 4 (n=8) got OVX, diet and corticosteroids (OVXDS). At months (M) -5M and -8M from euthanasia drill-holes (7.5mm diameter) were created in the iliac crest signifying 5M and 8M of healing period, respectively. Cellular metabolism was detected through TRAP and ALP enzyme-histochemical staining. Descriptive analysis reflected lower osteoclasts present at later stages of healing at 8M compared to the earlier stage at 5M in all groups. However, Osteoclasts were highest in the number and were remarkably high in the OVXDS at 5M compared to other groups and time points. ALP staining showed increase in osteoblast activity at 8M OVXDS. Interestingly, the osteoblast activity was lowest in the controls at 8M. Preliminary COLI staining identified non-osseous areas despite being mineralized mainly in the OVXD and OVXDS at 5M and 8M.

Currently, COLX, in addition to COLI, is being analyzed to investigate the discrepancies in repair and remodeling of osteoporotic bone corresponding to treatment. Furthermore, to quench the role of neovascularization in bone healing and its delay, α-SMA stain will be performed.

15F
Study design for comparison of healing patterns in metaphyseal and diaphyseal fracture gap in osteoporotic and non-osteoporotic female rats

Andreas Kaiser[2], Ulrich Thormann[1/2], Ursula Sommer[2], Seemun Ray[2], Francisca Alagboso[2], Christian Heiss[1/2], Volker Alt[1/2]

[1] Department of Trauma Surgery, University Hospital Giessen-Marburg GmbH, Campus Giessen, Germany
[2] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University, Giessen, Germany

Although most osteoporotic fractures are metaphyseal in nature, our knowledge in its healing process is mostly based on experimental studies focused on diaphyseal fracture healing. Detailed studies recapitulating real life situations are scarce, especially those comparing fractures at the diaphysis and the metaphysis.

48 healthy female rats with femoral metaphyseal and diaphyseal osteotomy and 48 female osteoporotic rats with femoral metaphyseal and diaphyseal osteotomy will be analysed for fracture healing. Functional solidity will be evaluated with biomechanical tests and co-related to the radiological findings. Evaluation of bone formation rate, cellular changes, and relative gene expression analysis of significant bone formation markers will be examined by histology, histomorphometry (BV/TV, Ob/Tb.Ar, Oc/Tb.Ar), enzymehistochemistry (ALP,TRAP), immunohistochemistry (BMP2, OPG/RANKL, OCN, ASMA) and RT-PCR (ALPL, Runx2, OPG, RANKL, Col10a1, Col1a1, OCN). TOF-SIMS will be used to analyze mineral composites and co-related to Mineral Apposition Rate using calcein double labelling.

A bony union healing in all groups is expected, with diminished bone stability in osteoporotic rats attributed to decrease of minerals and organic compounds thereby leading to a restricted healing pattern with simultaneous decrease in the bone formation markers, compared to the non-osteoporotic animals. A better healing potential in the diaphysis compared to the metaphysis is also assumed.

The results thus obtained would improve the healing outcome by understanding the underlying cellular and molecular differences between normal and osteoporotic rats and differences in the healing pattern of metaphysis compared to that of the diaphysis by recapitulating the conditions as seen in case of osteoporotic patients.

16F
Osteoblasts are target cells for activated complement in fracture healing after severe trauma

Stephanie Baur[1], Anna Kovtun[1], Thorsten Schinke[2], Michael Amling[2], Markus Huber-Lang[3], Anita Ignatius[1]

[1] Institute of Orthopaedic Research and Biomechanics, Centre of Musculoskeletal Research Ulm (ZMFU), University of Ulm, Ulm, Germany
[2] Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[3] Department of Traumatology, Hand-, Plastic-, and Reconstructive Surgery, Centre of Musculoskeletal Research Ulm (ZMFU), University of Ulm, Ulm, Germany

The complement system is an important trigger of posttraumatic inflammation. Especially the complement anaphylatoxin C5a can provoke strong pro-inflammatory effects. We have previously shown that the blockade of the C5a receptor (C5aR) attenuated the negative effects of post-traumatic systemic inflammation on fracture healing and that C5aR is expressed not only by immune cells but also by osteoblasts. Here we investigated whether osteoblasts are relevant target cells for C5a in fracture healing after severe trauma using mice with an osteoblast-specific C5aR overexpression (Col1a1-C5aR).
12-week old male Col1a1-C5aR mice and wildtype mice (WT) obtained a femur osteotomy, stabilized by an external fixator. Half of the mice received an additional thoracic trauma (TXT) to induce systemic inflammation. 21 and 25 days post-surgery, fractured femora were analyzed. Statistics: n=6-7, ANOVA, $p<0.05$.
21 days post-surgery Col1a1-C5aR mice exhibited impaired fracture healing compared to WT demonstrated by decreased flexural rigidity (EI, -40%), bone mineral density (BMD, -21%) and area of osseous tissue (-27%). Accordingly, cartilage was increased by 55%. Additional TXT impaired bone healing in WT mice, as expected. In Col1a1-C5aR mice the effect of the TXT was significantly stronger after 21 days compared to WT and was still visible after 25 days.
In Col1a1-C5aR mice regular fracture healing was compromised. Additional TXT further impaired the healing outcome, indicating that osteoblasts may be relevant target cells for C5a, especially after severe trauma.

SATURDAY, October 10th, 2015
7:45 – 8:45 pm

1S
Influence of nicotine, acetylcholine and BDNF to the osteoinductivity of calcium phosphate cement and collagen scaffolds

Sebastian Wilczek[1], Sven Knaack[2], Matthias Schumacher[2], Michael Gelinsky[2], Gabor Szalay[3], Christian Heis[1,3], Reinhard Schnettler[1], Katrin S. Lips[1]

[1] Laboratory for Experimental Trauma Surgery, Justus-Liebig University Giessen, Schubertstrasse 81, 35392 Giessen, Germany
[2] Center for Translational Bone, Joint and Soft Tissue Research, Medical Faculty and University Hospital, Technische Universität Dresden, Fetscherstrasse 74, 01307 Dresden, Germany
[3] Department of Trauma Surgery Giessen, University Hospital of Giessen-Marburg, Campus Giessen, Rudolf-Buchheim-Strasse 7, 35392 Giessen, Germany

An increase of osteoporotic fractures and bone defects is noticed in daily routine of orthopedics and trauma surgery. The defect healing can be stimulated by implantation of suitable bone substitute materials like calcium phosphate cement (CPC), collagen-tapes (CT), and pharmaceuticals, which shall improve osteogenic differentiation. The aim of the present study was to analyze whether brain-derived neurotrophic factor (BDNF), acetylcholine or nicotine at the interface of CPC and CT could improve osteogenic differentiation. Human multipotent stromal cells (hMSC) were isolated from human reaming debris, which was obtained during intramedullary nailing. The hMSC, which can differentiate into active osteoblasts, were cultured for 28 days at the interface of bone substitute materials and pharmaceuticals. Every second day media were changed and pharmaceuticals were renewed. Once a week cells were harvested, morphology was documented by light microscopy, cell number was determined and concentration of alkaline phosphatase was measured.

A reduced cellular proliferation and differentiation was determined at the interface of CPC compared with cells grown on blank cell culture plastic, while CT influenced differentiation positively, but not the proliferation. However, the application of BDNF together with bone substitute materials leads to an increased differentiation. Nicotine had partially positive effects on differentiation.

Our results demonstrated that the osteogenic differentiation capacity decreased at the interface of CPC, whereas CT had osteoinductive effects. In both cases addition of BDNF favored the osteogenic differentiation. BDNF could thus be suitable for the functionalization of bone substitute materials.

2S
Finite element modelling of bone growth stimulation and its suitability for therapeutic, biomedical applications

Wolfram Bosbach

University of Cambridge / Engineering Department, Cambridge, United Kingdom

Imposed strain fields can stimulate bone growth. The stimulation of bone growth could be beneficial in future biomedical devices e.g. around implants. The purpose of the presented in silico study was to investigate the mechanics of sintered metallic fibre networks embedded in surrounding bone tissue. The finite element (FE) simulations predicted the fibre deformation due to magnetic actuation and the imposed strain in the surrounding bone tissue.

The skeletons of six fibre network samples (fibre diameter ØF = 40µm, sample volume V = 4×4×4 mm3 or subsections) were run as FE simulations based on beam theory. The FE models were run locally and on the Cambridge High Performance Computing Cluster Darwin by the FE solver Abaqus.
The fibre skeleton geometries were acquired in a previously completed study by the application of a reduction algorithm to 3D computed tomography scans.

The obtained results predict an equivalent von-Mises strain ε_(v.Mises) of 0.001 which is the required magnitude for the stimulation of bone growth. All investigated samples exhibited this value locally for an experimentally achievable magnetic induction vector B of less than 1.00 T (see Figure 1). In principle, the suitability of the design for the intended purpose is predicted.

Aspects for future work will be the experimental confirmation of the obtained simulation results and an advanced non-homogeneous model for the bone tissue matrix.

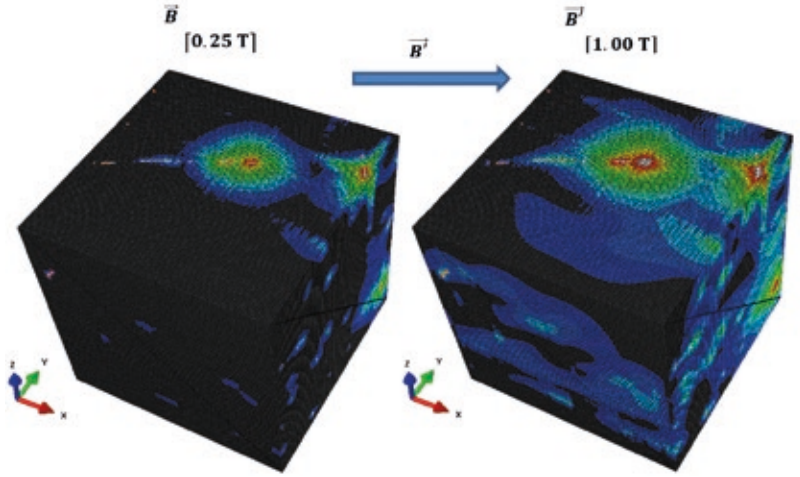

Figure 1: Strain field for magnetic induction vector $\vec{B}$ of 0.25 T and 1.00 T
Equivalent von-Mises strain ε_(v.Mises) of magnitude 0.001 in green

References:
Bosbach, W. The mechanical and magnetic behaviour of sintered fibre networks and their suitability for a therapeutic, biomedical application (PhD thesis, University of Cambridge), viva examination on 20th March 2015 positive

3S
Biomechanical comparison of a polyaxial angle-stable locking plate and a retrograde intramedullary nail for the fixation of distal femoral fractures.

Christopher Bliemel

Facharzt für Orthopädie und Unfallchirurgie, Notfallmedizin Oberarzt Zentrum für Orthopädie und Unfallchirurgie Universitätsklinikum Giessen und Marburg GmbH, Standort Marburg

Introduction:
Compromised bone quality and the need for early mobilization still lead to high rates of implant failure in geriatric patients with distal femoral fractures. With the newest generation of polyaxial locking plates and the proven retrograde femoral nails today two minimally invasive surgical procedures have been established. This study attempts to define the strength and failure mode of both surgical implants.

Materials and Methods:
An unstable AO/OTA 33-A3 fracture was established using a standardized fracture model. Eight pairs of human cadaver femora (mean age 79 years, range 63-100 years) with compromised bone quality were used. Osteosyntheses with eight locking plates and eight retrograde femoral nails were randomly performed. A materials testing machine (Instron 5566) was used to perform cyclic stress tests according to a standardized loading protocol, up to a maximum load of 5,000 N.

Results:
All specimens survived loading of at least 2,500 N. One plate and three nail constructs survived a maximum load of 5,000 N. The mean compressive force leading to failure was 4,429 N (CI 3,653-5,204 N) for plate osteosynthesis (p = 0.943) and 4,400 N (CI 4,122-4,678 N) for nail osteosynthesis. Proximal cutting out of the osteosynthesis was the most common reason for interruption in the plate and nail osteosyntheses. Significant differences between the plate and retrograde femoral nail osteosyntheses were seen under testing conditions for plastic deformation and stiffness of the constructs (p = 0.002 and p = 0.001, respectively).

Conclusion:
Based on our results, no statements regarding the superiority of either of the devices can be made. Even though the load to failure values for both osteosyntheses were much higher than the loads experienced during normal walking; however, because only axial loading was applied, it remains unclear whether both osteosyntheses meet the estimated requirements for postoperative full weight-bearing for an average heavy patient with a distal femoral fracture.

