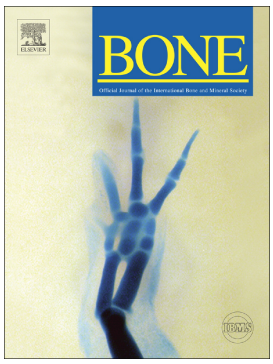

Meeting report from the 3rd IFMRS/KU Leuven Herbert Fleisch Workshop Brugge, Belgium, 17–19 March 2019

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MEETING REPORT FROM THE 3rd IFMRS / KU LEUVEN HERBERT FLEISCH WORKSHOP Brugge, Belgium, 17 – 19 March 2019

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The Third Herbert Fleisch Workshop was held in the beautiful city of Brugge, Belgium on March 17 to 19, 2019. The naming of this event as the Herbert Fleisch Workshop reflected its genesis as a support for young scientists in the field of bone and cartilage research, and the legacy of the late Herbert Fleisch: the Davos meetings that he organised over several decades until 2006 were notably supportive and encouraging of young scientists in the field. The first two such Workshops, held also in Brugge in 2014 and 2016 (for published reports, see www.ifmrs.org; http://www.ifmrs.org/wp-content/uploads/2018/01/2014-H-Fleisch-meeting-report.pdf; http://www.ifmrs.org/wp-content/uploads/2018/01/2016-H-Fleisch-meeting-report.pdf), were judged to be so successful that the International Federation of Musculoskeletal Research Societies (IFMRS) decided to support continuation of the event. Its maintained aim has been to address the needs of young investigators – PhD students, postdoctoral researchers and early faculty members – to give them a forum in which to present their work, having it evaluated and discussed by their peers and by a small group of senior, experienced investigators who would serve to encourage, inform and mentor them.

This was the approach pursued in Brugge in 2019, with the young investigators being strongly encouraged to submit their unpublished work – what is really interesting them right now and involving them as graduate students or early career researchers. The program had a base of plenary presentations by the group of senior scientists/mentors, highlighting what was the latest in the fields covered by their respective expertise, and driven also by the young investigator attendees presenting their work. Posters were available for discussion at all times throughout the meeting and in the evenings, with 23 of the participants invited to give oral presentations as well. The meeting design provided for the main aims of the Workshop, which were educational, and to inspire the young participants with maximum exposure of their work. This was achieved through the mentors' full engagement with the participants throughout the meeting, discussing their poster and oral presentations and career directions.

There were many positive features of the Brugge meeting. First, it was attended by a most enthusiastic group of around 85 young scientists from 16 countries, including several European countries, the USA, Japan, Australia and New Zealand. Second, they responded eagerly to the opportunity to interact with each other, and particularly, to get the close attention of the senior scientists as mentors and discussants. Third, the senior scientists fulfilled their mentoring roles gracefully and devotedly – they gave inspiring lectures and workshops, and vigorously interacted with the young participants during the oral and poster

sessions as well as informally over coffee breaks and meals. Finally, Brugge was a wonderful place to hold such a meeting – a most beautiful, ancient European city, small and attractive, and with a venue that made it easy to keep the Workshop participants together as a communal group. Herbert Fleisch would be the first to agree that it substitutes well for Davos, of course without the snow.

A popular feature of the meeting was the presentation of three methodological interactive workshops by some of the mentors. In her workshop on "*Studying and correctly identifying skeletal stem cells*" Pamela Robey (National Institutes of Health (NIH), Bethesda, Maryland, USA) discussed the methods, including their benefits and pitfalls, for identifying and studying skeletal stem cells, and the progress in the development of applications using such cells for tissue engineering. Marjolein Van der Meulen (Cornell University, Ithaca, New York, USA) conducted a workshop on "*Determining the mechanical performance and adaptation of the skeleton*" pointing the participants to the methodologies that can be used for mechanical loading experimentation, discussing their specificities and points of attention. The workshop by Peter Croucher (The Garvan Institute of Medical Research, Sydney, Australia) on "*Modelling malignancy in the skeleton*" was focussed on the importance of a deep understanding of bone cell interactions in studying how solid and hematological malignancies make their way in the bone microenvironment. He showed some beautiful new technical approaches to studying cancer cells in bone by intravital microscopy. These three workshops were each conducted twice in order to increase exposure, and were very enthusiastically appreciated by all.

