Bone Marrow Lesions in Osteoarthritis: What Lies Beneath

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Accepted Version:

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Short Title: BMLs What Lies Beneath

Word Count:

Submit to: Journal of Orthopaedic Research

Keywords: subchondral; microdamage; bone-cartilage crosstalk; Pain; TGF-b

Abstract

Osteoarthritis (OA) is the most common joint disease in the United States, affecting more than 30 million people, and is characterized by cartilage degeneration in articulating joints. OA can be viewed as a group of overlapping disorders which result in functional failure of the joint. However the cellular and molecular events within the joint, which precede these clinically observable changes, are neither well understood nor easily measurable. It is now clear that multiple joint tissues are important in this process. Cartilage tissue can be challenging to image clinically, however bone is more readily imaged and measured. Changes in subchondral bone have long been recognized as a hallmark of the condition, but this is typically at late stages of OA progression. However, bony changes which precede cartilage changes, in the form of Bone Marrow Lesions (BMLs as detected by MRI), are a relatively recent discovery – and their potential utility in predicting OA progression, or as a target for therapy, is not yet fully understood - nor is the mechanism by which these two distinct, yet connected, tissue compartments communicate. Here we will review the current state-of-the-art in this field under three broad headings: (1) BMLs in symptomatic OA: Malalignment, joint pain and disease progression (2) Biological considerations for bone-cartilage crosstalk in joint injury and disease and (3) Mechanical factors that may underlie BMLs and drive their communication with other joint tissues. Thus this review will provide insights on this topic from a clinical, biological and mechanical perspective.

Introduction

Osteoarthritis (OA) is the most common joint disease in the United States, affecting more than 30 million people [1], and is characterized by cartilage degeneration in articulating joints. This condition causes serious pain and/or restricted movement – and is extremely costly to national health systems. From a clinical perspective, OA can be viewed as a group of overlapping disorders which result in similar morphologic and clinical outcomes - namely functional failure of the joint. However the cellular and molecular events within the joint, which precede these clinically observable changes, are neither well understood nor easily measurable. However it is now clear that other joint tissues, in addition to the articular cartilage, are important in this process. While cartilage tissue can be challenging to image clinically, due to its small size and aqueous composition, bone tissue is more readily imaged and measured. Changes in subchondral bone (sclerosis) at late stages of OA, identifiable by simple plain film x-ray, have long been recognized as a hallmark of the condition. However, bony changes which precede cartilage changes, in the form of Bone Marrow Lesions (BMLs - detected by MRI), are a relatively recent discovery – and their potential utility in predicting OA progression, or as a target for therapy, is not yet fully understood. Here we will review the current state-of-the-art in this field from a clinical, biological and mechanical perspective.

BMLs in Symptomatic OA: Malalignment, Joint Pain and Disease Progression:

Understandably much of the early research into the pathophysiology of OA focused solely on articular cartilage, since it is primarily the status of this compartment that informs diagnosis and treatment. While this approach has yielded vast amounts of crucial information on the pathological processes involved in cartilage breakdown, it has not produced a targetable factor or strategy that

can be exploited to treat or prevent joint degeneration. Thus, more recently there has been a move to consider OA as a 'whole joint disease', which progresses with contributions from different tissues. Other such tissues that communicate with the joint - synovium, ligaments, menisci and periarticular muscles play a hugely important role in joint function, and thus are also likely candidates to play some role in its degeneration. However, communication between bone and cartilage (termed here 'bone-cartilage crosstalk') is of particular interest due to the intimate linking and common lineage of their cells and tissues.

Bone tissue has a particularly strong association with articulating joints, and unlike cartilage has the capacity to adapt rapidly [2]. Assessment of sclerosis and osteophyte formation in the subchondral and peripheral joint compartments, respectively, has long been part of the clinical imaging approach in late-stage OA. More recently BMLs have been presented as a useful clinically detectable phenomenon, which may inform treatment and management at early stages of disease. In the past, these have been referred to as bone marrow edema lesions or bone bruising and are defined as regions of hyperintense marrow signal on fluid-sensitive, fat-suppressed MR image sequences (Figure 1). BMLs have been associated with histological evidence of microscopic bone damage [3] and are related to malalignment [4], joint pain [5, 6] and to disease progression. While BMLs are indirectly related to the articulating joint compartment, and may be present long before its ultimate failure, there are precedents in other disease states for using such intermediate outcomes as treatment targets when the ultimate outcome occurs too infrequently (or too slowly) for it to be used directly. Examples include focusing on hypertension instead of the ultimate outcome of stroke, and the use of bone density to predict fracture. There is already evidence that

BMLs are strongly related to OA; they increase the risk of cartilage loss [1]; likelihood of OA progression [3], and of the development of knee pain [8, 9].