4S

Is there a benefit of proximal locking screws in osteoporotic distal radius fractures? – A biomechanical study

Benjamin Bockmann[1], Can Budak[1], Jens Figiel2, Philipp Lechler[1], Christopher Bliemel[1], Florian Debus[1], Tim Schwarting[1], Ludwig Oberkircher[1], Michael Frink[1]

[1] Center for Orthopaedics and Trauma Surgery, University Hospital Giessen and Marburg, Location Marburg, Baldingerstr., 35043 Marburg

[2] Department of Diagnostic and Interventional Radiology, University Hospital Giessen and Marburg, Location Marburg, Baldingerstr., 35043 Marburg

Introduction:
The distal radial fracture is a common fracture and frequently seen in geriatric patients. During the last years, volar plating has become a popular treatment option. While the application of locking screws at the distal fragment is widely accepted, there is no evidence for their use at the radial shaft.

Materials and methods:
In six osteoporotic pairs of matched human cadaver radii an extra-articular model creating an AO 23-A2.1 fracture was employed. Osteosynthesis were performed using the APTUS 2.5 Adaptive TriLock Distal Radius System (Medartis AG) with locking (LS) or non-locking screws (NLS) for proximal fixation. Biomechanical testing was performed in a staircase fashion: starting with 50 cycles at 200N, the load was continuously increased by 50N every 80 cycles up to a maximum force of 400 N. Finally, load to failure was analyzed with failure defined as sudden loss of force measured (20 %) or major deformation of the radii (10 mm).

Results:
At 200N, 250N, 300N, 400N and load to failure, the NLS group showed a higher degree of plastic deformation. In contrast, the LS group showed higher plastic deformation at 350N. Maximum force was higher in the LS group without reaching statistical significance. Reasons for loss of fixation were longitudinal shaft fractures, horizontal peri-implant fractures and distal cutting out. No difference was seen between the two groups concerning the development of the above mentioned complications.

Conclusion:
Our study did not show biomechanical superiority for distal radius fracture fixation when using locking screws in the proximal holes in an osteoporotic cadaveric model. At load to failure, longitudinal shaft fractures and peri-implant fractures seemed to be a more relevant problem rather than failure of the proximal fixation.

5S

Peek reconstruction plates for bridging of mandibular defect: comparative investigations under pro-inflammatory conditions in a human primary culture model of bone tissue

Andreas Schöbel

Department of Neuroanatomy and Molecular Brain Research, Faculty of Medicine, Ruhr-University Bochum, Germany

Human mandibular continuity defects can be accompanied by loosening or fracturing of the utilized titanium reconstruction plates. The synthetic material polyether-ether-ketone (PEEK) provides great homologies with human bone in terms of stability and elasticity and furthermore is characterized by a high degree in biocompatibility. AIM OF THIS PROJECT was to investigate the cellular compatibility of PEEK in a human primary cell culture model. For this purpose, cells (osteoblasts/fibroblasts) and materials (PEEK/titanium) were cultured under physiological and pro-inflammatory conditions induced by Lipopolysaccharide (LPS). LPS is known to induce a molecular state of local inflammation, as it is described for the periphery of oral implants in vivo. Data acquisition was carried out via confocal laser scanning microscopy, applied immunocytochemistry, scanning electron microscopy, real-time PCR, cell adhesion- and cytotoxicity-assays. Under physiological conditions, human osteoblasts/fibroblasts cultured on PEEK/titanium surfaces revealed similar adhesive qualities. In contrast to titanium, PEEK enforces a trajectory oriented growth pattern of both cell types. LPS induced pro-inflammatory conditions result in significant reduction of cell adhesive contacts of human osteoblasts and fibroblasts on titanium. Furthermore, the cell layers loose spatial orientation and structural integrity. In contrast, trajectory growth and density of the sensitive primary human osteoblasts remain unchanged due to the pro-inflammatory stimulus on PEEK. In comparison to titanium, affinity for human osteoblasts is also given by PEEK under peripheral pro-inflammatory conditions. This establishes a new perspective in osseointegration of implant materials in patients with critical immunological backgrounds or deficits in wound healing.

6S

Histological evidence of Staphylococcus aureus in an implant-associated infection rat model

Sabine Stötzel[1], Ursula Sommer[1], Ulrich Thormann[2], Walid Mohamed[3], Eugen Domann[3], Christian Heiß[2], Volker Alt[2]
[1] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University Giessen; 35392 Giessen, Germany
[2] Department of Trauma Surgery Giessen, Justus-Liebig-University Giessen; 35392 Giessen, Germany
[3] Institute of Medical Microbiology, Justus-Liebig-University Giessen; 35392 Giessen, Germany

Staphylococcus aureus (S.aureus) cause major infections in humans (e.g. bones, joints), especially methicillin-resistant strains are of serious clinical concern. Detection of solitary bacteria is a huge challenge as they are easily overseen in histological bone and joint specimens. Thus the main aim of the study is to detect the presence of S.aureus in an implant associated infection in a rat model. Male rats were implanted with an intramedullary implant in the tibia followed by an application of S.aureus suspension. The animals were euthanized one day after surgery. Scanning Electron Microscope (SEM) analysis was performed on the explanted intramedullary implant with or without rolling on agar plates. Toluidine-blue and Gram staining were used for histological examination.
SEM analysis of the implants after rolling out showed almost no bacteria. However, clusters of bacteria were found on all implants when no rolling out was performed before SEM analysis. Hematoma caused by the surgery, immigration of neutrophil granulocytes into the medullary space, the bone marrow, the cortical bone (degeneration/neoformation) and clusters of bacteria were distinguishable from each other by using toulidine-blue staining. Solitary bacteria were not visible with toluidine-blue staining. This was achieved using Gram staining which showed bacterial clusters as well as solitary ones in each sample.

SEM analysis should only be performed on the implants without any mechanical pretreatment. With toluidine-blue staining the reaction of cortical bone and bone marrow to the surgery and clusters of bacteria could be detected. However, Gram staining is necessary for detection of solitary bacteria.

7S

No alterations of the of inner organs and mRNA expression pattern at the interface of nanosilver (AgNP)-dopted implants compared to AgNP free controls in an experimental rat implantation model

Andre Worm[1], Sonja Hartmann[1], Ulrich Thormann[2], Linda Pauksch[1], Ursula Sommer[1], Reinhard Schnettler[1,2], Katrin Susanne Lips[1]

[1] Laboratory for Experimental Trauma Surgery, Justus-Liebig-University, Kerkraderstr. 9, Giessen, Germany
[2] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Campus Giessen, Rudolf-Buchheim-Str. 7, 35392 Giessen, Germany

Implant associated infections are a serious and rising problem in orthopedics and trauma surgery. Silver-nanoparticles (AgNP) are discussed for implant coatings to prevent implant associated infections. In vitro it could be shown that there is a small window where AgNPs hold antibacterial effectivity but does not induce cellular damage. In vivo, there is still a serious lack of information about the effectivity of AgNPs and its toxicity. Thus, the aim of our study was to analyze changes in the histology of the inner organs as well as alterations in cellular mRNA expression at the implant interface with real-time RT-PCR.
AgNP-dopted implants of polymethylmethacrylate (PMMA), mineralized collagen tapes (MCT), and titanium (TiAl6V4-Eli) were inserted in tibial medullary cavity of 13-weeks-old male Sprague-Dawley rats. After a post-operational period of 4 weeks and 6 months animals were euthanized, inner organs and tibia harvested. One slice of tibia was used for RNA isolation, cDNA synthesis, and real-time RT-PCR and subsequent statistical analysis using Kruskal-Wallis and Mann-Whitney tests. Liver, kidney, lung, spleen, heart, and brain were fixed with 4% buffered paraformaldehyde, embedded in paraffin, sectioned, stained, and evaluated with a light microscope.
Histological investigations did not show significant differences of the inner organs after insertion of AgNP-dopted implants compared to controls. In addition, mRNA expression was also not significantly changed at the interface of AgNP-dopted implants compared to control implants without AgNP.
In conclusion, the AgNP-dopted implants showed a suitable cellular compatibility that did not lead to morphological changes of the inner organs or to alterations in the mRNA expression level at the implant interface compared to the implants without AgNP.

Supported by BMBF FKZ 03X0103D.

8S

ToF-SIMS analysis of pharmaceutically modified cements from in vitro to in vivo

Anja Hens[1] , Peter Schmitz[1], Julia Kokesch-Himmelreich[1], Marcus Rohnke[1], Matthias Schumacher[2], Seemun Ray[3,4], Michael Gelinsky[2], Volker Alt[3,4], Jürgen Janek[1], Thomas Hanke[5]

[1] Institute for Physical Chemistry, Justus-Liebig-University of Giessen, Heinrich-Buff-Ring 58, 35392 Giessen, Germany
[2] Centre for Translational Bone, Joint and Soft Tissue Research, Technical University of Dresden, Fetscherstrasse 74, 01307 Dresden, Germany
[3] Laboratory of Experimental Trauma Surgery, University of Giessen, Kerkraderstrasse 9, 35394 Giessen, Germany
[4] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Rudolf-Buchheim-Str. 7, 35385 Giessen, Germany
[4] Institute of Materials Science TU Dresden, Fetscherstrasse 74, 01307 Dresden, Germany

Calcium phosphate based cements (CPC) are modified by the addition of pharmaceutically active compounds to enhance the osseointegration of the biomaterial and to stimulate the bone growth.

In this work we concentrate on strontium and bortezomib enriched CPCs since strontium is known to be an effective antiosteoporotic drug whereas borthezomib is used for the treatment of multiple myeloma. We present results from tracking the enriched calcium phosphate cements from in vitro to in vivo. Furthermore we demonstrate the potential of time of flight secondary ion mass spectrometry (ToF-SIMS) for use in the development of new bone implant materials. ToF-SIMS analysis of human osteoblast-like cells cultured on the strontium-modified cements prove clearly, that there is an uptake of Sr into the cells and Sr is incorporated into the mECM.
In an animal experiment the two biomaterials were used to fill defects in the femur of rats. After euthanasia the femura were embedded and sectioned. We imaged the fragments of the inorganic hydroxyapatite (Ca+) and the organic collagen ($C_4H_8N^+$) in the bone tissue as well as fragments of the particular drugs (Sr+ and BO+). Using ToF-SIMS the Sr release by the cement and the Sr distribution in the surrounding tissue was investigated. It was proven that Sr is localized in regions of newly formed bone but also within the pre-existing tissue. The BZCPC samples showed a uniform distribution of the BO+ in the entire tissue and implant area. An intensity gradient in or around the implant area could not be measured.

9S

Reaming debris as a source of mesenchymal progenitor cells for in vitro testing of new biomaterials

Katja Trinkaus[1], Katrin S. Lips[1], Olga Dakischew[1], Christian Heiß[1, 2], Reinhard Schnettler[3]

[1] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University Giessen, Schubertstraße 81, 35392 Giessen
[2] Department of Trauma Surgery, University Hospital of Giessen and Marburg, Rudolf-Buchheim-Straße 7, 35385 Giessen
[3] Professur für Infektiologie und Pathogenomik, Rudolf-Buchheim-Straße 6, 35385 Giessen

Cells applied for in vitro testing of new materials for bone substitution are immortalized cells and such not qualified to check the capability of osteogenic differentiation. In order to enhance validity of in vitro investigations cells are wanted to be most similar to those found at a fracture site in vivo. To establish an in vitro model which meets this demand cells cultivated from human reaming debris and from scar tissue were characterized by means of low density seeding, differentiation capacity and expression of CD-markers. Both tissues are residuals from surgery and a source of viable human cells which can be gathered without creating an additional intervention.

Primary cells cultivated from both sources show features of MSCs, moreover they express mesenchymal and hematopoietic markers concomitantly like already found in cells from adult bone marrow and from peripherical blood. Cells from scar tissue express CD73 in a much lesser extent and show a clearly inferior differentiation capacity than reaming debris.

In general a broad variation in differentiation capacity could be observed between different donors. This offers the possibility to evaluate a potential variability in biocompatibility in different patients, an aspect which had no relevance for in vitro testing of biomaterials up to now. Testing new bone substitutes in cells from donors in different stages of life and health will require more effort to get statistical significance in results however it will refine conclusions concerning biocompatibility by increasing the impact on information for realistic prospects of biological tolerance in vivo.