Osteoblast biology in health, disease and ageing of the skeleton

The meeting opened with a plenary lecture by Sundeep Khosla (Mayo Clinic, Rochester, Minnesota, USA) on "*Senescence of Bone Cells*". It was a most appropriate and timely starting point, since many of the submitted abstracts were concerned with bone cell origins and functions. Discussing the effects of aging offered ways of thinking about bone cell biology that could reveal new paths of study for the young scientists that could potentially lead to valuable translational research outcomes.

The morbidity and even mortality outcomes of the susceptibility to fracture of osteoporosis provide a huge and increasing public health problem. Existing treatments all have side effects

and their efficacies beyond 5 years have not been established. There is a need for new drugs that will build the skeleton as well as prevent its decay. An undesirable outcome of disease-specific treatment of the many age-related diseases is “polypharmacy”, - a situation in which elderly people are commonly exposed to multiple drug treatments. This provides an impetus to aim at manipulating broader principles of aging mechanisms and potentially affecting multiple common diseases simultaneously. A most promising direction is addressing the problem of cellular senescence, based on the hypothesis that targeting senescent cells could have rejuvenating effects on tissues. For bone, the potential gain of such new approaches could be considerable, especially given the fact that the dominant therapies for age-related bone loss have been anti-resorptive drugs, which don’t address the contribution of decreased osteoblast function to age-related bone loss [1].

Dr Khosla focused on how aging contributes to impaired bone formation through a relative reduction in bone formation at the cellular level. High throughput RNA sequencing was used to study iliac crest biopsy samples to identify genes and pathway changes associated with aging in young (mean age 30) and old (mean age 72) women [2]. Pathway analysis in that translational study revealed substantial changes in the skeletal transcriptome that included increased Notch and decreased Wnt signaling in bone, that could potentially contribute to decreased osteoblast function.

Cellular senescence was described as characterized by an irreversible replicative arrest and apoptosis resistance, associated with a senescence-associated secretory phenotype (SASP) that entails the release of inflammatory factors and proteolytic enzymes that function as inducers/mediators of senescence and tissue destruction. Cell senescence can be induced by several stresses, and is associated with the activation of pro-survival networks called senescent cell anti-apoptotic pathways (SCAPs) [3] [4]. Senolytics and senomorphics, compounds targeting the SCAPs and SASP, could thus potentially be beneficial in the treatment of multiple age-related comorbidities. The finding in genetically manipulated mice that cellular senescence and age-dependent deterioration of organs was associated with accumulation of $p16^{Ink4a}$ [5] led to the first pharmacological approach to age prolongation in mice [5]. In bone, the Khosla group showed that p16 expression in osteocytes increases upon advanced ageing, correlating with the occurrence of severe age-related bone loss, and that genetic elimination of senescent cells marked by $p16^{Ink4a}$ expression resulted in lower bone resorption with either maintained (trabecular) or increased (cortical) bone formation [6].

Interestingly, they arrived at a similar beneficial outcome on bone either with recently discovered senolytic compounds [7] that disable SCAPs, causing senescent cells to become susceptible to their own microenvironment and induce apoptosis, or by treatment with a JAK inhibitor as a senomorphic intervention inhibiting the proinflammatory secretome [4]. These treatments were effective in old mice, while they did not elicit effects in young mice [6]. Moreover, in addition to enhancing bone mass, clearing senescent cell burden was found to reduce frailty and increase lifespan [8].