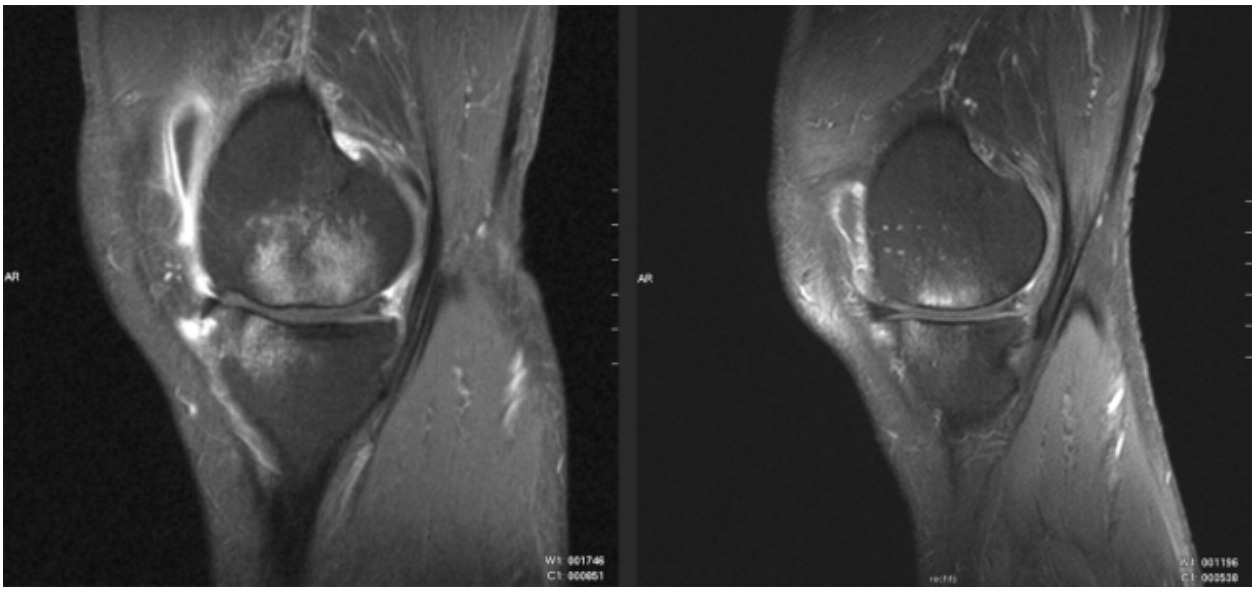

Figure 1: MR images in the sagittal plane of a knee joint with Bone Marrow Lesions (BMLs) in the subchondral compartments of the distal femur and proximal tibia. These are the regions of diffuse white signal within the bone compartment

The knee is a complex joint, with mechanical and biological functionalities that can be difficult to separate. However, knee malalignment is a common clinical issue and potent risk factor for OA progression, which serves as a useful natural experiment to study the effects of mechanical alteration on subsequent biological changes. Importantly, in this instance, one of the primary sequalae of knee malalignment is the formation of subchondral BMLs. Studies have shown a strong positive correlation between the extent of medial malalignment and the occurrence of BMLs in the medial joint compartment [4]. These data support the idea that altered mechanical forces across the joint lead to subchondral damage in the form of BMLs. Further validation of this was provided by demonstrating a similar relationship between lateral malalignment and the formation of BMLs in the lateral compartment. In those studies, which included > 250 patients, medial BMLs

were seen predominantly in patients with varus malalignment, while lateral lesions were mostly present in those with valgus changes. Approximately 36% of patients with medial BMLs showed medial progression, as compared with 8% of progressors without lesions (odds ratio for progression, 6.5 [95% CI, 3.0 to 14.0]). Around 69% of medial progressors had associated lesions, and lateral lesions increased risk for progression in that compartment. These increased risks were attenuated by 37-53% after adjustment for limb alignment.