10S

Negative influence of leptin receptor deficiency on murine bone architecture

Maren Stähle[1], Janet Beckmann[1], Wolfgang Floel[1], Mathias Kneffel[1], Lutz Dürselen[2], Anita Ignatius[2], Thomas Linn[3], Christian Heiss[4], Reinhard Schnettler[1,4], Katrin Susanne Lips[1]

[1] Laboratory for Experimental Trauma Surgery, Justus-Liebig University Giessen, Kerkrader Str. 9, 35394 Giessen, Germany
[2] Institute of Orthopaedic Research and Biomechanics, Center of Musculoskeletal Research, University of Ulm, Ulm, Germany
[3] Clinical Research Unit, Medical Clinic and Policlinic 3, Justus-Liebig University Giessen, Klinikstr. 33, 35392 Giessen, Germany
[4] Department of Trauma Surgery Giessen, University Hospital of Giessen-Marburg, Justus-Liebig University Giessen, Rudolf-Buchheim-Str. 7, 35392 Giessen, Germany

Adipocyte hormone-like peptide leptin is correlated with body fat mass and is involved in regulation of bone mass. Since plasma levels of adrenomedullin (ADM) and brain derived neurotrophic factor (BDNF) are altered in obese patients we asked whether these adipokines are regulated in bone in obesity resulting from functional deletion of leptin activity. We also wanted to reevaluate the controversially discussed effect of the leptin receptor on biomechanical bone quality and architecture. Femurs and vertebrae of 6, 12, and 18 week old mice with homozygous leptin receptor gene deficiency (db/db) were compared with heterozygous (db/+) control mice by means of three-point bending test, histology, and real-time RT-PCR. Homozygous db/db mice revealed significantly reduced bending stiffness compared to heterozygous db/+ mice. Bone histology did not show alterations between db/db and db/+ mice. On mRNA level, Cathepsin K and Connexin 43 expression was up-regulated whereas ADM was decreased. No regulation was observed for BDNF expression.

Thus, our results support the reports implying that bone mass and strength are reduced by leptin receptor deficiency. In contrast to patients with obesity where an increase in ADM plasma level was described, we measured a decline in ADM mRNA. Since decreased leptin concentrations correlate with proADM level during weight loss we suppose that leptin signaling holds a strong impact in ADM regulation of obesity. Therefore, the murine model of leptin receptor deficiency is well approved for studying the influence of leptin signaling on adipose tissue, bone remodeling and their correlation.

11S

Passage-depending modification in mesenchymal stem cells (MSCs) grown from cancellous bone for usage in tissue engineering

Elisa Kraus[1], Katja Trinkaus[1], Olga Dakischew[1], Ivonne Bergen[1], Reinhard Schnettler[1], Christian Heiss[1,2], Katrin Susanne Lips[1]

[1] Laboratory for Experimental Trauma Surgery, Justus-Liebig University Giessen, Germany
[2] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Campus Giessen, Germany

In the field of tissue engineering for synthetic bone substitute numerous kinds of cells have been used to test the biocompatibility of synthetic materials. The commonly used cell-types (osteosarcoma, fibroblasts, or osteoblasts) cannot accurately depict bone healing as they lack the osteogenic differentiation potential of MSCs. MSCs from cancellous bone have been cultured and differentiated with success, but the age-depending changes are still not fully known.

To evaluate the differentiation potential of MSCs, samples from 5 patients have been cultivated over 11 passages (approximately 6 months), differentiated into osteocytes, chondrocytes, and adipocytes, and investigated by real-time PCR, life cell observation and alkaline phosphatase (ALP) assay. By comparing the different passages and samples, it was shown that the growth potential of MSCs differed immensely between the different donors (10.000 to 60.000 cells per well (=1.9 cm^2)). The real-time PCRs showed not only significant variations in overall MSC-marker expression (2-3 fold differences in marker expression) between the samples, but also different expression developments over time: whereas some samples showed a steady increase in expression, others follow more of a parabola with strong expression in passages 3, 9 and 11 and weak expression in passages 5-7.
In conclusion not every sample can be regarded as suitable material for testing in tissue engineering, but some samples can be grown and repurposed over an extended period of time. However, a reliable prognosis of the differentiation potential of MSCs is necessary for usage as bone substitute or biocompatibility test.

12S

Functionalized and via 2-photon-polymerization (2PP) nanostructured biphasic implants as a treatment for circumscript osteochondral lesions in an ovine model

Christian Weyers[1], Iris Schleicher[2], Ursula Sommer[1], Michaela Marten[1], Lydia Heimann[3], Klaus Liefeith[4], Reinhard Schnettler[1], Katrin Susanne Lips[1]

[1] Justus-Liebig-Universität Giessen, Labor für experimentelle Unfallchirurgie, Giessen, Germany
[2] Universitätsklinikum Giessen und Marburg GmbH, Klinik und Poliklinik für Unfall-, Hand- und Wiederherstellungschirurgie, Giessen, Germany
[3] aap Biomaterials GmbH, Dieburg, Germany
[4] Institut für Bioprozess- und Analysenmesstechnik e.V., Heilbad Heiligenstedt, Germany

Treatment of large osteochondral lesions in weight bearing regions of a joint with autograft transfer has the disadvantage of donor site morbidity. A possible solution to this problem is the usage of synthetic biphasic implants. In this study biphasic functionalized implants with an osseous phase consisting of hydroxyapatite-granules and a via 2-photon-polymerization (2PP) technique nanostructured cartilaginous phase were prospectively tested to treat above-mentioned lesions in sheep.

Biopsies of articular cartilage were taken from sheep and cultured in vitro. After 6 weeks osteochondral defects with a size of 5.5 mm in diameter and 8.5 mm in depth were surgically created in the weight bearing as well as in the non-weight bearing region of the knee joint. Prior to press-fit insertion of the biphasic implants, calcium phosphate nanoparticles with Dickkopf-1 (DKK-1) siRNA were added into the defect. In-vitro cultured chondrospheres were added to the apical layer of the implant. The animals were euthanized after 6 and 12 weeks and the implants were collected for molecular and cell biological analysis. Histology, immunhistochemistry and transmission electron microscopy (TEM) were used for cell biological analysis. Molecular biological analysis was performed separately for the osseous and cartilaginous phase using real-time PCR. Statistical Evaluation was executed with SPSS Statistics using Kruskal-Wallis and Mann-Whitney Test.

Molecular biological analysis of the cartilaginous phase revealed a significantly higher expression of SOX-9, a transcription factor in chondrogenesis, in those implants with added chondrospheres ($p = 0.018$). The expression of type II collagen, the basis for hyaline cartilage, was detectable. However, there was no significant difference between the implants. Regarding the osseous phase a significantly higher expression of eNOS ($p = 0.008$), a marker for angiogenesis, and type I collagen ($p = 0.011$), a marker for osteoblasts, was measured for weight bearing samples. These results correlate well with the histological findings. After 6 weeks hydroxyapatite granules in the osseous phase were surrounded by granulation tissue. New bone tissue was formed after 6 weeks in non-weight bearing and after 12 weeks in weight-bearing samples. Analysis of the cartilaginous phase revealed type II collagen immunpositive granulation tissue. Regeneration of cartilage tissue was found to be further advanced in non-weight bearing samples.

The presented findings support the concept of 2PP nanostructured biphasic implants as a suitable treatment for large osteochondral lesions. The densely packed hydroxyapatite granules built a well integrated osseous phase and provided an appropriate fixation for the cartilaginous phase. Furthermore, molecular and cell biological analysis indicate the tremendous effect of eNOS expression and angiogenesis on both ossification and cartilage regeneration.

13S
Adipokines affect differentiation of osteoporosis spongiosa-derived mesenchymal stroma cells

L. Tsiklauri[1], K. W. Frommer[1], J. Werner [2], U. Müller-Ladner[1], S. Wenisch[2], E. Neumann[1]

[1] Justus-Liebig-University Giessen, Department of Internal Medicine and Rheumatology, Kerckhoff-Klinik, Bad Nauheim, Germany
[2] Justus-Liebig-University Giessen, Institute of Veterinary-Anatomy, -Histology and -Embryology, Clinic of Small Animals, Giesssen, Germany

Age-related bone loss and articular cartilage destruction are accompanied by increased bone marrow fat infiltration. Bone marrow adipocytes have high secretory activity including secretion of proinflammatory (e.g. adipokines) and matrix-degrading (matrix-metalloproteinases, MMPs) factors and may be involved in progressive bone loss as observed in osteoporosis. Adipocyte-derived factors most likely influence differentiation of mesenchymal stem cells (MSCs) into osteoblasts and adipocytes. Therefore, we analyzed the effects of resistin, leptin and visfatin on MSC differentiation and their distribution in bone. Spongiosa containing bone marrow from femoral heads of patients undergoing hip replacement after osteoporotic femoral neck fracture (n=10) or osteoarthritis (n=11) were collected. Primary spongiosa-derived mesenchymal stromal cells (hMSCs) as well as commercially obtained MSCs were cultured in adipogenic and osteogenic media 3 weeks with/without adipokines. mRNA expression of adipokines, TIMPs and MMPs of stimulated hMSCs/MSCs and of bone samples were evaluated by real time PCR.

Significantly higher expression of visfatin and significantly reduced MMP2 expression in bone was observed, whereas MMP13 was unchanged. Leptin was significantly elevated in osteoporotic bone. MSC stimulation with visfatin significantly increased expression of MMP13 during adipogenic differentiation (n=3) but not leptin and resistin. During osteogenic differentiation, variability between samples was high. However, a reduction of MMP2 was visible.
In contrast to resistin and leptin, visfatin may locally influence cell metabolism due to visfatin-induced MMP production which may promote bone degradation at the adipose tissue/bone interface. Increased leptin levels in osteoporotic bone might be involved in the etiology of osteoporosis.

14S
Alteration in bone status of adult female nicotinic acetylcholine receptor subunit alpha 9 and 10 knockout mice

Lisa Baumann[1], Vivien Kauschke[1], Anna Kovtun[2], Lutz Dürselen[2], Katrin Susanne Lips[1]

[1] Laboratory for Experimental Trauma Surgery, Justus-Liebig-University, Giessen, Germany
[2] Institute of Orthopedic Research and Biomechanics, University of Ulm, Ulm, Germany

Acetylcholine (ACh) is an important neurotransmitter in the nervous system and a widespread signaling molecule in non-neuronal cells, e.g. bone cells, where it is involved in regulation of proliferation, differentiation and apoptosis. ACh acts via binding to muscarinic and nicotinic acetylcholine receptors (nAChR). nAChR are ligand gated ion channels that consists of 5 subunits.

Subunit α9 is able to form homopentamers as well as heteropentameric receptors in combination with subunit α10. nAChR α9/10 is permeable for bivalent cations like calcium in addition to sodium ions. In the present study we asked whether deletion of nAChR α9 or nAChR α10 might lead to alteration in bone strength, microstructure, and expression profile.

Vertebrae, femora, tibiae, and humeri of female bone-adult nAChR α9 and 10 knockout mice (KO) and their corresponding wildtype mice (WT) were harvested and processed for biomechanical testing, µCT-analysis, histology, cell- and molecularbiological methods.

Left femur was used for biomechanical testing that revealed a significant decline in bending stiffness ($p = 0.001$) and breaking force ($p = 0.001$) of nAChR α9 KO compared to their corresponding WT. No statistically significant differences were measured for nAChR α10 in comparison to their WT.

Preliminary results revealed a loss of bending stiffness and breaking force of the left femora of nAChR α9 KO mice in comparison to their WT. Thus, further analysis must be made to characterize the role of nAChR α9 in bone and how it might have an impact on bone turnover.

Funded by SFB/TRR 79 and LOEWE NNCS.

15S

Cement Interdigitation and Bone-Cement Interface after augmentation of vertebral compression fractures – a cadaver study

Ludwig Oberkircher, Christopher Bliemel, Steffen Ruchholtz, Antonio Krüger
Center for Orthopaedics and Trauma Surgery, University Hospital Giessen and Marburg GmbH, Location Marburg

Introduction:
The treatment of painful vertebral compression fractures via transpedicular cement augmentation is well accepted. There is still uncertainty about long- and midterm effects of the PMMA in the trabecular bone of the vertebral bodies. Preservation of the trabecular structures, as well as interdigitation of the cement with the surrounding bone, therefore has been gaining increasing attention. Interdigitation of cement is likely relevant for biological healing and the biomechanical augmentation process. In this study a cutting and grinding technique was used to evaluate the interdigitation for 4 augmentation techniques.

Materials and Methods:
One fresh frozen human cadaver spine was used. The bone density measurement showed a T-score of -5,31 representing substantial osteoporosis. The spine was dissected into single vertebral bodies, the surrounding soft tissue, the laminae and spinal processes were removed. Altogether 13 undamaged vertebral bodies were prepared (from the 3rd thoracic to the 3rd lumbar vertebral body). The endplates were embedded in Technovit. Using a standardized protocol wedge compression fractures were created (Instron 5566). The axial load was continuously increased until the hight of the anterior edge of the vertebral body was reduced to 50%. The load was maintained for 15 minutes. After creation of the wedge fractures the vertebral bodies were assigned into four similar groups concerning size and needed force to produce the fracture. The four groups were randomized to different cementing techniques. The treatment options were Balloon-Kyphoplasty (Kyphon, Medtronic), StabiliTTM RF-Kyphoplastie (DFine), Shield-Kyphoplasty (Soteira) and Vertebral Body Stenting (VBS, Synthes®). All procedures were performed by the same surgeon using an image intensifier. To attain a relevant result clinical judgement was used to proceed or stop the cement injection. After the procedure all vertebral bodies were fixed in 10% formalin and cut in 2mm slices using a diamond band saw (EXACT).