The important general question arising from this work is whether cellular senescence can be targeted safely to prevent bone fragility and other age-related disorders, especially in light of the thought that cellular senescence may have evolved as anti-cancer mechanism [3]. Relevant specific questions for the young scientists at the Workshop centered upon the changes that take place with senescence in bone cells.

Microbiome and Bone Health

Another plenary lecture that brought attention to the effects on bone cells of a new regulatory system was that by Claes Ohlsson (University of Gothenburg, Sweden) on "*Microbiome and Bone*", starting by pointing out that the gut microbiome constitutes a large additional organ, with 10 times the number of human cells and 300-fold more genes than the rest of the body. Evidence for a role of the gut microbiome has been obtained for a large number of disorders, primarily through effects on immune status and host metabolism. These include inflammatory bowel diseases, obesity, and metabolic disorders including diabetes as well as psychiatric disorders (e.g. [9]). These effects might reflect the view that gut-derived bacteria may influence immune responses taking place in distant organs [10]. The influence on host physiology depends on the type and number of bacteria in the microbiome [11]. Many diseases are associated with reduced diversity of the gut microbiota, and typically, the gut microbiome diversity is reduced in the elderly.

A number of recent studies indicated effects of the microbiome on skeletal physiology and showed that the gut microbiota can influence bone mass, possibly through immune mechanisms [12, 13]. Dr Ohlsson even introduced the term "Osteomicrobiology" to refer to this new pathway of investigation [12] [14]. Dr Ohlsson illustrated with examples from the literature that the effects of the gut microbiota on bone are complex and can vary depending

on the duration of the colonization, the age and strain of the mice, the diet, and microbiome composition. In their original work on this topic, they found that germ-free mice had greater trabecular and cortical bone mass than conventionally raised mice. The changes in the germ-free mice could be reversed by reconstitution of the gut microbiome from conventionally raised mice [15]. There were no differences in the levels of sex hormones, calcium, PTH or vitamin D, but germ-free mice showed fewer CD4+ T-lymphocytes in the bone marrow than conventionally raised mice. In line with reduced T cells, germ-free mice showed decreased levels of the inflammatory cytokines IL-6 and TNFα in their bones and a reduced number of osteoclasts [15].

Altered immune status has long been known to be associated with the bone loss following ovariectomy (OVX). Connection with the microbiome was investigated by treatment with probiotics, viable microorganisms that alter the composition and metabolic activity of gut microbiota [16], and enhance epithelial barrier function [17]. Most interestingly, the Ohlsson group and others [18-20] found that probiotic treatment protected mice from OVX-induced bone loss, by inhibiting bone resorption, associated with reduced immune cell expression of cytokines such as TNFα, IL-1β, and RANKL [21]. The importance of sex steroids in gut permeability has been stressed [18, 21] and proposed as a contributory factor to the findings in OVX mice. The model proposes that increased intestinal permeability due to sex steroid-depletion enhances the passing of antigens through the intestinal barrier and reaching antigen-presenting cells, leading to activation of the immune system, increased production of osteoclastogenic cytokines by $CD4^+$ T cells, and increased bone resorption (e.g. [13, 18]). The capacity of the gut microbiota to influence immune processes may be contributed to by their effect on the gut permeability; probiotic treatment could reduce intestinal permeability and possibly thereby exert a bone-protective effect in mice with a sex steroid deficiency induced by OVX [18, 21]. The immune connection between the microbiome and bone has been repeatedly confirmed in experiments with mice.

The mouse data is compelling enough to identify a need for much clinical and translational research to place the gut microbiome in perspective for its potential in regulating human bone mass through immune and other mechanisms. Studies are being conducted that analyze the gut microbiota composition in osteoporosis and osteopenia patients, as well as the potential effects of probiotic supplements on bone health [22-24]. For the audience of this Workshop a lesson from this work is the need to think of this biology, how the microbiome influences

bone cell function, and to be able to identify changes in bone cell behavior that originate from changes in the gut microbiota.

