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WORKSHOP ABSTRACTS

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W1.1
Decellularised Tissues

Shu Barylak
USA

The use of biologic scaffolds composed of extracellular matrix has found widespread use in multiple body systems including the musculoskeletal system, gastrointestinal tract, lower urinary tract, central nervous system, and cardio-vascular system, among others. Such ECM scaffold materials are prepared by decellularization of source tissues. The mechanism by which these inductive scaffold tissues facilitate constructive tissue remodeling include recruitment of endogenous stem cells, modulation of the host innate immune response, and the temporary mechanical substrate for tissue reconstruction. The presentation will include methods of scaffold preparation, mechanisms of action, and clinical applications with a focus upon musculoskeletal repair.

W1.2
Talking to Cells: Surface Topography as Tool to Evoke Cellular Responses

Jan de Boer
Laboratory of Cell Biology-inspired Tissue Engineering, MerIn Institute, Maastricht University, The Netherlands

Research in our laboratory is dedicated to understanding and applying basic cell biological principles in the field of biomedical engineering, in particular in the regeneration of bone tissue. The research program is characterized by a holistic approach to both discovery and application, aiming at combining high throughput technologies, computational modeling and experimental cell biology to streamline the wealth of biological knowledge to real clinical applications. In my seminar I will present our latest work on controlling the interaction of cells with biomaterials through design of surface topography. For instance, we are interested in the bone-inducing properties of a subset of porous calcium phosphate ceramics and show how through reverse engineering, we are uncovering an interesting and complex response of cells to materials. Inspired by this, we have started to design high throughput screening strategies of biomaterials libraries, and in particular libraries of surface topographies. Using a design algorithm, we have generated numerous different patterns, which can first be reproduced on a silicon mold and then imprinted onto polymers using microfabrication. After cell seeding, we use quantitative high content imaging and machine learning algorithms to characterize the response of the cells to the thousands of different surfaces and learn more about the relation between surface topography and cell response. For instance, we have identified surfaces which stimulate osteogenic differentiation of mesenchymal stem cells and we are currently testing whether these surfaces can be applied in orthopedic surgery.

W1.3
Engineering of 3D Vascularized Human Tissues

Heike Walles
Germany

Regenerative Medicine is a multidisciplinary field that combines engineering, physical and biological sciences and medicine with the overall goal to restore or to replace damaged tissues or organs. In order to reach this goal there are several strategies such as the application of cell suspensions or biomaterials. Furthermore the use of tissue engineering constructs, which are produced by a combination of both cells and scaffolds, is a more sophisticated approach. Therefore, it is necessary to isolate cells from the patient that are cultured together with the scaffold in vitro before this construct can be implanted into the patient. Vascularization is a major challenge in creating tissues *ex vivo*. Complex tissue engineered constructs exceeding a thickness of 100-200 µm need a vascular system in order to supply the cells with oxygen and nutrients and moreover remove waste products. This restricts generation of tissues with an appropriate size for clinical application and complex tissues such as the bone. We developed 3D vascularized tissues based on decellularized porcine small bowl segments and preserved tubular structures of the capillary network within the collagen matrix which is functional associated with one small vein and artery (biological vascularized scaffold - BioVaSc). This vascularized matrix enables the generation of a functional artificial vascular network and vascularized tissues as trachea, bone, skin, fatty tissue, intestine and liver. Possible application of this technology is the so called ATMPs - advanced therapy **medicinal** products. We are in preclinical and clinical testing of different vascularized implants. During the talk will be shown that it is possible to use the BioVaSc platform technology to generate autologous human transplants wich can be connected to the recipient vascularization. An overview of the initiated phase I/II clinical TraVaSc trial (tracheal reconstruction), the preclinical trial for the treatment of critical bone size defect (BoneVaSc) and the application as SkinVaSc and AdiVaSc will be shown. At the end of the presentation an overview of non-destructive methods to characterize the complex implants will be given. Our Team is focusing on the impedance and Raman spectroscopy for these applications.

