1) „Treating Spinal Fractures: Biomechanics, Biomaterials or Biology“

Prof. Dr. Stephen J. Ferguson
ETH Zurich

The spine is truly the backbone of our musculoskeletal system, providing both mobility and load-carrying capacity. Spinal pathologies, and back pain in general, are some of the most costly challenges facing our society. Age-related changes to the structural composition of the bone tissue expose the spine to a higher risk of injury in response to acute loading. Current treatments do not specifically address the root cause of the problem and are not adequately adapted to an ageing patient population. Furthermore, research in the past has been too narrowly focused on the mechanics of fracture treatment, often ignoring the complex link between biomechanics and biology in the injury and healing processes. In this talk, examples will be given of how medical engineering principals can be applied to better understand the patho-mechanism of spinal injury and to develop more effective treatments. For the prevention or treatment of osteoporotic fragility fractures, a combination of computer simulation methods and new biomaterials allows the minimally invasive repair and reinforcement of weakened or compromised bone. A link between traumatic fractures of the spine and subsequent intervertebral disc degeneration is explored and explained. The problem of intervertebral disc degeneration and structural failure of the disc is approached by studying the mechanobiological response of the whole organ to acute overloading. Combining knowledge of the mechanics and biology of the disc at multiple scales, new methods to repair damage to the disc and simultaneously suppress inflammatory response have been developed. In summary, treatment of a spinal fracture requires a broader focus, considering the vertebra and disc as a closely integrated, functional organ, and combining knowledge of biomechanics, biomaterials and biology to achieve the optimal outcome.

2) „The bone inducing principle: from bench to clinic“

Prof. Dr. Franz E. Weber
University Hospital Zurich

Hippocrates, the father of medicine, noticed around 400 B.C. that bone heals without scarring. It wasn't until the year 1965 that Marshal Urist recognized that underlying molecules concentrated in a crude extract derived from demineralized bone have the same capability as bones. In the years to come initial clinical trials were performed in the orthopedic field using this rather crude extract, focusing mainly on the treatment of non-unions from the femur or the tibia. These trials were proof of the potential of the "bone inducing principle" for bone formation and healing purposes in the medical field. In 1988, Bone morphogenetic proteins (BMPs),

the proteins responsible for the bone inducing principle, were isolated, cloned and shown to be key cytokines in bone formation and repair. The potential of BMP to induce bone formation and repair has been demonstrated in preclinical trials using numerous animal model systems. In humans, however, huge amounts of BMP are needed to show effect or to measure up to results normally achieved by the use of autologous bone. From 6 clinical trials reported in the dental field, only 2 showed an improvement upon BMP treatment, especially in high BMP dosage groups. Since high BMP dosages could induce side effects and increase the cost of treatment, several strategies have been employed to reduce the amount of BMP needed for clinical applications: optimization of the BMP release by the delivery system, inhibition of BMP antagonists and by enhancing BMP activity by small chemicals or other means. In the first part of our presentation, we will report on BMP-delivery systems based on hydrogels, on calcium phosphates and titanium scaffolds.
In the second part, the effects of the BMP enhancer N-methyl-pyrrolidone on osteoblasts and osteoclasts will be presented. In addition to in vitro and preclinical trials on NMP and other BMP enhancers, we will also report on the outcome of 2 clinical trials on bone regeneration and bone augmentation using the NMP releasing membrane in the dental field. Since NMP enhances BMP activity and reduces the maturation and activity of osteoclasts, it could become a versatile tool for the treatment of patients suffering from osteoporosis.

3) „Modulation of extracellular matrix proteins by serine protease HtrA1 and its influence on bone formation"

Dr. Peter J. Richards
University of Zurich

Mammalian HTRA1 is a secreted member of the trypsin family of serine proteases, which can degrade a variety of bone matrix proteins and as such, has been implicated in musculoskeletal development. In the present study, we have investigated the role of HTRA1 in mesenchymal stem cell (MSC) osteogenesis and suggest a potential mechanism through which it controls matrix mineralization by differentiating bone-forming cells. Osteogenic induction resulted in a significant elevation in the expression and secretion of HTRA1 in MSCs isolated from human bone marrow (hBMSCs), mouse adipose tissue (mASCs), and mouse embryonic stem cells (mESCs). Recombinant HTRA1 enhanced the osteogenesis of hBMSCs as evidenced by significant changes in several osteogenic markers including integrin-binding sialoprotein (*IBSP*), bone morphogenetic protein 5 (*BMP5*) and sclerostin (*SOST*), and promoted matrix mineralization in differentiating bone-forming osteoblasts. These stimulatory effects were not observed with proteolytically inactive HTRA1 and were abolished by small interfering RNA (siRNA) against *HTRA1*. Moreover, loss of HTRA1 function resulted in enhanced adipogenesis of hBMSCs. HTRA1 Immunofluorescence studies showed co-localization of HTRA1 with IBSP