Molecular and Cellular Mechanisms Regulating Skeletal Remodeling

The plenary lecture by Sakae Tanaka (Tokyo University, Japan), "*Mechanisms of Regulation of Osteoclast Differentiation*", took us first through the discovery of the tumour necrosis factor (TNF) family members, receptor activator of nuclear factor κB ligand (RANKL) and its receptor, RANK, in promoting osteoclast formation from hematopoietic precursors, and its inhibition physiologically by the soluble TNF receptor, osteoprotegerin (OPG). This system provides the basis for the therapeutic application of RANKL blockade using denosumab, a fully human monoclonal antibody against RANKL. The efficacy of denosumab in inhibiting resorption has been thoroughly demonstrated in osteoporosis [25] and in bone metastases in prostate and breast cancer [26, 27]. In two double-blind, placebo-controlled trials in patients with rheumatoid arthritis with methotrexate treatment, addition of denosumab resulted in inhibition of resorption-induced structural damage that was assessed either by the magnetic resonance imaging (MRI) erosion score [28] or radiologically [29].

These promising findings certainly focus attention on the RANKL/RANK pathway, but it was clear from Dr Tanaka's presentation that there is much to be learned yet about this process, with a number of transcriptional regulatory mechanisms influencing it. An example given is the mechanism by which TGFβ significantly enhances RANKL-induced osteoclast formation in vitro [30, 31]. This was explained with the finding of Yasui et al [32] that the binding of TGFβ-induced SMAD 3 to the TRAF-TAB1-TAK 1 complex is crucial for RANKL-induced osteoclastogenesis. That is what makes TGFβ indispensable for the process.

There is a long list of cytokines whose genetic ablation or overexpression results in severe bone phenotypes, either osteopetrosis or osteoporosis, from inadequate or excessive osteoclast formation respectively. From the evolutionary point of view, it identifies the importance for survival of the tightly regulated processes of bone resorption and formation in bone remodelling, such that skeletal strength and form can be maintained. It is not surprising

therefore, that in addition to factors enhancing regulated osteoclast formation, there should be effective inhibitors.

T cells are the source of a number of such inhibitors. Some examples include interferon (IFN)γ, which inhibits bone resorption in organ cultures [33] and osteoclast formation from precursors [34] by rapidly inducing degradation of the RANK adaptor protein TRAF 6, thus preventing RANKL-induced transcriptional activation. Some other T cell-derived inhibitors are IL-4 [35, 36] and IL-18, which inhibits osteoclast formation independent of its ability to increase IFNγ production, but produces its effect by promoting T cell production of GM-CSF [37].

The concept of inhibitory control of osteoclastogenesis from within the osteoclast itself was introduced with identification of IFNβ as a product of RANKL stimulation in monocytic osteoclast precursors, inhibiting osteoclast formation by preventing RANKL-induced expression of c-FOS [38]. IFNα was not induced by RANKL under conditions of IFNβ induction, and studies of the RANKL induction of IFNβ promoter indicated also that the response is specific to IFNβ.

Dr Tanaka further addressed this concept of inhibitory regulation from within the osteoclast lineage by discussing interferon regulatory factor 8 (IRF8). This transcription factor expressed in immune cells was down-regulated in osteoclasts in response to RANKL treatment, and deficiency in IRF8 resulted in severe resorption-induced bone loss in mice [39]. The IRF8 effects on osteoclastogenesis were found to be independent of any IFNγ effect, nor was IFNβ expression affected in IRF8-deficient cells. Osteoclast differentiation is stabilised by methylation of IRF8, mediated by DNA methyl transferase 3a (DNMT3a), resulting in suppression of IRF8 expression [40], leading to recognition of the role of epigenetic regulation in osteoclast formation.

