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Animal Model Workshop Presentations

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WS1
The Rat as A Model for Osteoporosis

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Disorders of remodelling are central to many diseases of bone, but the factors that initiate and co-ordinate remodelling *in vivo* are not completely understood. Osteoporosis is the most common of these diseases, affecting older people with a combined lifetime risk of fracture of over 40%. A large variety of animal species, including rodents, rabbits, dogs, and primates, have been used as animal models for osteoporosis research. Although many researchers prefer the laboratory rat, this model must be used with the knowledge of its advantages and limitations. These are not always well understood. For example, trabecular bone in the rat undergoes secondary remodelling, but in the absence of an appropriate stimulus, cortical bone does not. The rat has been used in many experimental protocols leading to bone loss, including hormonal interventions (ovariectomy, orchidectomy, hypophysectomy, parathyroidectomy), immobilization, and dietary manipulations. However, because many long bones grow well in to adulthood, age-related bone loss is best studied in rats of at least 6–8 months of age, otherwise effects on growth, and not ageing, confound interpretation. This presentation will examine the ovariectomized rat and its advantages as an appropriate model, and provide information about the most relevant age and bone site selection according to the goals of an experiment. Several methods for evaluating bone mass will be discussed, as well as the use of biochemical markers, densitometry, histomorphometry, and bone mechanical testing. These will highlight effective use of the rat model for evaluation of preventive or therapeutic strategies for preventing or treating osteoporosis.
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WS2
In Vivo Bone Imaging - From Dynamic Morphometry To Systems Biology

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A systems biology approach to understanding biological systems demands the development of *in vivo* imaging methods allowing hierarchical assessment of bone structure and function, from micro to macro and from cell to organ. A number of new microstructural imaging modalities have been put forward recently allowing quantification with high precision and high resolution. Although biomedical *in vivo* imaging technology is now readily available, few attempts have been made to expand the capabilities of these systems by adding quantitative analysis tools and by exploring structure function relationships in a hierarchical fashion over the different length scales. Nevertheless, such quantitative endpoints have become an important factor for success in basic research and the development of novel therapeutic strategies in biomedicine and clinical practice.
Computed tomography is key to these developments being an approach to image and quantify trabecular bone in three dimensions and providing multi-scale biological imaging capabilities with isotropic resolutions ranging from a few millimeters down to one hundred nanometers. With the recent development of time-lapsed *in vivo* imaging allowing the assessment of not only static but also dynamic morphometric indices in small animals, it is now possible to identify and monitor local remodeling events and to calculate bone formation and resorption rates in bones. As part of the presentation, new strategies for advanced hierarchical quantification of bone in live animals and its structure function relationships will be presented. The focus will be on *in vivo* imaging from dynamic morphometry to the use of systems biology approaches to combine the imaging of bone remodeling with local gene expression.
Such an approach is expected to improve our understanding of structure function relationships in bone down to the cellular level and with that to allow improved quality control and more successful outcomes in the pharmacological treatment of bone.

WS3
Histomorphometry Analyses: Preclinical and Clinical Application

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Bone histology and histomorphometry have played essential role in the study of bone pathphysiology and are important in the diagnosis and management of metabolic bone diseases. In osteoporosis research, bone histomorphometry is a unique technique that provides critical data for understanding tissue level mechanisms of action and plays an important role in documenting the biological effects and possible side-effects of new drug treatments.
With proper *in vivo* double labeling schedule, the level of bone turnover and rate of bone formation can be determined, and

possible mineralization abnormalities can be identified. Tetracycline families are commonly used for human while calcein, xylenol orange, alizarin complex and tetracycline can all be used for preclinical studies. These chemicals have spontaneous fluorescence with distinguish colors and bind to actively forming bone surfaces.

Static histomorphometric analyses allow to quantitate the bone volume, microstructure, connectivity, bone cell (osteoblast, osteoclast and osteocyte) and osteoid at different bone surfaces. Polarized microscopy evaluation can detect the collagen orientation so to identify the woven or lamellar bone in nature. Dynamic bone histomorphometric analyses of bone turnover rate, activation frequency, mineralizing surface and mineral appositional rate are the most important parameters to access the drug working mechanism and efficacy. Due to the importance of cortical component contributes to bone strength, quantitative evaluation should not only limit to trabecular surface, and it can be conducted on cortex of human iliac crest and animal long bones.

Bone sections of in situ hybridization histochemistry (ISHH) can reveal cellular sources and different gene expressions of specific biomolecules involved in bone metabolism, i.e., cytokines, matrix proteins, and receptor species. With the improving embedding and staining techniques, qualitative and quantitative analyses of immunohistochemistry (IHC) on undecalcified bone sections are doable. IHC assesses the metabolic state and bone formation and resorption markers of bone cells by visualizing protein production surface and cell specifically.

In conclusion, bone histomorphometry provides unique information precisely on the tissue level qualitative and quantitative changes of bone structure, matrix, turnover, and cellular characteristics. Together with other methods such as circulating biomarkers, densitometry, biomechanical test, we can evaluate the bone of material property, morphology, tissue level cellular activities and quality.

WS4

Using CreER System for Lineage Tracing and Functional Studies

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In recent years the CreER transgenic approach has been widely used for lineage tracing and functional studies. Using *Osx-CreER* and *Nes-CreER* transgenic mice, distinct osteoblast progenitor cell populations have been identified during specific stages of skeletal development. Our lab has recently generated *Col2-CreER* transgenic mice. Using these mice we have identified specific Col2-expressing cell populations in bone and these cells include growth plate and articular chondrocytes in long bones, inner annular fibrosus (AF) cells and cartilage endplate chondrocytes in intervertebral disc, and TMJ condyle cells in temporomandibular joint (TMJ). Using *Col2-CreER* transgenic mice, we have specifically deleted β-*catenin* and *Sox9* genes or activated β-*catenin* gene in Col2-expressing cells and gained significant insights into functions of β-catenin and Sox9 in Col2-expressing chondrocytes. These studies demonstrate that CreER system is a useful tool for lineage tracing and functional studies.

WS5

Murine Models of Arthritis and Their Phenotypic Analyses by *in Vivo* Imaging Technologies

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Arthritis, including rheumatoid arthritis and osteoarthritis, is a chronic, painful and disabling disease affecting millions of people worldwide. Murine models of arthritis are an important tool for both the preclinical study of arthritis pathogenesis and the efficacy of anti-arthritis drugs, which have significantly advanced our understanding of arthritis. In the first part of my presentation, I will introduce 3 mouse models of rheumatoid arthritis including TNF transgenic mice, the K/BxN arthritis and Collagen-induced arthritis, and a Meniscal Ligamentous Injury-induced mouse model of osteoarthritis. These models have been widely used in pre-clinical studies in many labs. I will focus on applications, advantages and limitations of individual model. Because longitudinal translational outcome measures of disease progression or interventional therapy in mouse models of arthritis are essential for prototypical preclinical investigations of drug effects on inflammation and tissue damages, in the second part of my presentation, I will introduce several *in vivo* imaging techniques that are recently developed by our group and others. These techniques include contrast-enhanced magnetic resonance imaging, near-infrared lymphatic imaging, Power Doppler ultrasound, and near-infrared dye based molecular imaging, which allow us to assess disease activity and progression *in vivo* in arthritic mice longitudinally in preclinical arthritis study. I will present examples that we used these technologies in TNF transgenic mice, the K/BxN arthritis and a Meniscal Ligamentous Injury-induced mouse model of osteoarthritis. Thus, molecular imaging technologies will greatly enhance the utility of murine models of arthritis in biological research.