W2.1

Abstract not available

W2.2

Abstract not available

W2.3

Pharmacological Management of Sarcopenia

William Evans
Muscle and Health Division, KineMed, Emeryville, CA and Division of Geriatrics, Duke University, Durham, NC

Sarcopenia is a life-long process of loss of muscle mass and strength. The causes of sarcopenia are complex and multifactorial. Among the many factors involved are insulin resistance, reduced physical activity, decreased dietary protein intake along with an age-related increase in dietary protein needs, loss of motor units, and decreased anabolic factors such as IGF, testosterone, and an increase in myostatin levels. Exercise has a profoundly positive effect even in very old and frail people. In particular, resistance exercise results in a large increase in strength, improved balance, increased bone density, and improved functional capacity. However, extreme loss of muscle mass and strength occurs in many older people during hospitalization and bed rest. In addition, muscle wasting associated with chronic disease (such as CHF, COPD, Cancer, and CKD) in elderly people may accelerate sarcopenia. Under these circumstances exercise and diet may play a very limited role. A new generation of anabolic therapies may rescue elderly people from conditions of rapid muscle wasting and improve functional capacity sufficiently to allow elderly people who suffering muscle wasting to recover more rapidly and decrease the risk of disability. These therapies include Selective Androgen Receptor Modulators, TGF-beta superfamily targets such as anti-myostatin anti bodies, ActRIIb, FLRG, and ghrelin. These anabolic agents stimulate the rate of muscle protein synthesis by activating mTOR in muscle. Protein synthetic pathways appear to be the most promising targets rather than anti-catabolic therapies. In addition, most of the drugs that stimulate muscle protein synthesis have been demonstrated to also increase bone density. Data will be presented on preclinical and clinical trials, with particular attention of aging.

W3.1

Regulation of Bone Remodelling by Semaphorins and Sensor Innervations

Shu Takeda
Japan

It was believed that cytokines and hormones are main regulators of bone remodeling. However, this view has been challenged. Organ network has been shown to play a major role in homeostasis, recently. Bone is not the exception. Clinically, it is well known that head trauma accelerates fracture healing. Advances in molecular genetics revealed that neurons and neuropeptides, including sympathetic nervous system, are intimately involved in bone remodeling. Semaphorin 3A (Sema3A) is a diffusible axonal chemorepellent that plays an important role in axon guidance. Previous studies have demonstrated that Sema3A is an osteo-anabolic autocrine and, accordingly, Sema3A-KO mice develop a low bone mass due to decreased bone formation. However, recently, we demonstrated that mice lacking Sema3A in neurons had low bone mass similar to Sema3A-KO mice, indicating that neuron-derived Sema3A is responsible for the bone abnormalities independent of the local effect of Sema3A in bone. Indeed, sensory innervations of trabecular bone were significantly decreased in neuron-specific Sema3A-KO. Moreover, ablating sensory nerves decreased bone mass in wild-type mice, whereas it did not deteriorate low bone mass phenotype in neuron specific Sema3A-KO mice, further indicating the essential role of sensory nervous system in normal bone homeostasis. Thus, we demonstrated that sensory nervous system is also a critical regulator of bone remodeling.

W3.2

Control of Bone Metastases by the Sympathetic Nervous System

Florent Elefteriou
USA

We have shown that activation of the sympathetic nervous system (SNS) alters the bone marrow environment and causes bone loss in mice, via some of the same signaling molecules that have been implicated in breast cancer metastasis to bone. In addition, chronically depressed patients do not seem to benefit from newly developed treatments for cancer and present with shorter survival. Because severe depression and chronic stress stimulate sympathetic outflow, we hypothesized that SNS activation induced by psychosocial factors remodels the bone marrow environment to make it a fertile “soil” for breast cancer cells, thereby promoting disseminating cancer cell establishment in the skeleton and leading to reduced survival. A series of experiments in preclinical models of bone metastasis supported this hypothesis, identified RANKL as a SNS-induced cytokine promoting breast cancer cell migration and establishment in bone, and showed that the beta-blocker propranolol could reduce the skeletal dissemination of cancer cells. Retrospective clinical studies also suggest a beneficial effect of sympathetic blockade in term of less advanced disease at diagnosis, lower cancer-specific mortality, longer disease-free survival and reduced metastasis development and tumor recurrence, particularly in patients that have taken beta-blockers before diagnosis. Therefore, beta-blockers or therapies normalizing sympathetic tone might be beneficial as early adjuvant therapies to limit skeletal metastases and to improve prognosis in patients with breast cancer.