These findings outlined by Dr Tanaka in his keynote lecture all served to highlight the links between osteoclast biology and the immune system and illustrate further the importance of endogenous regulation within the osteoclast lineage, including epigenetic regulation.

Cancer and Bone

There is something special about the microenvironment of bone that favours the establishment and growth of tumour cells, as known for many years for breast cancer with the 'seed and soil' hypothesis postulated by Stephen Paget in 1989 [41], as well as for other solid tumours including prostate, lung, kidney and thyroid, but especially also for multiple myeloma. These events are challenging to investigate, and were discussed by Peter Croucher (The Garvan Institute of Medical Research, Sydney, Australia) in his plenary lecture, "*Cancer and Bone*", with considerations of the evolution of skeletal invasion, the molecular programming of events, their temporal progression and how the process in its various stages is influenced by the diverse types of bone cells.

Tumour-bone microenvironment interactions have long ago been postulated to center on the establishment of a 'vicious cycle' in which tumour cells produce factors that stimulate osteoclastic bone resorption as well as inhibitors of osteoblastic bone formation. Targeting these events in the bone environment (e.g. with bisphosphonates, RANKL inhibition, anti-Dkk1 or anti-sclerostin) can prevent bone disease or effectively reduce tumour burden. However, it was stressed that the current vicious cycle model represents a relatively simplified view that does not fully capture today's expanded knowledge on the heterogeneity of the bone microenvironment. There are many distinct cells and compartments to be considered in the microenvironment of bone, and their complexity needs to be taken into account. For instance, what used to be referred to as the "osteoblast" is now known to not simply constitute one homogenous cell type but rather a range of osteoblast lineage cells in distinct states and differentiation stages, exhibiting differing phenotypes, and residing in different locations within the bone [42]. Mature, bone-synthesizing osteoblasts might even be the least likely members of the osteoblast lineage to contribute dynamically to the control of tumour cell behaviour in bone. Thus, a valuable message given was that we need to keep in mind that there are very many aspects of bone biology that could influence cancer growth in the dynamic multicellular bone and bone marrow environment [42]. Having said that, of all the metastatic environments to investigate, bone might be the most amenable, because there have been so many insights developed into how the cells of bone function and communicate with each other.

Experimental approaches to bone metastasis have until recently been heavily biased towards the terminal stage of the process, whereas there is much to be learned about the colonisation of bone by tumour cells. The evidence is convincing that the bone microenvironment

profoundly influences tumour cell behavior, likely already from the earliest time a tumour cell arrives in bone, when it could be activated to grow instantly, or – perhaps more commonly – be quiescent and require activation to form a metastatic lesion. Quiescent (non-dividing) cells are recognised to be resident in bone in breast and prostate cancer as well as in multiple myeloma [43]. The extreme of the quiescent state is those cells that remain dormant for long periods of time. How do they manage to remain dormant for long periods in their niche and then be "awakened" to become first micro-metastases and then (macro-)metastases? Why they are present but not activated to grow is not clear, but it may suggest that besides an intrinsic ability to become activated, the activation events may require extrinsic signals or changes in the environment that are critical determinants of tumour development. As mentioned above, in the case of bone there are many cell types that need to be considered as potential influences. Dr Croucher summarised a novel approach to this question that they have applied in the study of dormancy in myeloma. When cells were labelled with a dye, DiD, the resulting red colour was lost when dormant cells resumed the capability to divide. Intravital 2-photon microscopy was used in the mice to follow the label-retaining dormant cells through their reactivation [44]. In a mouse model of multiple myeloma they showed that dormant cells are resistant to chemotherapy that targets dividing cells, and that dormancy is a reversible state that is maintained in bone, possibly by the cells that constitute the "lining" component of the osteoblast lineage [44]. When they promoted osteoclast formation and bone remodelling with sRANKL treatment, dormant myeloma cells were reactivated, leading to the proposal that switching on the dominant cells required activation processes that might be related to osteoclast activation in endosteal remodelling.