Dehydration and Degassification was performed in a precooled desiccator. The probes were embedded in Technovit 7200 using blue light polymerization. The polymerized tissue blocks were removed from the embedding mould and trimmed to the needed size with a diamond band saw (EXACT). The blocked surface of the embedded tissue probe was grinded with the EXACT micro grinding machine. After the probes were grinded to an appropriate thickness staining was performed. A protocol for histologic analysis was designed. From the center of the cement a actinomorphic grid was placed on the specimens (24 radiuses, 15°). The relation between length of radius and preserved cancelous bone was evaluated. Additionally the distance between the cement and trabecular bone (bone/cement interface) was ascertained (Leitz Aristomet) and calculated using the Image-Pro-Plus Software.

Results:
For the void-creating procedures, the distance between bone and cement was 341.4 ± 173.7 µm and 413.6 ± 167.6 µm for vertebral stenting and balloon kyphoplasty, respectively. Specifically, the trabecular bone was condensed around the cement, forming a shield of condensed bone. The procedures with-

out a balloon resulted in shorter distances of 151.2 ± 111.4 µm and 228.1 ± 183.6 µm for RF and shield kyphoplasty, respectively. The difference among the groups was highly significant (P ‹ .0001). The percentage of interdigitation was higher for the procedures that did not use a balloon: 16.7% ± 9.7% for balloon kyphoplasty, 20.5% ± 12.9% for vertebral stenting, 66.45% ± 12.35% for RF kyphoplasty, and 48.61% ± 20.56% for shield kyphoplasty. The difference among the groups was highly significant (P ‹ .00001).

Summary:
The protocols for creating wedge fractures, as well as the cutting-and-grinding technique and microscopic evaluation, led to reproducible results and effects. The interdigitation of cement and trabecular bone seems better for the procedures that do not create a void before augmentation.

16S
Histomorphometrical investigations of bone turnover of M3R and BDNF knockout mice

Fee Willscheid[1], Vivien Kauschke[1], Christian Heiss[2], Katrin Susanne Lips[1]

[1] Laboratory for Experimental Trauma Surgery, Justus-Liebig University Giessen, Kerkraderstr. 9, 35394 Giessen, Germany

[2] Department of Trauma Surgery Giessen, University Hospital of Giessen-Marburg, Campus Giessen, Rudolf-Buchheim-Str. 7, 35392 Giessen, Germany

Recently it has been shown that muscarinic acetylcholine receptor M3 (M3R) expression is regulated in a rat osteoporosis model. In addition a decrease in bone strength was reported for male adult M3R knockout mice (KO). Most prominent ligand of muscarinic receptors is acetylcholine, a well-known neurotransmitter in the nervous system and an important signalling molecule in non-neuronal tissues. Similar to ACh the neurotrophin brain-derived neurotrophic factor (BDNF) has an important role in the nervous system and non-neuronal tissues. BDNF is expressed in osteoblasts and up-regulated during human and murine fracture healing. Thus, we asked in the present study if BDNF and M3R-KO mice depict alterations in bone turnover by means of histomorphometrical analysis.

Female 16-weeks-old heterozygote BDNF and homozygote M3R-KO mice were euthanasized, vertebrae L3 (M3R) and left femur (BDNF) were harvested, demineralized, embedded in paraffin, sectioned and stained routinely with hematoylin-eosin (HE). In addition, enzyme histochemical incubations were performed for detection of osteoblasts (alkaline phosphatase, ALP) and osteoclasts (tartrate-resistant acidic phosphatase, TRAP). The sections were digitally imaged, analysed with the software programs GIMP 2.8.2. and Inkscape 0.48 and subsequently compared with the statistical tests of Kruskal-Wallis and Mann-Whiteny using the software SPSS.

In female M3R-KO mice the histomorphometrical analysis of TRAP staining revealed a significant increase (p ‹ 0.001) in relative osteoclast number of M3R-KO mice compared to wildtype mice (WT). The histochemical staining of ALP resulted in an increase of ALP positive labelled trabecular surface in M3R-KO mice compared to WT (p ‹ 0.001). Regarding the BDNF-KO mice no statistically significant changes could be determined compared to the corresponding WT.

In summary, we found an increase in the number of osteoclasts and osteoblasts in M3R-KO mice. In consideration of additional analysis of BMD, microarchitecture by µCT, biomechanical loading, real-time RT-PCR, and transmission electron microscopy where also an increased bone resorption was provided, we assume that M3R-KO mice generate a phenotype looking similar to high turnover osteoporosis in human. The impact of BDNF on bone remodelling needs to be investigated in further studies.

Supported by DGF TRR79 project B7.

17S
Interactions of titanium surfaces with simulated body fluid

S. Groeger[1], C. Ganß[2], R. A. Düring[3], N. Krämer[4], J. Meyle[1]

[1]Department of Periodontology, Dental School, Justus-Liebig-University, Giessen, Germany

[2]Department of Conservative Dentistry, Dental School, Justus-Liebig-University, Giessen, Germany

[3]Institute of Soil Science and Soil Conservation, Justus-Liebig-University, Giessen,

[4]Department of Pedodontic Dentistry, Dental School, Justus-Liebig-University, Giessen, Germany

The ankylotic anchorage of titanium (Ti) based materials in the human bone is of crucial importance for successful implant treatment. Dental implants perforate the mucosal surface and extend into the bacterially contaminated oral cavity. Similarly to natural teeth they connect the outer milieu with the inner body.

Like on human teeth the titanium surface exposed to the oral microbiome is easily colonized and a bacterial biofilm forms, which elicits a local immune reaction and can result in peri-implantitis a major threat to the long-term success for the implant. For further improvement of the anchorage of hard and soft tissues on the titanium surface, it is essential to understand the interactions between the material and the surrounding milieu.
The aim of this in vitro study was to analyze possible changes of the implant surfaces as well as to test the solubility of titanium using implants with different surface modifications, which were exposed to simulated body fluid (SFB).

In different independent experiments titanium implants with 4 types of surface modifications were incubated 4h, 12h, 24h and 4d in simulated body fluid containing 1. Hank´s buffered salt solution (HBSS) 100%, 2. HBSS containing 25% human serum (HS) and 3. HBSS containing 50% HS.
The titanium surfaces were analyzed before/after incubation by scanning electron microscopy (SEM) to detect nano-crystalline precipitates and by X-ray Photoelectron Spectroscopy (XPS) for the analysis of the chemical composition of the precipitates on the surfaces.

The supernatants oft he incubation media were subjected to analysis by inductively coupled plasma mass spectrometry (ICP) for the determination of the concentrations of titanium (Ti), calcium (Ca), and phosphorus (P).

Incubation in serum free medium induced a further formation of crystal-like plates on the surfaces of the modified implants and a crystal cover on the non-modified implants, both types containing Ca and P. The presence of serum led to a dissolution and the development of a protein coverage.
Titanium was clearly dissolved from all implant surfaces into the surrounding fluid containing serum, in a time and concentration dependent manner.
Human serum is a very corrosive medium, which consists of a high number of different amino acids, peptides and proteins as well as lipids and other substances. The term “inertness” should no longer be used with regards to the behavior of implant materials under conditions in the human body.

This study was supported by a grant from Dentsply Implants

FREE PAPER

Neovascularisation of the critical size defect in osteoporotic bone: a morphometric micro-CT study

Marian Kampschulte[1,2], Gabriele A. Krombach[1,2], Dana Richards[2], Jonas Sender[1,2], Katrin Lips[3], Ulrich Thormann[3,4], Thaqif El Khassawna[3], Seemun Ray[3], Volker Alt[3,4], Alexander C. Langheinrich[2,5]

[1] Department of Diagnostic and Interventional Radiology, University Hospital Giessen and Marburg GmbH, Giessen
[2] Laboratory of Experimental Radiology, Justus-Liebig-University Giessen
[3] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University Giessen
[4] Department of Trauma Surgery, University Hospital Giessen and Marburg GmbH, Giessen
[5] Department of Diagnostic and Interventional Radiology, BG Trauma Hospital Frankfurt/Main

Neovascularisation is essential for bone regeneration. This study aimed to investigate the microvascular morphology and distribution in the non-injured femur and the neovascularisation of the metaphyseal critical size defect in a small animal model of osteoporosis. Rats (n=7) were ovariectomized (OVX) and received a multideficiency diet. Three month after OVX, a 5 mm wedge shaped critical size defect was cut at the distal femoral metaphysis and stabilized with a T-shaped mini-plate. Animals were euthanized six weeks after the surgical procedure. Fracture consolidation and microvascularisation were assessed by micro-CT. No fracture consolidation was observed. In the non-injured bone, micro-vessels showed an spatial arrangement, thereby enabling a differentiation between epi-, meta-and diaphysis . Micro-CT based morphometry revealed a significant reduction of the vascular volume fraction (VVF, $p < 0{,}001$) as well as the vascular diameter (St.Th., $p < 0{,}001$) in the critical size defect compared to the intact contralateral side. Convexity versus concavity of vascular surfaces (fragmentation index), blood volume related vascular surface (object surface/volume) and object number increased significantly ($p<0{,}001$). Thereby, the vessel-tissue interface area (object surface density) was hold steady. Micro-CT based vascular morphometry of the bone marrow and critical size defects shows significant differences between epi- meta and diaphysis in the non injured bone as well as differences between the critical size defect an the non injured metahysis. As angiogenesis is a crucial prerequisite that precedes osteogenesis, our results may influence further evaluation of osteoconductive or osteogenic biomaterials in this small animal model of osteoporosis.

Optimization of the electrospinning process for production of mesenchymale stem cell (MSC) seeded, nanofiber scaffolds for bone regeneration.

Karl-Friedrich Schüttler[1],Vanessa Ketter[1], Roland Dersch[2], Andreas Greiner[2], Jürgen RJ Paletta[1]

[1] Center for Orthopaedics and Trauma Surgery / University Hospital Giessen and Marburg GmbH, Location Marburg, Germany
[2] Department of Chemistry, Faculty of Biology, Chemistry and Earth Sciences University Bayreuth, Germany

Nanofiber scaffolds are suitable tools for bone tissue engineering. Mimicking the extracellular matrix, they allow for cell growth and differentiation of human mesenchymal stem cells towards osteoblasts. However, in large 3D scaffolds, uniform cell colonization presents an unsolved problem. A direct incorporation of living cells into nanofiber scaffolds during electrospinning could be a promising approach for producing cell-containing scaffolds. One possibility is the combination of electrospraying of cells with electrospinning of nanofibers. The intention of this project is to build up and optimize cell seeded scaffolds for bone replacement. This should be achieved by a combination of coaxial- or multi-jet electrospinning of different nanofibers and electrospraying of MSC.

Based on results achieved by means of a prototype spinning device a multi-jet coaxial multi-jet spinning apparatus was constructed, allowing for iteration of different variables influencing scaffold architecture. Under different conditions cells were incorporated into the scaffolds. The resulting constructs were incubated and analyzed with regard to cell survival, growth and differentiation.

Increasing the number of either polymer or cells jets let to a non proportional increase in scaffold mass or cell count. With respect to cell survival, spinning voltage, temperature, humidity, and different culture media had a minor influence while spinning duration, solvent of the polymer, flow rate of cells as well as the use of a liquid counter electrode had an important impact on cell survival.

The constructed multijet apparatus allows for incorporation ov vital MSC into nanofiber scaffolds. Iteration of spinning variables increases cell survival.

Effect of M3 muscarinic acetylcholine receptor deficiency on collagen antibody-induced arthritis

Katrin Susanne Lips[1], Nicole Dittmann[1], Iris Schütz[1], Jochen Klein[2], Janet Beckmann[1]

[1] Laboratory of Experimental Trauma Surgery, Justus-Liebig University, Giessen, Germany
[2] Department of Pharmacology, School of Pharmacy, Goethe-University Frankfurt, Frankfurt am Main, Germany

Rheumatoid arthritis is one of the most common inflammatory joint diseases affecting 1% of the world's population. Recent studies reported the involvement of cholinergic receptors in development of arthritis. Activation of the muscarinic M3 receptor (M3R) induces proliferation of fibroblasts and can have pro-inflammatory effects. Thus, we asked in the present study if M3R-deficiency could protect from experimental arthritis in mice.
12-week old M3R knockout mice (M3-KO) and their corresponding wildtypes (WT) were injected with a cocktail of monoclonal antibodies against collagen-2 (Chondex, Redmond, WA, USA) and lipopolysaccharide (LPS) to induce rheumatoid arthritis. Control animals were only treated with LPS. Severity of arthritis was assessed by daily scoring of the paw swelling. After 10 days mice were sacrificed and samples were analyzed using real-time RT-PCR, FACS, ELISA, and electron microscopy.