W3.3

Hypothalamic Control of Bone and Fat

Paul A Baldock
Australia

Skeletal research is currently undergoing a period of marked expansion. One aspect in particular is the relationship between bone and fat metabolism. Emerging evidence indicates that bone and adipose activity are co-regulated and inter-dependent. Signals from fat cells are known to regulate bone mass, with prominent adipokines such as leptin and adiponectin. One bone/ fat-active pathway is controlled by neuropeptide Y

(NPY), most prominently in the hypothalamus, but also in the osteoblast. NPY is a critical downstream mediator of leptin actions, and is fundamental to the skeletal response resulting from hypothalamic leptin signaling. Elevation in central NPY expression produces powerful stimulation of appetite and adipose tissue production, but also inhibition of osteoblast activity, through neural relays from the brain to the bone. Interestingly, signals produced by bone cells are now being identified that are capable of regulating fat cells, both directly and through central hypothalamic signaling. Osteocalcin, a protein secreted by osteoblasts, is capable of regulating of energy and glucose homeostasis, reducing fat mass and increase insulin sensitivity. We have recently, identified a novel central loop for osteocalcin signalling to regulate bone, but also capable of regulating adipose and glucose homeostasis. In conclusion, the link between energy and bone homeostasis is far more complex than previously appreciated, with multiple axes of control, involving both central and direct signalling pathways. These signalling axes reveal a classic, hypothalamic pattern of feedback and efferent neural/endocrine control.

W4.1
Nanomedicine: What is it?

Robert Chapman, Molly M. Stevens
London, UK

The ability to engineer the structure of materials on the nanoscale has enabled access to extraordinary optical properties, the compartmentalisation and release of molecular components on demand, and the ability to interact with biological systems in unprecedented ways [1-2]. The design and use of these materials to produce a medical effect, a field known as nanomedicine, is poised to have a profound impact on healthcare [3]. The power of nanomedicine can be observed in a number of ways. The unique optical properties of inorganic nanoparticles and quantum dots have enabled the development of a range of new biosensors for diagnosis of disease. By linking small changes in the surface chemistry to dramatic and visible changes in bulk properties, the ease and sensitivity of detection of a range of protein and nucleic acid biomarkers has been greatly improved. Nanoparticles have also enabled new bioimaging techniques, through their ability to enhance Raman scattering allowing rapid imaging of chemical information within cells, and their use as targeted imaging agents *in vivo*. Patterning of surfaces with nanoscale precision has allowed the investigation of biological interfaces and processes *in vitro*. Nanomaterials that can compartmentalise molecular components, such as liposomes, exosomes and self-assembled polymeric nanoparticles have also been widely exploited as drug delivery vehicles. Targeting these materials towards particular receptors and linking their properties to external triggers such as light, pH, temperature or enzymatic activity, has enabled the delivery of therapeutics to particular locations. This talk will provide an overview of the field of nanomedicine and discuss the use of nanoparticles in biosensing, biomedical imaging, diagnostics and therapy, with examples from our work.

References:

[1] Howes PD, Rana S, Stevens MM, *Chem. Soc. Rev.* **2014**, 43, 3835.
[2] Howes PD, Chandrawati R, Stevens MM, *Science* **2014**, 346, 53.
[3] Doane TL, Burda C, The unique role of nanoparticles in nanomedicine: imaging, drug delivery and therapy. *Chem. Soc. Rev.* **2012**, 41, 2885.