This concept of an "endosteal niche" for myeloma cell dormancy is an appealing one to investigate in solid cancers such as breast and prostate. The methods of intravital microscopy and tracking of dormant cells that have been developed for myeloma [44] could readily be applied for this purpose. This three-dimensional intravital microscopic method has also been very instrumental in the study of hematopoiesis [45] and the colonisation of bone by leukemic and myeloma cells [46, 47] and will undoubtedly be applied for many purposes and help clarify the complexity of the bone and bone marrow environment in the near future, along with the current speedy developments of broad-scale and in-depth molecular analysis methods such as single cell sequencing technologies.

This plenary lecture provided a beautiful example of how these novel techniques and the study of bone cell function can be applied to a major disease mechanism.

Skeletal Stem and Progenitor Cells (SSPCs) for Bone Growth and Regeneration

In her plenary lecture, "*Stem cells and bone*", Pamela Robey (NIH, Bethesda, Maryland, USA) summarised how this field has developed historically to what is understood today. The concept that remodelling and regeneration must depend on the existence of a stem cell goes back well over a century. A tissue-specific stem/ progenitor cell in bone from either the periosteum or from marrow was suggested as early as the 1860s in studies on bone regeneration, and the concept of a stem cell was further crystalized in the late 1800s through the early 1900s [reviewed by Robey [48]]. The hypothesis was that the ability of a tissue to maintain itself throughout the lifetime of an organism is based on the existence of a "stem" cell that would remain primarily quiescent and undifferentiated, but also be able to regenerate the functional parenchyma of its tissue of origin following injury or the need for tissue rejuvenation (tissue turnover). The defining features of a stem cell are that it is able to self-renew and that the offspring of a single cell reconstitutes the functioning parenchyma of the tissue of origin [48].

Originally, stem cells were thought to exist only in tissues with high rates of turnover, such as blood, skin and the gastrointestinal tract; however, even tissues never thought to turnover or repair, such as brain, were found to contain stem cells [see https://stemcells.nih.gov/info/basics/1.htm]. It was subsequently thought that virtually every tissue in the body contains some type of a stem/progenitor cell. The concept of stem cells has had a major impact in terms of understanding the dynamics of tissue homeostasis in health and disease, and large enthusiasm has been instigated by the premise that they could be useful in tissue regeneration.

When bone-free fragments of hematopoietic marrow were transplanted under the kidney capsule, the marrow could be regenerated, but only after the formation of bone [49]. It was Friedenstein, later in collaboration with Maureen Owen, who determined that the origin of bone in those marrow fragments, was in fact a sub-population of non-hematopoietic cells of the stroma upon which hematopoiesis occurs. Much is owed to the work of Friedenstein and Owen [50]. Their seminal work identified the existence of osteogenic stem cells within the bone marrow stroma. This was achieved when intraperitoneal transplantation of diffusion chambers containing bone marrow cells in rabbits gave rise to a mixture of tissues, including bone and cartilage [50]. When single colonies were transplanted in conjunction with a

collagen sponge under the kidney capsule with complete access to the vasculature, some colonies were able to form not only bone, stroma and marrow adipocytes of donor origin, but also supported the formation of hematopoietic tissue of recipient origin (an ectopic bone/marrow organ). Friedenstein and Owen proposed the existence of the first multipotent tissue-specific stem/progenitor cell from a solid connective tissue, which they termed a bone marrow stromal stem cell, able to differentiate into four different phenotypes: chondrogenic, osteoblastic, stromogenic and adipogenic [51], stressing that the stroma created by these stem/progenitor cells is required to transfer the hematopoietic microenvironment [52].