In arthritic M3-KO the number of neutrophils was enhanced. The levels of neutrophil chemoattractant CXCL2 and pro-inflammatory IL-6 were already strongly increased in mice with low cumulative arthritis score (CAS), whereas WT only showed induced expression of these markers when reaching high CAS. Arthritic M3-KO displayed stronger collagen-2 degradation in the articular cartilage and histopathological evaluation revealed more severe bone destruction in arthritic M3-KO. This was confirmed, as in M3-KO gene expression of markers for bone degradation (MMP13, cathepsin K and RANKL) were also already increased in mice with low CAS.

In conclusion, our results showed an increased inflammation and bone degradation in M3-KO. Transferring these observations to the clinical situation, one might speculate that treatment of patients with M3R agonists might possibly have protective effects for rheumatoid arthritis.

Supported by LOEWE Hessen Germany (NNCS, project B2).

Dendritic glycopolymers and their polyelectrolyte complexes as efficient drug delivery systems for retarded release of bortezomib from calcium phosphate cements

Bettina Mamitzsch[1,2], Christin Striegler[1,2], David Vehlow[1,3], Martin Müller[1,3], Matthias Schumacher[4], Michael Gelinsky[4], Brigitte Voit[1,2], Dietmar Appelhans[1]

[1] Leibniz-Institut für Polymerforschung Dresden e.V., Hohe Strasse 6, D-01069 Dresden, Germany
[2] Technische Universität Dresden, Organische Chemie der Polymere, D-01062 Dresden, Germany
[3] Technische Universität Dresden, Physikalische Chemie polymerer Materialien, D-01062 Dresden, Germany
[4] Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Zentrum für translationale Knochen-, Gelenk- und Weichgewebeforschung, Fetscher Str. 74, D-01307 Dresden, Germany

The disease multiple myeloma leads to lesions in the bone tissue. As local therapy there are varieties of bone graft substitutes, e.g. calcium phosphate cement (CPC). The use of CPC provides crucial advantages, such as osteoconductivity, biodegradability and the potential drug loading. Though it lacks retarded drug release for short-/long-term treatment due to the free diffusion of small molecules through the micropores in the CPC.
Thus we present dendritic glycopolymers (DG) consisting of poly(ethylene imine) (PEI) decorated with oligo(glutamic acid) and/or maltose and maltotriose, respectively. Those core-shell architectures and their polyelectrolyte complexes (PEC) with cellulose derivatives are used as nanocarriers for the proteasome inhibitor bortezomib (BZM). In aqueous solution the drug delivery systems exhibit a sufficiently high drug uptake, while a significant retarded BZM release from DG/CPC composite is determinable. This has been observed with different polymer/drug ratios. At high polymer concentrations the mechanical and morphological properties of the bone substitute are not influenced by the DG. Moreover biocompatibility of the GD was tested by lactate dehydrogenase (LDH)- and alkaline phosphatase (ALP)-activity. The GD do not alter/hamper the cell proliferation/differentiation of hMSC. Concluding the results CPCs loaded with BZM complexed by the GD or PEC are promising materials for bone reconstruction in terms of short-/long-term treatment of cancer damaged bones.

Key words: dendritic glycopolymers, drug delivery, controlled release, multiple myeloma

Biomaterials for myeloma bone lesions – In vitro release of bortezomib from bioactive calcium phosphate-containing silica/collagen xerogels and in vivo effect on bone remodeling

Sina Rößler[1], Benjamin Kruppke[1], Anja Seckinger[2], Seemun Ray[2], Sascha Heinemann[1], Dirk Hose[2], Volker Alt[3], Thomas Hanke[1]

[1] Max-Bergmann-Zentrum für Biomaterialien, Technische Universität Dresden, Germany
[2] Universitätsklinikum Heidelberg, Medizinische Klinik V, Sektion Multiples Myelom, Heidelberg, Germany
[3] Klinik und Poliklinik für Unfall-, Hand- und Wiederherstellungschirurgie Universitätsklinikum Giessen und Marburg, Justus-Liebig-Universität Giessen, Germany

Biocompatibility and mechanical stability are basic requirements for bone substitute materials. Looking forward to clinical application the issue of drug loading reaches necessity. The local therapy of multiple myeloma is investigated as a part of the Collaborative Research Centre TRR 79. In addition to the systemic therapy against myeloma cells in bone lesions the local release of the proteasome inhibitor bortezomib from a bone substitute material is necessary. Therefore, the knowledge of the materials degradation and its effect on drug release is essential. The material preparation of calcium phosphate-containing silica/collagen xerogels is based on a sol-gel process. A rise of pH causes gel formation, when mixing a buffered collagen fibril suspension with a pre-polymerized silicic acid. This allows the incorporation of calcium phosphate phases and the addition of active agents. Drying of the composite material leads to the formation of a compact and yet nano-porous material. A controlled release of bortezomib from degradable three-phase xerogels in different solutions and time regimes was shown in vitro. The bioactivity of xerogels led to a deposition of apatite from physiological medium on the surface which slightly affects the release during the observation period of 14 days. The required bortezomib concentration in xerogel was analysed in vitro using cell culture of myeloma cell lines. A concentration-dependent decrease in myeloma cells was detected. The effect of locally applied bortezomib-loaded silica/collagen xerogel granules was investigated in vivo in healthy rat bone. An optimal drug load was identified in histological sections by means of an increase in bone growth by bortezomib-loaded xerogel granules in comparison to plain ones. Finally, it can be concluded that bioactive calcium phosphate-containing silica/collagen xerogels can be modified by bortezomib and a release can be adjusted to relevant concentrations. The biomaterial is suitable as a local drug supplier.

Unravelling the biocompatibility and new bone formation capabilities of bortezomib-loaded biomaterials

Francisca Alagboso[1], Seemun Ray[1] , Ulrich Thormann[1,2] , Ursula Sommer[1] , Marcus Rohnke[3] , Anja Henss[3] , Michael Gelinsky[4] ,Reinhard Schnettler[1,2] , Christian Heiss[1,2] , Volker Alt[1,2]

[1] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University, Giessen, Germany
[2] Department of Trauma Surgery, University Hospital Giessen-Marburg GmbH, Campus Giessen, Germany
[3] Institute of Physical Chemistry, Justus-Liebig-University, Giessen, Germany
[4] Center for Translational Bone, Joint, and Soft Tissue Research, University Hospital, Dresden University of Technology, Germany

Osteolytic bone destruction is the most common clinical feature of multiple myeloma. Bortezomib has been shown to regulate bone remodelling by inhibiting osteoclastogenesis and stimulating osteoblastogenesis. In skeletal disorder, local delivery of biologically active molecules via biomaterial implants has been shown to enhance bone healing, but this treatment approach is yet to be tested in multiple myeloma. Therefore, we plan to investigate the new bone stimulating effects of bortezomib delivered locally via composite biomaterial into defect created in the metaphyseal area of the femur.

Female Sprague-Dawley rats will be randomized into control and test groups (n=16/group). A 2.5 mm drill hole will be created in the metaphysis of the left femur. The defect will either be filled with novel biomaterial substitutes (test group) or left empty (control group). After 6 weeks, bone parameters (e.g. tissue and bone mineral density) will be quantitatively and qualitatively evaluated and correlated to the biomechanical strength of the bone. Subsequently, immunohistochemical staining for bone formation and resorption markers (e.g. osteocalcin osteoprotegerin and dickkopf-1) and the expression levels of certain genes by qPCR (e.g. collagen-10 and alkaline phosphatase) to demonstrate the effects of bortezomib and the biomaterials on new bone formation. Secondary ion mass spectrometry will be used to assess the release kinetics of bortezomib from the implant to the surrounding tissues. Our preliminary results revealed an indirect correlation between concentration and healing. At lower concentration bortezomib was well tolerated and produced significant increase in bone formation in the defect area than at higher concentrations.

Preservation of bone ultrastructure using high pressure freezing and microwave assisted chemical fixation

Diaa Eldin S. Daghma[1], Thaqif El Khassawna[1], Christian Heiss[1,2]

[1] Laboratory for experimental trauma Surgery, Justus-Liebig University of Giessen, Germany
[2] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Giessen, Germany

Bone metabolism and bone healing involve series of interdependent cellular events. Many studies described physiological and molecular aspects of bone homeostasis and regeneration. However little is known about ultrastructure during this process. The study aims at achieving a close-to-native and cryo preservation of bone to enable its ultrastructure analysis and imaging. Thus, enhance the understanding of cell-cell and cell-matrix interaction, mineral composition correlation, and three-dimensional organization. Tissue chemical fixation protocols available up to date are performed at room temperature and result in ultrastructure alteration of bone. This impacts our understanding of diseased bone as the reference ultrastructure of healthy bone is not to standard.

Methodology:
Rat bone samples were divided into two groups, the first was subjected to microwave assisted chemical fixation, while the other were fixed by high pressure freezing (HPF) followed by freeze substitution. Further, Human biopsies were also fixed by HPF.

Results:
Microwave fixation has shortened the process of bone samples preparation for TEM investigation and allowed better fixing penetration improving ultrastructure preservation. Interestingly, HPF processed samples exhibited a close to genuine ultrastructure preservation.

Conclusion:
Microscopic diagnostics are crucial approaches to identify discrepancies between diseased and healthy bone. Cellular activity may vary due to the ultrastructural component and not the cell numbers. Despite its importance, ultrastructual differentiation of bone diseases has not been yet addressed. However, the fixation protocols at hand do not allow optimal differentiation of ultrastructural cellular discrepancies.

ToF-SIMS analysis of lipids in hMSCs harvested from osteoporotic and control bone – comparative study of cells from adipogenic, osteogenic and basal culture conditions

Kaija Schaepe[1], Tessa Bartges[1], Janina Werner[2], Marcus Rohnke[1], Sabine Wenisch[2], Jürgen Janek[1]

[1] Institute of Physical Chemistry, Justus-Liebig-University Giessen, Heinrich-Buff-Ring 58, 35392 Giessen, Germany.
[2] Institute of Veterinary Anatomy, Histology and Embryology, Justus-Liebig-University Giessen, Frankfurter Strasse 98, 35392 Giessen, Germany.

Time-of-Flight secondary ion mass spectrometry (ToF-SIMS) is a surface sensitive technique which provides information about a sample's chemical composition. In contrast to optical microscopy it is possible to image specific chemical components by plotting the intensity distribution of an analyte signal through its mass-to-charge ratio.

The goal of this study was to identify and visualize lipids in human bone-derived mesenchymal stromal cells (hMSCs) harvested from an osteoporotic patient in order to generate highly adapted bone substitutes for osteoporotic bone.

Therefore, hMSCs isolated from osteoporotic and healthy bone were cultivated within basal, osteogenic and adipogenic media.

After cultivation three samples per group were fixed with 4% paraformaldehyde (PFA) and were investigated by von Kossa and Oil Red O staining. Additionally, another three samples per group were fixed with a 1% glutar- and 2% paraformaldehyde mixture and were stored in Milli-Q water for ToF-SIMS analysis. Principle Component Analysis (PCA) was performed to reveal differences in the lipid composition of the plasma membranes.

Osteogenic data indicates increasing levels of fatty acids like FA (20:4), FA (18:2) and FA (20:3) throughout osteogenic differentiation. In comparison to the controls the osteoporotic cells show very low levels of FA (18:1). Additionally, the osteoporotic cells were not able to form a mineralized matrix, as shown by von Kossa and also by ToF-SIMS measurements. This might be caused by their limited differentiation capacity.
Adipogenic differentiated cells reveal high contents of fatty acids like FA (16:0), FA (16:2) and FA (14:0).

AIOD DEUTSCHLAND

Themen

Humerus
- Marknagelung proximaler Humerusfrakturen
- Bewegungsfixateur am Ellenbogen
- Humerusmarknagelung retrograd

Unterarm
- Fixateur externe bei Radiusfrakturen

Femur
- Verriegelungsnagel bei pertrochantärer Femurfraktur
- Verriegelungsnagel bei Femurschaftfraktur
- Retrograde Femurmarknagelung bei distalen Femurfrakturen

Tibia
- Marknagelung bei Tibiafrakturen
- Fixateur externe bei proximalen Tibiafrakturen
- Sprunggelenksarthrodese mit Nagelung

OP-Pflege aktuell

Problemfeld
- Zentralsterilisation
- Mitarbeitereinsatz im Zentral OP (von der Sectio zum Polytrauma)
- Lagerung
- Tischorganisation
- Team-Timeout
- Blutsperre
- uvm.

Die Termine finden Sie auch auf **www.aiod-akademie.de**

Information & Anmeldung:
AIOD Deutschland e.V. · Postfach 210135 · 45065 Essen
Fon: +49 201 8945162 · Fax: +49 201 4260240 · info@aiod-ev.de · www.aiod-deutschland.de

Evaluation and establishment of a sheep model of osteoporosis – an insight in the T-Value standard

David Weisweiler[1,2], Lisa Schröter[2], Jakob Schmidt[2], Markus Engelhardt[1], Wolfgang Böcker, Christian Heiss[1, 2], Thaqif El Khassawna[2]

[1] Klinik und Poliklinik für Unfallchirurgie, Universitätsklinikum Giessen-Marburg GmbH, Standort: Giessen
[2] Labor für Experimentelle Unfallchirurgie, Justus-Liebig-Universität Giessen
[3] Klinik für Allgemeine, Unfall-, Hand- und Plastische Chirurgie am Klinikum der Ludwig-Maximilians-Universität (LMU)

Triple therapy with ovariectomy, glucocorticoids and a low vitamin D and calcium diet should induce an osteoporotic bone status in sheep and evaluated by dual energy x-ray absorptiometry (DEXA). For sustaining t-values another young healthy reference group is included in addition to age-matched z-values. Aim of this study was the establishment of a standardized large animal model (sheep).