protein in osteogenic mASC spheroid cultures and was confirmed as being a newly identified HTRA1 substrate in cell cultures and in proteolytic enzyme assays. A role for HTRA1 in bone regeneration *in vivo* was also alluded to in bone fracture repair studies where HTRA1 was found localized predominantly to areas of new bone formation in association with IBSP. These data therefore implicate HTRA1 as having a central role in osteogenesis through modification of proteins within the extracellular matrix.

4) „Investigating the role of Toll-like receptor 2 in intervertebral disc degeneration and inflammation“

Dr. Karin Würtz
ETH Zurich

Although inflammatory processes play an essential role in painful intervertebral disc (IVD) degeneration, the underlying regulatory mechanisms are not well understood. The objective of this study was to investigate the expression, regulation and thus the role and importance of specific Toll-like receptors (TLRs) in inflammation of the IVD, with a focus on TLR2

A comprehensive, step-wise approach was used to characterize the TLRs expression/regulation in the degenerated human IVD. Basal mRNA expression of TLR1-10 was investigated and then correlated to the degree of degeneration. In a next step, regulation of correlated TLRs by typical inflammatory mediators present in the IVD (IL-1β or TNF-α) was investigated. In addition, the effects of IL-1β and TNF-α on the expression of possible endogenous TLR2 ligands (HSP60, HSP70 and HMGB1) as well as the inflammatory properties of these endogenous ligands was analyzed.

In summary, we were able to show expression of multiple TLRs in the human IVD (TLR1-TLR6, TLR9, TLR10) with TLR1, TLR2, TLR4, TLR6 and TLR10 showing a correlation to the degree of degeneration. Stimulation with IL-1β and TNF-α predominantly resulted in increased mRNA expression of TLR2, which could be confirmed on the protein level. Furthermore, typical TLR2 target genes (IL-6, IL-8) were up-regulated upon stimulation with IL-1β and TNF-α. However, inhibition experiments clearly demonstrated that neither IL-1β nor TNF-α act as TLR2 ligands. IL-1β and TNF-α also did not increase the expression of HSP60, HSP70 or HMGB1, which could potentially act as endogenous TLR2 ligands. Although described in cartilage, HSP60, HSP70 or HMGB1 did not induce the expression of the TLR2 target genes IL-6 and IL-8.

This project clearly demonstrated the importance of TLRs in inflammatory IVD disease, with TLR2 playing a dominant role. However, IL-1β and TNF-α do not act directly as TLRs ligands. Furthermore, HSP60, HSP70 and HMGB1 – which were not elevated by IL-1β and TNF-α - have no effect on the expression of typical TLR2 target genes.

However, we were able to show in another project that hyaluronic acid fragments cause increased expression of IL-6 by activating TLR2. In future experiment, we will test IL-1β and TNF-α induce expression of hyaluronic acid degrading enzymes (i.e. hyaluronidases) in IVD cells, which has been described for other cell types.

5) „Imaging technologies and their specific applications“

Prof. Dr. Patrick R. Kircher
University of Zurich

With the implementation of modern diagnostic imaging technologies into Veterinary Medicine, new expectations toward the veterinary radiologists arose. Not only in clinical situations, where Computed Tomography and Magnetic resonance imaging slowly but steadily become routine diagnostic tools. But also in research the demands grow toward more and more sophisticated technologies, such as functional and molecular imaging possibilities. As a vast amount of biological and medical research projects are jacked on animal trials, veterinary diagnostic imaging is forced to enter this field, also for the sake of the animals used. The concept of the 3Rs shall here be mentioned.
Besides imaging technologies used in laboratories, such as micro-CT, ultra-high field MRI (>7T), the clinical modalities are in focus more and more, as animals such as dogs, pigs and sheep play an important role. Imaging protocols to show physiology (MR-spectroscopy, BOLD-imaging, MR-angiography, PET) or that have the potential to show changes on molecular basis (receptor targeted contrast media) are under strong investigation, also in Veterinary medicine in order to replace invasive sampling techniques. If this aim will once be reached remains an open question.
A very important field is image processing. The datasets acquired in today's modalities are large and reconstructions and calculations within them is a research field in itself. Therefore big efforts have to be made to fuse the knowledge of researchers in all fields touching diagnostic imaging.