W4.2
Molecular Imaging Using Nanoparticles

Fabian Kiessling
Germany

Nanoparticles are frequently used as carriers for therapeutic drugs but can also be used as part of imaging compounds for various imaging modalities. In this talk, an overview will be given on the use of nanoparticles for imaging and theranostics. Most nanoparticles are characterized by either long blood half-lifes or by rapid uptake by the RES. In addition, they tend to selectively accumulate in tissues with high vessel leakiness due to EPR effects. Thus, they are favourably suited as drug carriers in oncology. The large surface of nanoparticles enables to generate different functionalities in the same probe and adding an imaging marker to such a therapeutic probe results in a theranostic agent that can be used for patient selection and for controlling probe accumulation. Another important indication for diagnostic nanoparticles is imaging of the RES as it is used clinically to detect tumors in liver and lymph nodes using iron oxide nanoparticles. Since nanoparticles are internalized strongly by cells they can also be used for cell labelling and in vivo cell tracking experiments. Alternatively, in the emerging field of tissue engineering nanoparticles can be used to label scaffolds in order to localize the transplants and to monitor their resorption and remodelling. For targeted imaging, however, except for very small renally cleared nanoparticles, nanoparticles are critical since their penetration into tissues is low and high background signal is generated due to EPR effects. If targeted nanoparticles are designed for intravascular imaging, however, one may consider designing them larger (up to micrometer size) in order to avoid the unspecific retention in the interstitial space. In summary, although there are clear indications for the use of nanoparticles as diagnostic probes they are no magic bullets for imaging purposes and their use should be carefully considered taking into account alternative strategies such as the use of small molecules.

W4.3
Targeted Nanomedicine and Nanotheranostics

Gert Storm
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Since the introduction of the liposomal doxorubicin formulation Doxil®/Caelyx® on the market 20 years ago, a number of targeted nanomedicine formulations have become part of treatment regimens in the clinic. An update will be given of problems encountered and current strategies to tackle them. The preclinical use of nanomedicine formulations for thera-

peutic and diagnostic applications is increasing exponentially. Many different systems and strategies have been developed for drug targeting to pathological sites, as well as for visualizing and quantifying important (patho-) physiological processes. In addition, ever more efforts have been undertaken to combine diagnostic and therapeutic properties within a single nanomedicine formulation. These so-called nanotheranostics are aimed to provide valuable information on drug delivery, drug release and drug efficacy, and they are considered to be highly useful for personalizing nanomedicine-based (chemo-) therapeutic interventions.

W5.1

Next Generation Sequencing

Uwe Kornak
Germany

Next generation sequencing (NGS) has begun to dramatically change research and diagnostic approaches. Compared to conventional Sanger sequencing the price per sequenced base has dropped several thousand-fold leading to a shift of the bottleneck from sequence data generation to bioinformatic interpretation. After an introduction into the principles of NGS different research applications centered on gene regulation and expression signatures will be discussed. Next, the upcoming role in the clinics will be highlighted. The possibility of parallel sequencing of hundreds of genes up to the whole exome in targeted gene panel approaches or even whole genome sequencing has shifted the paradigm in differential diagnosis of rare skeletal disorders to a "sequence first" strategy. But also in seemingly common skeletal disorders the parallel sequencing of many candidate genes offers unprecedented insight into the underlying disease etiologies. In a pilot study on early onset osteoporosis we found evidence for monogenic inheritance in a significant portion of patients. With increasing numbers of sequenced patients and appropriate available databases probably also more complex oligogenic inheritance patterns can be elucidated. Given the relevance of this genetic information for personalized prognosis and treatment it is to be expected that increasingly rapid genotyping at dropping costs will become a standard tool in many fields of medicine.

W5.2

Systems Dissection of Pluripotency

Frank Buchholz
Dresden, Germany

Pluripotent stem cells are important to understand early mammalian development and hold great promise for future regenerative therapies. The identification of factors required to maintain pluripotency is a pivotal step to fully develop these cells for applied purposes and to decipher embryonic differentiation. I will describe the combined use of genome-scale RNAi screens targeting protein coding and long non-coding RNAs with genetic interaction, protein localization and protein-level dependency studies to delineate connectivity between factors that control ES and EpiSC identity. Examples of newly identified protein coding, and long non-coding RNAs that impact on pluripotency will be presented.