31 female merino land sheep with an average age of 5,5 years were divided into four groups: control-group (C; n=8), the bilaterally ovariectomized OVX-group (OVX; n=7), diet-group received an additional deficient diet (OVXD; n=8) and triple-therapy-group combining diet and OVX and the application of methylprednisolone (2/ week 500mg i.m.; OVXDS; n=8) and reference group (n=28) for calculating the t-values.
Bone density of lumber vertebrae (LV) and left proximal femur were performed after intervention, after 3 and 8 month.

On contrast to literature neither did the OVX and the OVXD groups with no significant changes concerning t and z-values. OVXDS group showed a significant reduction of BMD at 3M (Femur: average z-value -2,5; t-value -2,0; LV average z/t-value -2,2) and 8M (Femur: average z-value -4,7; t-value -2,8; LV average z-value -3,6; t-value -2,9) compared to 0M and reference group ($p \leq 0.001$).

The represented study convey a triple therapy as crucial in achieving osteoporotic bone status with t-values below 2,5 measurable in the gold standard testing method DEXA. For the first time an additional reference group could quantify the reduction of BMD and distinguish between a single loss of BMD and a statistical established t-value.

Influence of mast cells on fracture healing in mice

Jochen Kroner[1], Anna Kovtun[1], Sebastian Seitz[2], Thorsten Schinke[2], Anne Dudeck[3], Anita Ignatius[1]

[1] Institute of Orthopaedic Research and Biomechanics, Center of Musculoskeletal Research (ZMFU), Ulm University, Germany
[2] Institute of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Germa
[3] Institute of Immunology, Medical Faculty Carl Gustav Carus, Technical University Dresden, Germany

Mast cells (MC) are pro-inflammatory sensor and effector cells of the immune system, which modulate immune responses. MC also influence bone metabolism, since patients with systemic mastocytosis develop osteopenia and exhibit an increased fracture risk. It was also shown that MC are present in the fracture callus during bone healing, however, their function has not yet been investigated so far. Therefore, the aim of this study was to specifically examine the role of MC in fracture healing using a MC deficient mouse model. Male MC deficient mice (Mcpt5-Cre R-DTA, 12 weeks) received a standardized osteotomy of the femur, stabilized by an external fixator. Fracture healing was analyzed at day 1, 3, 7, 14 and 23 by histomorphometry and immunohistochemistry. At day 23, biomechanical testing and micro-computed tomography (µCT) was performed. Osteoclasts were evaluated using tartrate-resistant acid phosphatase (TRAP) staining. Statistics: Student's t-test.

Immunohistochemical staining revealed the presence of MC during the entire healing period in wildtype mice. 23 days after fracture, MC deficient mice showed a significantly increased bending stiffness (+147%) compared to MC competent mice due to an increased relative bone volume in the callus (BV/TV, +23%). Histomorphometric analysis confirmed a significantly increased bone fraction at day 23 (+47%). Additionally, decreased numbers of osteoclasts (-22%) were detected in the peripheral callus of MC deficient mice.
In conclusion, we showed that MC deficiency increased the bone content in the peripheral fracture callus. This might be caused by a diminished remodeling due to a reduced osteoclast activity indicating that MC may regulate bone resorption.

Regulation of biological processes in fracture repair

D. Malhan[1], K. Schmidt-Bleek[3], G.N. Duda[3,4], T. El Khassawna[1], C. Heiss[1,2]

[1] Laboratory of Experimental Trauma Surgery, Justus-Liebig University, Giessen, Germany
[2] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Giessen, Germany
[3] Julius Wolff Institute and Center for Musculoskeletal Surgery, Charité-Universitätsmedizin Berlin, Germany
[4] Berlin Brandenburg Center for Regenerative Therapies (BCRT)

The healing of skeletal fractures involves the series of interdependent cellular events. This study aims to deepen understanding of molecular networks orchestrating these events.

Standard mid diaphyseal closed fracture in the left femur in male 8-10 weeks old C57BL/6N mice were analyzed at (day = D) D3, D7, D10, D14, D21&D28 post fracture (N = 5 / time point). Data normalization and analysis was performed using the "R" program. The thresholds for filtering the differentially expressed genes (DEG) were set at FC (fold-change) ≥ |2| and p-value ≤ 0.01. Functional enrichment analysis was performed using NCBI-DAVID to identify genes of immune system response, mitochondria and ribosome.

Compared to Do control, 215,202,178,145,21,14 genes were differentially expressed on D3,D7,D10,D14,D21,D28 respectively. Genes representative to the targeted biological process were selected by functional enrichment analysis. As a marker for adaptive immune response (ADR) B2M was downregulated at D3&D7 while upregulated at D10&D14. This suggests that the role of ADR is less crucial at the beginning and end of the repair process. Similarly, the ribosome related gene Pdia3 was downregulated at the initial stages indicating stress before its up-regulation at D10 to support cell survival. Expression of Oxct1 gene was downregulated at D3&D7 which is found to be involved in the ketone bodies catabolism.
Currently, the identification of the candidate genes involved in these biological processes and their pathways are being investigated. However, the results are indicating promising regulation of B and T cell mediated immune response along with power house of the cell.

Influence of neutrophil granulocyte depletion on fracture healing in mice

Anna Kovtun[1], Stephanie Baur[1], Rebecca Wiegner[1], Markus Huber-Lang[2], Anita Ignatius[1]

[1] Institute of Orthopaedic Research and Biomechanics, Centre of Musculoskeletal Research Ulm (ZMFU), University of Ulm, Ulm, Germany
[2] Department of Traumatology, Hand-, Plastic-, and Reconstructive Surgery, Centre of Musculoskeletal Research Ulm (ZMFU), University of Ulm, Ulm, Germany

Fracture healing is tightly regulated by the immune system. An excessive inflammatory response, induced by a severe trauma can disturb the early inflammatory phase of fracture healing and impair the healing outcome. Neutrophils are the most abundant cells in early fracture hematoma, however their specific role is so far poorly understood, especially in the context of severe trauma.

In the present study, we used 12-week old C57BL/6J male mice, which obtained a femur osteotomy stabilized with an external fixator. Half of the mice additionally received a thoracic trauma (TxT) to induce systemic inflammation. Neutrophil depletion was performed with a specific anti-Ly-6G-antibody 24h before surgery. Mice were sacrificed at different time points and fracture calli were analyzed biomechanically, histologically and micro-computed tomographically (µCT). The concentrations of inflammatory mediators were determined via ELISA. Statistics: Kruskal-Wallis or two-way ANOVA, $p<0.05$.

Neutrophil depletion led to a significant decrease of the bending stiffness of the fractured bone after 21 days (-35%). Also TxT alone worsened the healing outcome (bending stiffness: -52%), as well as the combination of both TxT and neutrophil depletion (bending stiffness: -63%). These data were confirmed by µCT and histomorphometric analysis. Immunohistological and molecular analysis of fracture callus revealed increased cytokine concentrations and macrophage infiltration in antibody-treated animals.

Thus, our data demonstrated that a balanced activation of neutrophils might be essential for successful fracture healing, as their depletion led to an impairment of mechanical and structural bone properties and worsened the healing outcome both after osteotomy and in case of polytrauma.

Comparison of metaphyseal fracture defect healing between osteoporotic and non-osteoporotic bone of the rat

Thormann Ulrich[1,2], Ray Seemun[2], El Khassawna Thaqif[2], Sommer Ursula[2], Dürselen Lutz[3], Kampschulte Marian[4], Schnettler Reinhard[2], Alt Volker[1,2]

[1] Department of Trauma Surgery, University Hospital Giessen-Marburg GmbH, Campus Giessen, Germany
[2] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University Giessen, Germany
[3] Institute of Orthopedic Research and Biomechanics, University Ulm, Germany
[4] Department of Radiology, University Hospital Giessen-Marburg GmbH, Campus Giessen, Germany

Osteoporotic fractures represent a growing problem in the fast overaging societies in the industrial countries. Detailed information about the different bone healing in osteoporotic bone in this clinical relevant situation rarely exists.

Initially we induced an osteoporotic bone status by bilateral ovariectomy and multi-deficiency diet (OVX) in 14 female Sprague-Dawley rats. We compared them with a control group of 14 female rats, who underwent a sham surgery and got standard diet (SHAM). After three month DEXA showed a statistically significant reduction of bone density. Subsequently we created a 3 mm wide triangular defect at the metaphyseal area of the distal femur. We stabilized the fracture defect with a mini-T-plate. After 42 days µCT, biomechanical and histological analysis was performed.

In both groups new bone formation was seen in the histological analysis, whereupon in the SHAM-group a complete consolidation was detected. In the OVX-group mainly woven bone was detected at the area of the endost.
At µCT we saw in both groups bone healing between all cortices. Biomechanical analysis detected a reduction of bending-stiffness of the metaphysis at the SHAM animals of 7.4% (112.7 ± 34.7 N/mm) in contrast to the OVX animals with a reduction of 54% (55.8 ± 25.8 N/mm) after six weeks. The direct compromise between OVX and SHAM animals showed a significant reduction of bending-stiffness of 50% in the osteoporotic bone status.
Metaphyseal defect fractures in the area of the distal femur with a size of 3 mm in osteoporotic bone show a delayed fracture healing with less biomechanical stability in comparison to a normal bone status after six weeks.

Impact of CLP-induced sepsis on bone in a murine animal model

Wolfgang Floel[1], Janet Beckmann[1], Lutz Dürselen[2], Marian Kampschulte[3], Juliane Ott[4], Konstantin Meyer[4], Markus A. Weigand[5], Christian Heiss[1,6], Katrin Susanne Lips[1]

[1] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University Giessen, Giessen, Germany
[2] Institute of Orthopaedic Research and Biomechanics, Center of Musculoskeletal Research, University of Ulm, Ulm, Germany
[3] Laboratory of Experimental Radiology, Justus-Liebig-University Giessen, Giessen, Germany
[4] Universities of Giessen and Marburg Lung Center, Justus-Liebig-University Giessen, Giessen, Germany
[5] Department of Anesthesiology, University of Heidelberg, Heidelberg, Germany
[6] Department of Trauma Surgery, University Hospital Giessen and Marburg, Campus Giessen, Giessen, Germany

Hospitalisations with sepsis nearly doubled between 2000 and 2008. Patients who survived sepsis showed partially a prolonged reduced health condition combined with increased rate of fractures. Therefore, we posed the question whether the increase in fracture incidence is caused by muscle weakness or by an altered bone quality – and – if fatty acids are protective in systemic sepsis concerning bone metabolism and structure. To investigate these questions we used an experimental model of murine sepsis.

Sepsis was induced by caecal ligation and puncture (CLP) in male transgenic FAT-1 mice (producing n-3 poly unsaturated fatty acids (pufa)) and C57Bl6/J wild-type (WT) mice with administration of an n-3 pufa precursor. After 2, 5 and 8 days postoperatively, bones were harvested for biomechanical testing, micro computed tomography (µCT), histology and real-time-RT-PCR analysis. For statistical evaluation, the Kruskal-Wallis and the Mann-Whitney U test were employed.
Biomechanical testing resulted in a significant decrease of bending stiffness ($p < 0.05$) 2 days postoperatively in FAT-1 mice compared to WT-mice. In addition, µCT analysis of bone mineral density showed a significant ($p < 0{,}005$) reduction in FAT-1 mice compared to WT mice, whilst no difference between WT with or without sepsis could be measured. Using real-time-RT-PCR mRNA expression of osteocalcin and cathepsin K mRNA was reduced significantly during sepsis.

In conclusion, bone metabolism seems to become inactive during the progress of systemic sepsis, as both osteocalcin and cathepsin K expression was reduced. Concerning bending stiffness and bone structure analysed with µCT, systemic sepsis may only have influence on transgenic mice with altered fatty acid metabolism.