6) „Aneurysm treatment – from bench to bedside“

Dr. Zsolt Kulscar
Hirslanden Hospital Zurich

The incidence of cerebral aneurysm rupture is only about 10 in every 100,000 persons per year, although epidemiological data show that 2 to 4 % of the western population carries a cerebral aneurysm. It is therefore of outmost importance to identify the aneurysms at risk of rupture, which will require treatment. Besides clinical studies on rupture risk assessment based on aneurysm location and size, it

becomes more and more accepted that morphological characteristics and intra-aneurysmal hemodynamics play also an important role in risk assessment, requesting focused research. Our research including computerized flow dynamic simulations (CFD) aim the picturing of intra-aneurysmal hemodynamics, and provide an additional tool to the understanding of transduction of hemodynamic factors into biological signal.
Once an aneurysm requires preventive treatment to avoid (re)rupture, endovascular therapy is the most convenient therapy of choice due to its minimally invasive nature and safety profile. Flow diversion treatment became in the last years a very promising reconstructive modality and repair of the diseased vessel segments. With all shortcomings of flow diversion unravelled, our research in this topic is focusing on understanding hemodynamic changes induced by flow diverters, and on the development of new flow diverter devices with better safety and effectiveness profile.
We have shown, that spontaneous or induced intra-aneurysmal thrombosis may have a detrimental effect on the aneurysm wall, leading to rupture. Engineering clot formation, and influencing clot formation in a way to avoid the destructive effects is our research goal. Our plan is to combine computerized simulations with pathophysiological processes for better understanding the effects of thrombosis.

7) „The interaction of pharmacological treatment and mechanical loading in a mouse model of postmenopausal osteoporosis“

Dr. Gisela Kuhn
ETH Zurich

Postmenopausal osteoporosis, the most common form of osteoporosis in women, is characterized by a high bone turnover with a negative remodeling balance. This results in a loss of bone mass and an increased fracture risk. Most patients are treated with bisphosphonates which decrease bone remodeling and therefore bone loss. Bone formation can be stimulated with parathyroid hormone (PTH). However, this therapy is expensive and less accepted by patients, because of the daily injections needed. An alternative treatment option is vibration therapy. It simulates mechanical stimulation which increases bone mass in healthy bone. However, it is currently unclear whether pharmacological intervention counteracts adaptation to mechanical loading.
In our study, we investigated the interaction of pharmacological treatment with bisphosphonate or PTH combined with mechanical loading in a mouse model of postmenopausal osteoporosis. Estrogen depletion due to ovariectomy (OVX) induces high turnover bone loss in the mouse, mimicking postmenopausal osteoporosis. Mice were treated with bisphosphonate (Zoledronate, 100 µg/kg once) or PTH (hPTH 1-34 80µg/kg daily), or the respective vehicle for 4 weeks and subjected to mechanical loading of the 6th caudal vertebra with 8N or 0N starting 5 or 11 weeks

after OVX. Bone morphometric parameters were assessed by time-lapsed in vivo micro-CT before surgery, at treatment start, and twice during treatment.
After OVX, mice lost approximately 20% of the bone mass in the 6th caudal vertebra. Trabecular bone volume density decreased by 30% over the time course, which was caused by a loss in trabecular number and thickness. Bisphosphonate treatment decreased bone resorption rate and helped to preserve the existing microarchitecture. No further trabeculae were lost. PTH led to a strong increase in both bone formation and bone resorption, with an overall positive remodeling balance. This resulted in an increase in bone volume density which was caused by thickening of the trabeculae. However, the loss of trabeculae could not be stopped. Loading alone led also to a positive remodeling balance. Loss of trabeculae could not be counteracted, however the remaining structures thickened. Combination of loading with both treatments increased the gain in bone mass compared to loading or pharmacological treatment alone.
We conclude that the positive effect of mechanical loading is not counteracted by the most commonly used pharmacological treatments for osteoporosis. Therefore an increase in physical activity might be beneficial for all osteoporotic patients under treatment, independent of the type of pharmacological intervention.