These and subsequent studies using rigorous differentiation assays and CFU-F cells to show multipotency, identified a bona fide skeletal stem cell (SSC) cell subset within bone marrow stroma that is able to self-renew and capable of forming skeletal tissue, and that likely resides in the bone microenvironment as pericytes lining skeletal blood vessels[53]. SSCs are very rare in the marrow stromal cell population and need to be defined by rigorous clonal and differentiation assays. Indeed, searching for SSCs in such mixed marrow cell populations is a formidable task, with an estimate of 1 stem cell per 10 million cells. Broad and non-specifically identified populations of non-hematopoietic adherent cells of the bone marrow became known commonly as "mesenchymal stem cells" (MSCs) [54, 55], a term that needs to be urgently revised or abandoned [56]. Such cells are often claimed to be capable of forming osteoblasts *in vitro*, but there remain technical reservations about this. The International Society for Cellular Therapy (ISCT) recommended the term "multipotent mesenchymal stromal cells" that reflects only their *in vitro* properties, without clonal analyses or *in vivo* studies [57]. Yet, mesenchyme as classically defined by developmental biologists denotes an embryonic connective tissue, in other words 'mesenchymal' refers to a prenatal state – no postnatal stem cell could make mesenchymal tissue that can functionally form connective tissues, blood and blood vessels. Caveats about this terminology were summarized in a recent review [58] and commentary [56]. A more appropriate terminology for the mixed cell population obtained from marrow might be "Bone Marrow Stromal Cells" (BMSCs). To indicate that a population contains but is not limited to bona fide SSCs, the overarching terminology could be "Skeletal Stem and Progenitor Cells" (SSPCs).

Dr Robey emphasized that whatever cells from bone marrow are claimed to be stem cells that can form bone, they must be shown to do so by the *in vivo* demonstration of formation of bone with identifiable cells and the support of formation of blood cells of recipient origin. In

contrast to the hematopoietic stem cell field, in which studies on the development of differentiated progeny from multipotent stem cells is facilitated by the many available consensus cell surface markers and the readily recognizable phenotypes of progeny, there are no single markers for SSCs or their progeny. Yet, a combination of markers, together with exclusion of hematopoietic and endothelial markers, can be useful to identify the cells under study. In tracing the identification of SSCs and their progression through the osteoblast lineage, Dr Robey was drawing attention to the need for rigorous identification of phenotypic properties of the cells under study. For validation of SSC status though, the requirement always remains to show *in vivo* capability of generating bone and hematopoietic compartments.

Dr Robey also elaborated on roles that SSCs/BMSCs play in the pathophysiology of skeletal diseases and in some hematological disorders, and discussed about developments in regenerative medicine, where SSCs are being used to establish cell therapy applications for bone reconstruction through tissue engineering. Given the diverse and important roles that SSCs play in diseases and regenerative therapies, their origins and life cycle need to be clearly understood.

Mechanosensing and Bone

For those working in bone biology and the cells of bone, the ultimate phenotypic properties are the strength of bone, how well it withstands physical stress, and how well it responds to changes in loading by mechanosensing. These were all topics considered by Marjolein Van der Meulen (Cornell University, Ithaca, New York, USA) in her plenary lecture, "*Mechanosensing and Bone*".

Understanding loading response mechanisms is directly relevant to many approaches for the prevention of fragility-related fractures. Loading of bone increases bone mass in a number of ways as shown in mice, rats and dogs. Progress in the study of effects of loading on bone began with the experiments of Dr Lance Lanyon on the turkey wing in the 1980's. The methodology allowed continuous or intermittent loading, which showed that a daily intermittent, but not a static load, resulted in a substantial increase in bone mass [59, 60]. After it had become clear from that and other work that the beneficial effect of loading on bone was optimally achieved by short bursts of activity [61, 62], a method of cyclic loading

of mouse tibia in vivo was developed [63], based on a non-invasive method used in rats [63-65]. Cyclic loading of the tibia when compared with the unloaded tibia in the same mouse, increased cancellous bone volume substantially [63]. Further, such loading prevented the cancellous bone loss that occurred following orchidectomy [66]. This method in the mouse offers special advantages, since it can be so readily applied to genetically manipulated mice and allow questions to be addressed concerning specific pathway effects on bone mass and strength. As such, the role of ERα in adaptation to mechanical loading could be investigated, as has been done with mature osteoblast-specific ERα knockout mice, generated by using an osteocalcin-Cre strain [67].