Intracellular Staphylococcus aureus in osteoblasts can be killed via TLR9-mediated induction of oxidative stress

Walid Mohamed[2, 3], Eugen Domann[3], Trinad Chakraborty[3], Christian Heiss[1, 2], Reinhard Schnettler[1, 2], Volker Alt[1, 2]

[1] Department of Trauma Surgery Giessen, University Hospital of Giessen-Marburg, Campus Giessen, 35385 Giessen, Germany
[2] Laboratory of Experimental Trauma Surgery Giessen, Justus-Liebig-University Giessen, 35394 Giessen, Germany
[3] Institute of Medical Microbiology, University Hospital of Giessen-Marburg, Campus Giessen, 35392 Giessen, Germany

Staphylococcus aureus is the principle causative pathogen of osteomyelitis and implant-associated bone infections. S. aureus is able to invade and proliferate inside osteoblasts thus avoiding antibiotic therapy and the host immune system. Therefore, development of alternative approaches to stimulate host innate immune responses could be beneficial in prophylaxis against S. aureus infection. One of the largest and most extensively studied groups of innate immune response receptors are the Toll-like receptors (TLRs). TLR9 is the intracellular receptor which recognizes unmethylated bacterial CpG-DNA and activates immune cells. Although TLR9 are mainly expressed in immune cells, it was also detected in osteoblasts. Synthetic CpG-motifs containing oligodeoxynucleotide (CpG-ODNs) mimics the stimulatory effect of bacterial DNA. Osteoblast-like SAOS-2 cells were pretreated with 250 nanomole of a CpG-ODN type-A 2216, type-B 2006, or negative CpG-ODN 2243 (negative control) 4h before infection with S. aureus EDCC5055. Intracellular bacteria were plated out on BHI plates 4h and 20h after infection. ODN2216 as well as ODN2006 but not ODN2243 were able to significantly inhibit the intracellular bacterial growth. RT-PCR analysis of cDNAs from SAOS-2 cells showed that pretreatment with ODN2216 or ODN2006 stimulated the expression of TLR9. Induction of oxidative stress inside SAOS-2 cells was examined after treatment with different ODNs. Pretreatment of SAOS-2 cells with ODN2216 or ODN2006 but not ODN2243 managed to induce reactive oxygen species (ROS) production inside osteoblasts as measured by flow cytometry analysis. Moreover, treating SAOS-2 cells with the antioxidant Diphenyleneiodonium (DPI) obviously reduced S. aureus killing ability of TLR9 agonists mediated by oxidative stress. In this work we demonstrated for the first time that CPG-ODNs have inhibitory effects on S. aureus survival inside SAOS-2 osteoblast-like cell line. This effect was attributed to stimulation of TLR9 and subsequent induction of oxidative stress. Pretreatment of infected SAOS-2 cells with ROS inhibitors resulted in the abolishment of the CPG-ODNs killing effects.

Microbiological analysis of antibacterial effectivity of silver-nanoparticle (Agnp)-dopted bone substitute materials in an experimental rat model

Alexander Lampe[1], Sonja Hartmann[1], Ulrich Thormann[2], Olga Dakischew[1], Ursula Sommer[1], Reinhard Schnettler[1], Katrin Susanne Lips[1]

[1] Laboratory for Experimental Trauma Surgery, Justus-Liebig-University, Schubertstr. 81, 35392 Giessen, Germany
[2] Department of Trauma Surgery, University Hospital of Giessen-Marburg, Campus Giessen, Rudolf-Buchheim-Str. 7, 35392 Giessen, Germany

Implant-assoziated infections are a serious problem and rising problem in orthopedics and trauma surgery. Some strain of bacteria establish biofilms and complex antibiotic resistance. As a possible method against such infections there has been proved an antibacterial effectivity of silver nanoparticle (Agnp) on culture medium. The study´s aim is to prove the antibacterial potency of Agnp in a complex in-vivo rat model.

16 weeks old male rats received an Agnp-dopted implant in the tibia and an injection of 104 CFU of gentamicin resistant MRSA bacteria. Implants consisted of a) polymethyl methacrylate-cement (PMMA) charged with gentamicin and AgNP, b) PMMA with gentamicin but without Agnp, c) PMMA with glycine and Agnp, d) titanium with Agnp, e) titanium without Agnp, f) collagen-scaffolds with Agnp, and g) collagen-scaffolds without Agnp. Per implant a group of 7 rats were chosen that were sacrificed 4 weeks after implantation. One slice of each tibia was extracted, homogenized, exposed on nutrient blood agar, cultivated, and finally enumerated. Another slice was embedded in paraffin, sectioned, and incubated with an antibody against Staphylococcus aureus. Bacteria were visualized and counted with a laser scanning microscope and subsequently statistically analysed. The microbiological analysis of specimen supplied no significant disparity between Agnp charged materials and common materials. There was not even a significant difference between

each material. Falsification of the findings caused by possible contamination other microbes was eliminated by morphological view and additionally by Maldi-Tof research. Analysis of immune fluorescence labelling of Staphylococcus aureus also did not attest a significant disparity between the groups.

In conclusion, Agnp charged bone substitute materials did not induce a significant antibacterial effect on gentamicin resistant MRSA bacteria in the presented model. Potential effect of Agnp in different models has to be analysed in up-coming studies.

Development of new beta-Ti alloys for hard tissue replacement

Romy Schmidt[1,2], Stefan Pilz[1,2], Arne Helth[1], André Reck[2,3], Mariana Calin[1], Martina Zimmermann[2,3], Jürgen Eckert[2], Annett Gebert[1]

[1] Leibniz Institute for Solid State and Materials Research Dresden, Germany
[2] Institute of Materials Science, Dresden University of Technology, Germany
[3] Fraunhofer Institute for Material and Beam Technology, Dresden, Germany

To meet the increasing demand for suitable Ti-based alloys for non-degradable hard tissue implants further development of their bio-functionality is required. Efforts are focussed on the adjustment of the mechanical properties of the implant material to those of the human bone, as well as on tailoring functional surfaces to induce accelerated bone healing and improved anchoring. This is particularly challenging for systemically diseased bone tissue. Beta-titanium alloys are increasingly used in orthopaedics owing good mechanical properties and superior corrosion resistance. A promising candidate is the highly biocompatible Ti-40Nb alloy possessing a Young's modulus of 62 GPa and a compressive strength of above 1200 MPa in the solution treated state.

Presently, a thermo-mechanical processing scheme, based on warm- and cold rolling as well as on heat treatments, is developed to increase of the strength-to-Young's modulus ratio of the alloy. The influence of several surface conditions obtained by mechanical and chemical treatments, on the osteogenic differentiation and the metabolic activity of human mesenchymal stromal cells was investigated by comparative tests with cp-Ti and Ti-6Al-4V. Since the stress conditions for an implant in the human body are quite complex, fatigue studies of the material are carried out. Furthermore, a powder-metallurgical processing route is under development, for the production of fine-grained porous samples with very low stiffness by utilizing hot pressing and sintering.

These efforts can yield new implant materials with an optimal combination of high biomechanical compatibility and suitable surface properties for improving the osseointegration.
This project is funded by the DFG in the framework of the SFB-Transregio 79, project M1.

Role of Osteocyte in Implant Degradation And Bone – Implant Anchorage

Seemun Ray[2], Thaqif El Khassawna[2], Ulrich Thormann[1,2], Ursula Sommer[2], Marcus Rhonke[3], Anja Henns[3], Michael Gelinsky[4], Thomas Hanke[5], Reinhard Schnettler[1,2], Christian Heiss[1,2], Volker Alt[1,2]

[1] Department of Trauma Surgery, University Hospital Giessen-Marburg GmbH, Campus Giessen, Germany
[2] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University, Giessen, Germany
[3] Institute of Physical Chemistry, Justus-Liebig-University, Giessen, Germany
[4] Center for Translational Bone, Joint, and Soft Tissue Research, Medical Faculty of Technische Universität Dresden, University Hospital Carl Gustav Carus, Dresden, Germany
[5] Institute of Materials Science Technische Universität Dresden, Germany

Objectives:
Osseo-integration and implant degradation are the main problems in developing biomaterials for systemically diseased bone. Osteocytes are crucial for bone tissue homeostasis and mechanical integrity. This study examines osteocyte distribution and biology in an ovariectomized rat model with a critical size metaphyseal defect.

Methodology:
60 osteoporotic female Sprague-Dawley rats with a 4mm- wedge shaped metaphyseal osteotomy were randomized into five groups: SrCPC(n=15);CPC(n=15);ScB30(n=15); ScB30Sr20(n=15) and empty defect(n=15). After six weeks, osteocyte morphology and networks were detected using silver-staining. ECM proteins and cellular populations were investigated through immunohistochemistry and enzyme-histochemistry respectively. Mineralization was assessed using time of flight secondary ion mass spectrometry (TOF-SIMS).

Results:
Osteocytes morphology was enhanced in SrCPC when compared to other groups. An increased osteocytic activity was also seen in ScB30Sr20 when compared to SCB30 alone. A regular pattern of osteocyte distribution was also seen in case of the Sr substituted cements. Whereas in case of the ScB30 degenerated osteocytes with a comparatively irregular arrangement were seen. Interestingly, osteocytes were also localized near the blood vessels within the newly formed woven bone. Osteocytes allocation at bone-implant interface and on the implant surface were qualitatively better in the Sr substituted groups. This correlates with healing enhancement and implant retention results obtained from the histomorphometry and immunohisto-chemistry. Sr supplemented biomaterials showed a lower expression of sclerostin with simultaneous up-regulation of matrix extracellular phosphogly-coprotein compared to the Sr free ones.

Result/Conclusion:
Sr enhances osteocytic activity which plays a key role in fracture healing process of the surrounding bone around implants.
Keywords: CPC: Calcium Phosphate Cement; SrCPC: Strontium modified Calcium Phosphate Cement ScB30: macroporous silica/collagen scaffold; ScB30Sr20: strontium-enriched macroporous silica/collagen scaffold

Bone Morphogenetic Protein 7 (BMP-7) Influences Tendon-Bone Integration In Vitro

Tim Schwarting, Philipp Lechler, Johannes Struewer, Marius Ambrock, Thomas Manfred Frangen, Steffen Ruchholtz, Ewgeni Ziring, Michael Frink

[1] Center for Orthopaedics and Trauma Surgery, University of Giessen and Marburg, Marburg, Germany

Objective:
Successful graft ingrowth following reconstruction of the anterior cruciate ligament is governed by complex biological processes at the tendon-bone interface. The aim of this study was to investigate in an in vitro study the effects of bone morphogenetic protein 7 (BMP-7) on tendon-bone integration.

Methods:
To study the biological effects of BMP-7 on the process of tendon-bone-integration, two independent in vitro models were used. The first model involved the mono- and coculture of bovine tendon specimens and primary bovine osteoblasts with and without BMP-7 exposure. The second model comprised the mono- and coculture of primary bovine osteoblasts and fibroblasts. Alkaline phosphatase (ALP), lactate dehydrogenase (LDH), lactate and osteocalcin (OCN) were analyzed by ELISA. Histological analysis and electron microscopy of the tendon specimens were performed.

Results:
In both models, positive effects of BMP-7 on ALP enzyme activity were observed ($p<0.001$). Additionally, similar results were noted for LDH activity and lactate concentration. BMP-7 stimulation led to a significant increase in OCN expression. Whereas the effects of BMP-7 on tendon monoculture peaked during an early phase of the experiment ($p<0.001$), the cocultures showed a maximal increase during the later stages ($p<0.001$). The histological analysis showed a stimulating effect of BMP-7 on extracellular matrix formation. Organized ossification zones and calcium carbonate-like structures were only observed in the BMP-stimulated cell cultures.

Conclusion:
This study showed the positive effects of BMP-7 on the biological process of tendon-bone integration in vitro. Histological signs of improved mineralization were paralleled by increased rates of osteoblast-specific protein levels in primary bovine osteoblasts and fibroblasts. Our findings indicated a role for BMP-7 as an adjuvant therapeutic agent in the treatment of ligamentous injuries, and they emphasized the importance of the transdifferentiation process of tendinous fibroblasts at the tendon-bone interface.

AWARDS

The Scientific Committee identified the 3 most highly ranked Posters and acknowledge their achievement with a Poster Award (300 Euro for each rewarded poster). Further the 3 most highly ranked Free Paper presentations will be awarded with a Free Paper Award (500 Euro for each rewarded paper). The Award Presentation Ceremony will take place after the final session of the symposium on Saturday, October 10, 2015, 12:45 am.

SPONSORED BY

von behring|röntgen| stiftung

SFB/TRR79

Premium Sponsors

AIOD DEUTSCHLAND E.V.

ZIMMER BIOMET Your progress. Our promise.

aap

Gold Sponsors

stryker

Arthrex

Sponsors

DePuy Synthes

Medtronic

Acelity

ORS GmbH Orthopädietechnik / Sanitätshaus

AMGEN

Lilly

AIOD DEUTSCHLAND

AIOD Deutschland e.V.

Die **AIOD** ist ein Gremium für den nationalen und internationalen Erfahrungsaustausch im Bereich Unfallchirurgie und Orthopädie. Die Aufgabe ist die Förderung von Forschung und Lehre im Bereich der Unfallchirurgie und Orthopädie sowie die Verbesserung von Frakturen und Erkrankungen des Bewegungsapparates.