W5.3

Abstract not available

W6.1

The Evaluation of Bone Quality in Healthy and Diseased Human Cortical Bone

Robert O. Ritchie
USA

As one of the most important natural materials, cortical bone is a composite material comprising assemblies of tropocollagen molecules and nanoscale hydroxyapatite mineral crystals, forming an extremely tough, yet lightweight, adaptive and multi-functional material. Bone has evolved to provide structural support to organisms, and therefore its mechanical properties are vital physiologically. Like many mineralized tissues, bone can resist deformation and fracture from the nature of its hierarchical structure, which spans molecular to macroscopic length-scales. In fact, bone derives its fracture resistance with a multitude of deformation and toughening mechanisms that are active at most of these dimensions. Here we examine ways to quantity the "quality" of bone in terms of its basic mechanical properties of strength, ductility and most importantly resistance to fracture (toughness). We show that bone's strength and ductility originates primarily at the scale of the nano to submicron structure of its mineralized collagen fibrils and fibers, whereas bone toughness is additionally generated at much larger, micro- to near-millimeter, scales from crack-tip shielding associated with interactions between the crack path and the microstructure. We further how the effectiveness with which bone's structural features can resist fracture at small to large length-scales can become degraded by biological factors such as aging and disease, which affect such features as the collagen cross-linking environment, the homogeneity of mineralization, and the density of the osteonal structures. In this regard, we specifically examine the effects of various diseases, such as vitamin D deficiency, *osteogenesis imperfecta* and Paget's disease, on bone quality, and present the results of preliminary experiments on the effects of bisphosphonate treatments as a possible cause of atypical femoral fractures.

W6.2

Bone Fragility Beyond Bone Strength: The Clinical Hip Fracture as A Challenge to Basic Understanding

Jonathan Reeve
Oxford, UK

Every hip fracture begins with a microscopic crack that enlarges explosively. Most hip fractures in the elderly occur on falling from standing height, usually sideways or backwards. The typically moderate level of trauma very rarely causes fracture in younger people. This growing fragility may follow the decline of multiple protective mechanisms at many length scales from nanometres to that of the whole femur. With normal aging, the femoral neck asymmetrically and progressively loses bone tissue precisely where the cortex is already thinnest and is compressed in a fall. At the microscopic scale of the basic remodelling unit (BMU), increased numbers of actively remodelling BMUs associated with reduced mechanical loading in a typically inactive old age augments the numbers of mechanical flaws in the structure potentially capable of initiating cracking. Menopause and

over-deep osteoclastic resorption are associated with incomplete BMU refilling leading to excessive porosity, cortical thinning and dis-connection of trabeculae. In the femoral cortex, replacement of damaged bone or bone containing dead osteocytes is inefficient, impeding the homeostatic mechanisms that match strength to mechanical usage. In consequence the participation of healthy osteocytes in crack-impeding mechanisms is impaired. Observational studies demonstrate that protective crack deflection in the elderly is reduced. At the most microscopic levels attention now centres on the role of tissue aging, which may alter the relationship between mineral and matrix that optimises the inhibition of crack progression, on the role of osteocyte aging and death that impedes tissue maintenance and repair and on one newly revived and one quite new topic: the potentially key role of citrate in binding crystal to matrix and regulating crystal growth; and the potential role of repeated moderate trauma to cause de-lamination of cortical bone.

W6.3
Preclinical Methods for Assessing Bone Quality

Eleftherios P. Paschalis
Austria

Bone is a composite material consisting of mineral, organic matrix, and water. Its resistance to fracture is determined by its amount and its quality, the latter an umbrella term encompassing its structural and material properties. Metabolic bone diseases manifesting fragility fractures (such as osteoporosis) are routinely diagnosed based on bone mineral density (BMD) and biochemical markers measurements. Although clinically useful, it is nowadays well accepted that these measures do not fully account for fracture incidence. The emergence of bone quality led to the development of a plethora of analytical techniques (analysing bone tissue at both the macro and the micro scale) that provide information on all 3 components of bone (mineral, organic matrix, and water). Nevertheless, it is unlikely that a single outcome is responsible for fracture occurrence, as fracture is believed to be the culmination of a series of events. As a result, a combinatorial approach is preferred when possible. In this presentation, the most widely used techniques for preclinical determination of bone quality will be presented. For each, what is actually measured and what is inferred will be discussed, and emphasis will be placed on potential pitfalls. Finally, the example of Idiopathic Osteoporosis will be presented so as to highlight the importance of bone quality determination in the understanding of the pathophysiology of fragility fractures.