Illustrating the versatility of this *in vivo* tibia loading method, it can also be used to assess cartilage mechanobiology. Chondrocytes can respond to direct biomechanical perturbation by increasing production of inflammatory cytokines. Dr Van der Meulen discussed how the loading model can be used to reproduce key aspects of osteoarthritis. A single cyclic compressive loading session in mice initiated osteoarthritis-like cell changes in the articular cartilage and subchondral bone [68]. When cyclic loading is applied with this method, the classical changes take place of articular cartilage degeneration and subchondral bone thickening, as well as osteophyte formation that can be seen as early as 2 weeks and can be quantitated [69, 70]. This experimental method for the study of osteoarthritis is a particularly promising and attractive one (reviewed with other methods in [71]), given the availability of so many genetically manipulated mice with relevant engineered defects, and given also its suitability for pharmacological studies [72].

History of bisphosphonates: celebrating 50 years of bisphosphonates

An additional plenary lecture that was most appropriate for the occasion was given by Graham Russell (University of Sheffield and Oxford University, UK) on the history of the bisphosphonates. As one who began with Herbert Fleisch on the progression from pyrophosphate findings to the synthesis and effects of the stable analogues, bisphosphonates, and who has continued to contribute so much to the impact of these drugs over the last 50 years, he was ideally situated to inform this group on the impact of bisphosphonates on the bone field. The progressive development of more potent bisphosphonates and the recognition of the inhibitory action of nitrogen-containing bisphosphonates on the mevalonate pathway

[73, 74] were put into historical perspective. These drugs have had a massive impact on the field from the time of the first major clinical trial of alendronate in osteoporosis [75] to the recent finding of a very prolonged prevention of fractures by injections each 12- or 18-months of zoledronate [1, 76, 77]. The efficacy in cancer-related bone disease was also reviewed, as were newer possibilities for bisphosphonate treatment targets.

POINTS FOR SUMMARY

It was easy to see how presentations of the plenary speakers provided for the addition of wisdom to the massive collection of facts and knowledge that must be dealt with. Importantly for the objectives of the workshop though, the topics and the approaches used by the plenary speakers highlighted for the young scientists ways in which basic studies of the biology of bone cells could be directed along a number of different pathways that could lead to translational outcomes. Some of these are relatively new pathways (cell senescence, microbiome), others have been thought about and have received attention for some years (cancer/bone, osteoclasts, stem cells, biomechanics), but are refreshed now with exciting new approaches and concepts.

The meeting was, as before, complemented by a social programme stimulating networking and companionship among the Workshop attendees, including an evening close to the Beguinage and the swans of Brugge for a group dinner including local beers. We were also very warmly welcomed on behalf of the mayor of the city in the medieval City Hall of Brugge where the chief of protocol, the deputy mayor and Roger Bouillon briefed us on the long commercial and cultural history of “het Brugse vrije” (fee state of Brugge). Our group thus reinforced only modestly the more than 5 million visitors per year. This was followed by a picture taking event (Figure) and a reception in the historic building with, indeed, a glass of famous “Brugge’s fool” beer. On the final day, the delegates paid for a short guided tour of the 12th Century hospital (Memling museum) and the highlights of the city such as the Begijnhof, the Belfry and the Church of our Lady, where we could see hidden in the renovation of the church, the statue Pieta by Michelangelo.

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Figure 1. Group picture of the 3rd Herbert Fleisch Workshop attendees.

Figure 2. Travel grant awardees supported by various international bone research societies to attend the 3rd Herbert Fleisch Workshop.

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