Die **AIOD** bietet Kurse und Fortbildungen im Bereich Arthroskopie, Fußchirurgie, Wirbelsäulenchirurgie uvm. für Ärzte und OP-Personal an. Unsere Kurse bieten Implantatübungen am Knochenmodell oder an anatomischen Präparaten und decken jeden Fort-und Weiterbildungsanspruch ab.
Mit der Teilnahme an den Kursen nehmen Sie automatisch an der Zertifizierung der Landesärztekammern teil.

Wir machen uns für die Ausbildung junger Kolleginnen und Kollegen stark und bieten Hospitationen und Klinikbesuche in unseren Partnerkliniken an.

Besuchen Sie uns auf **www.aiod.deutschland de**.

Termine **2015**

27.09.–02.10.	Sylt	Inselkurs der AIOD, Herbstkurs
08.10.–09.10.	Hamburg	OP-Workshop für OP-Personal
05.11.–06.11.	Gießen	OP-Workshop für OP-Personal
09.11.–10.11.	Leipzig	Arthroskopie-Kurs mit Workshop: Knie / Sprunggelenk
20.11.	Murnau	Zugspitzsymposium
28.11.	Gießen	Handchirurgisches Symposium
03.12.–04.12.	Erfurt	OP-Workshop für OP-Personal

Termine **2016** (1. Jahreshälfte 2016)

12.02.–13.02.	Hannover	Intensivkurs Ellenbogengelenk - Operationstechniken am anatomischen Präparat
18.02.–19.02.	Köln	OP-Workshop für OP-Personal
02.03.–04.03.	Gießen	36. Handchirurgischer Operationskurs
07.03.–08.03.	Leipzig	Arthroskopie-Kurs mit Workshop: Schulter
07.03.–11.03.	Frankfurt	Microsurgery Course
10.03.–11.03.	Lübeck	Fußchirurgischer Hands on-Kurs
14.03.–18.03.	Frankfurt	Microsurgery Course
17.03.–18.03.	Köln	Intensivkurs Ellenbogengelenk - Operationstechniken am anatomischen Präparat
17.04.–21.04.	Sylt	Inselkurs der AIOD, Frühjahrskurs
06.06.–10.06.	Alpbach, Österreich	Traumakurs Alpbach
23.06.–24.06.	Murnau	OP-Workshop für OP-Personal

Information & Anmeldung:
AIOD Deutschland e.V. · Postfach 210135 · 45065 Essen
Fon: +49 201 8945162 · Fax: +49 201 4260240 · info@aiod-ev.de · www.aiod-deutschland.de

Alagboso, Francisca
Justus-Liebig-University Giessen
Laboratory of Experimental
Trauma Surgery
Giessen, Germany

Prof. Dr. Dr.
Alt, Volker
University Hospital Giessen and
Marburg, Campus Giessen
Department of Trauma Surgery
Giessen, Germany

Prof. Dr.
Aspenberg, Per
Linköping University
Orthopedics
Linköping, Sweden

Prof. Dr.
Baumgart-Vogt, Eveline
Justus-Liebig-University Giessen
Department of Anatomy and Cell
Biology II, Division of Medical Cell
Biology
Giessen, Germany

Bohl, Friedrich
von-Behring-Röntgen-Foundation
Marburg, Germany

Prof. Dr.
Cheung, Louis
The Chinese University of Hong Kong
Department of Orthopedics and
Traumatology,
Hongkong, China

Dr.
Daghma, Diaa Eldin S.
Justus-Liebig-University Giessen
Laboratory for Experimental
Trauma Surgery
Giessen, Germany

Prof. Dr.
Domann, Eugen
Justus-Liebig-University Giessen
Medical Microbiology Molemlar
Diagnostics
Giessen, Germany

Prof. Dr.
Drees, Philipp
University Medicine Mainz
Center of Orthopedics and
Trauma Surgery
Mainz, Germany

Prof. Dr.
Ebeling, Peter
Monash University
Monash Medical Center,
Campus Clayton
Monash, Australia

Dr.
El Khassawna, Thaqif
Justus-Liebig-University Giessen
Laboratory for Experimental
Trauma Surgery
Giessen, Germany

Ernst, Manuela
AO Research Institute Davos
Biomedical Services
Davos Platz, Switzerland

Floel, Wolfgang
Justus-Liebig-University Giessen
Laboratory of Experimental
Trauma Surgery
Giessen, Germany

Prof. Dr.
Frink, Michael
University Hospital Giessen and
Marburg, Campus Marburg
Center for Orthopedics and
Trauma Surgery
Marburg, Germany

Dr.
Geissler, Sven
Charité University Medicine
BORT/ JWI
Berlin, Germany

Dr.
Hanke, Thomas
Technical University Dresden
Institute of Materials Science
Dresden, Germany

Prof. Dr.
Heiss, Christian
University Hospital Giessen and
Marburg, Campus Giessen
Department of Trauma Surgery
Justus-Liebig-University Giessen
Laboratory of Experimental
Trauma Surgery
Giessen, Germany

PD Dr. Dr.
Hose, Dirk
University Hospital Heidelberg
Medical Clinic V
Heidelberg, Germany

Prof. Dr.
Ignatius, Anita
University of Ulm
Institute of Orthopedic
Research and Biomechanics
Ulm, Germany

Dr.
Kampschulte, Marian
University Hospital Giessen and
Marburg, Campus Giessen
Department of Diagnostic and
Interventional Radiology
Giessen, Germany

Prof. Dr.
Kann, Peter Herbert
University Hospital Giessen and
Marburg, Campus Marburg
Department of Endocrinology and
Diabetology
Marburg, Germany

Dr.
Kovtun, Anna
University Hospital Ulm
Institute of Orthopedic Research
and Biomechanics
Ulm, Germany

Kroner, Jochen
University Hospital Ulm
Institute of Orthopedic Research
and Biomechanics
Ulm, Germany

Lampe, Alexander
Jutus-Liebig-University Giessen
Laboratory for Experimental
Trauma Surgery
Giessen Germany

Dr.
Lechler, Phillip
University Hospital Giessen and
Marburg, Campus Marburg
Center for Orthopedics and
Trauma Surgery
Marburg, Germany

Prof. Dr.
Lehmann, Wolfgang
University Hospital
Hamburg-Eppendorf,
Trauma Surgery
Hamburg, Germany

Prof. Dr.
Lips, Katrin S.
Justus-Liebig-University Giessen
Laboratory of Experimental
Trauma Surgery
Giessen, Germany

Prof. Dr.
Lohoff, Michael
Philipps-University Marburg
Institute of Medical Microbiology
and Hospital Hygiene
Marburg, Germany

Malhan, Deeksha
Justus-Liebig-University Giessen
Laboratory of Experimental
Trauma Surgery
Giessen, Germany

Mamitzsch, Bettina
Technical University Dresden
Leibniz Institute of Polymer Research
Dresden, Germany

Dr.
Mohamed, Walid
University Hospital Giessen and
Marburg, Campus Giessen
Institute of Medical Microbiology
Giessen, Germany

Dr.
Möhler, Thomas
University Hospital Heidelberg
Laboratory of Myeloma Research
Heidelberg, Germany

PD Dr.
Moter, Annette
Deutsches Herzzentrum Berlin
Center of Biofilm
Berlin, Germany

Prof. Dr.
Mukherjee, Joybrato
Justus-Liebig-University Giessen
President
Giessen, Germany

Prof. Dr.
Neubauer, Andreas
University Hospital Giessen and
Marburg, Campus Marburg
Clinic of Hematology,
Oncology, Immunology
Marburg, Germany

Prof. Dr.
Pohlemann, Tim
University Hospital of Saarland
Clinic of Trauma-, Hand- and
Reconstructive Surgery
Homburg/Saar, Germany

Dr.
Ray, Seemun
Justus-Liebig-University Giessen
Laboratory for Experimental
Trauma Surgery
Giessen, Germany

Prof. Dr.
Roschger, Paul
Hanusch-Hospital
Ludwig Boltzmann-Institute of
Osteology
Vienna, Austria

Rössler, Sina
Technical University Dresden
Max-Bergmann-Center for
Biomaterials
Dresden, Germany

Prof. Dr.
Ruchholtz, Steffen
University Hospital Giessen and
Marburg, Campus Marburg
Center of Orthopedics and
Trauma Surgery
Marburg, Germany

Ph.D.
Sahota, Surinder
University of Southampton
Cancer Sciences Unit
Faculty of Medicine
Southhampton, United Kingdom

Schaepe, Kaija
Justus-Liebig-University Giessen
Institute of Physical Chemistry
Giessen, Germany

Prof. Dr.
Schäfer, Helmut
University Hospital Giessen and
Marburg, Campus Marburg
Faculty of Medicine
Marburg, Germany

Prof. Dr.
Schäffler, Andreas
University Hospital Giessen and
Marburg, Campus Giessen
Department of Internal Medicine III
Giessen, Germany

Prof. Dr.
Schieker, Matthias
University Hospital Munich
Clinic of General Medicine,
Trauma-, Hand- and Plastic Surgery
Munich, Germany

Prof. Dr.
Schmidmaier, Gerhard
University Hospital Heidelberg
Clinic of Orthopedics and
Trauma Surgery
Heidelberg, Germany

Schmidt, Romy
Leibniz Institute for Solid
State and Materials Research
Dresden
Institute for
Complex Materials
Dresden, Germany

Dr.
Schüttler, Karl-Friedrich
University Hospital Giessen and
Marburg, Campus Marburg
Center for Orthopedics and
Trauma Surgery
Marburg, Germany

Dr.
Schwarting, Tim
University Hospital Giessen and Marburg, Campus Marburg
Center for Orthopedics and Trauma Surgery
Marburg, Germany

Dr.
Scott, David
Monash University
Monash Medical Centre, Clayton Campus
Monash, Australia

Dr.
Seckinger, Anja
University Hospital Heidelberg
Medical Clinic V
Heidelberg, Germany

Prof. Dr.
Seeger, Werner
University Hospital Giessen and Marburg, Campus Giessen
Medical Clinik and Policlinic II
Giessen, Germany

Dr.
Thormann, Ulrich
University Hospital Giessen and Marburg, Campus Giessen
Trauma Surgery
Giessen, Germany

Prof. Dr.
Weidner, Wolfgang
University Hospital Giessen and Marburg, Campus Giessen
Faculty of Medicine
Giessen, Germany

Weisweiler, David
University Hospital Giessen and Marburg, Campus Giessen
Department of Trauma Surgery
Giessen, Germany

PD Dr.
Ziller, Volker
University Hospital Giessen and Marburg, Campus Marburg
Clinic of Gynaecology and Obstetrics
Marburg, Germany

EDITORS

Univ.-Prof. Dr.
Christian Heiss
University Hospital Giessen and Marburg, Campus Giessen
Department of Trauma Surgery
Justus-Liebig-University Giessen
Laboratory of Experimental Trauma Surgery
Giessen, Germany

Univ.-Prof. Dr.
Steffen Ruchholtz
University Hospital Giessen and Marburg, Campus Marburg
Center of Orthopedics and Trauma Surgery
Marburg, Germany

Ungehindert im Leben bewegen –
mit den Profis der ORS

ORS-GmbH | Orthopädie-Technik | Sonderbau | Sanitätshaus

Die ORS Orthopädie- und Rehatechnik GmbH steht für gewachsene Kompetenz und zertifizierte Qualität in den Bereichen Orthopädie-Technik, Sonderbau und Sanitätshaus.

Überzeugen Sie sich persönlich in einer unserer 6 Filialen in Gießen, Lich und Wetzlar von dem ORS-Komplettservice für Gesundheit und Mobilität.

Frankfurter Straße 1
35390 Gießen
Telefon (0641) 97258-0

Am Klinikum · Forsthausstraße 5
35578 Wetzlar
Telefon (06441) 30989-0

ORS GmbH
Orthopädietechnik / Sanitätshaus

info@ors-gmbh.de · www.ors-gmbh.de

NOTES

AIOD
DEUTSCHLAND

Ankündigung

AIOD-Frühjahrskurs, Sylt
16.04. – 21.04.2016

Thema:
Spezielle Traumatologie
von Rumpf, Wirbelsäule
und Becken, Septische Chirurgie

Themenbezogene Vorträge, Workshops
am Kunstknochen, Falldiskussionen,
Intensivseminare

AIOD-Herbstkurs, Sylt
02.10. – 07.10.2016

Thema:
· Extremitätentraumatologie
· Kindertraumatologie
(anerkannt durch die DGUV:
Kindertraumatologie für den D-Arzt)

Themenbezogene Vorträge, Workshops
am Kunstknochen, Falldiskussionen,
Intensivseminare

Außerdem zum Herbstkurs 2016:
BG-Gutachten Basiskurs

Information & Anmeldung:
AIOD Deutschland e.V.
Postfach 210135 · 45065 Essen
Fon: +49 201 8945162 · Fax: +49 201 4260240
info@aiod-ev.de · www.aiod-deutschland.de