Another important parameter relating to OA that can be clinically assessed is that of pain. In assessing pain it is instructive to consider its potential sources in the joint. One tissue which is not involved is the articulating cartilage itself, which lacks any nerve supply. The periosteum has rich sensory nerve supply and sympathetic innervation, and also has the densest innervation of the tissues around the joint. Internally, the bone marrow also has a rich sensory nerve supply while subchondral bone has a high volume of neural innervation [7]. Synovial thickening, effusions, periarticular lesions and bursitis may also be sources of joint pain. A recent review of this issue reported an odds ratio (OR), linking pain with subchondral BMLs, ranging between 2.0 (no Confidence Interval (CI) reported) and 5.0 (CI: 2.4 to 10.5). The OR of pain with effusion or synovitis ranged between 3.2 (CI: 1.04 to 5.3) and 10.0 (CI: 1.1 to 149). The levels of evidence between other MRI features and pain were either limited and/or conflicting [6]. An important caveat to consider here is that cross-sectional studies of OA patients are inherently limited because many pathological features co-exist, thus isolating one feature amongst them, and assigning causality, can be challenging. Longitudinal studies in joints with one pathological feature, which can be monitored with changes in pain over time, are the ideal way to approach this issue. Furthermore, incorporating targeted interventions in such studies would improve their impact

further still. Recent data from such studies have shown an intriguing link between BMLs and joint pain. Zhang et al. (2011) demonstrated that resolution, or decreased severity, of knee pain was associated with shrinkage of BMLs [8]. A randomized trial from one of the authors' laboratory (DTF) showed that a knee brace designed to specifically target knee pain and BMLs in OA of the patella-femoral (PF) joint, significantly reduced pain. Furthermore, the PF joint brace reduced BML size in the PF joint, but not in the tibio-femoral joint – indicating a compartment specific effect.

Biochemical treatments targeting BMLs in OA and joint pain have also started to show promising results. In a study by Laslett et al (2012) the bisphosphonate Zoledronic Acid (ZA) was shown to reduce BML size and also overall joint pain [9]. In that study, patients with knee OA and associated BMLs were randomized to receive either ZA or placebo. Pain was measured using a visual analogue scale (VAS). VAS pain scores were reduced by ZA after 6 months (-14.5 mm, 95% CI -28.1 to -0.9). Reduction in total BML area was greater in the ZA group after 6 months (-175.7 mm^2, 95% CI -327.2 to -24.3) with a trend after 12 months (-146.5 mm(2), 95% CI -307.5 to +14.5). A greater proportion of those in the ZA group achieved a clinically significant reduction in BML size at 6 months (39% vs 18%, p=0.044).

In summary, clinical studies suggest that BMLs represent some form of mechanical damage in sub- chondral bone, and are related to joint degeneration. BMLs may also be a source of pain, and they appear to respond to mechanical (bracing) and biological (bisphosphonate) interventions. Thus, targeting BMLs with novel treatments is a promising future strategy for OA.

Biological Considerations for Bone-Cartilage Crosstalk in Joint Injury and Disease

The clinical findings associating BMLs with joint disease are intriguing and compelling. In this section, we will discuss the current understanding of micro- structural, mechanical, and biochemical properties at the osteochondral interface, this will help characterize and harness this new information toward new treat- ments and targets. First, if subchondral events such as BMLs can influence articular cartilage (patho)- physiology then diffusion/molecular-transport across the osteochondral interface is one mechanism by which this might occur. This kind of direct solute transport is not easily measured using standard histological tools, since it is not associated with observ- able microstructural changes. Another cross-talk mechanism may involve the local microvasculature. In other instances of inter-tissue crosstalk local this is an important method of communication. In this case, changes in neovasculature can be measured directly based on observed microstructural change. Finally, certain mechanical changes in the subchondral bone may induce a significant biological response if they occur in specific regions near the osteochondral inter- face. This point will be discussed more detail in the next section.

Before addressing these issues, a brief review of the subchondral mineralized compartment microanatomy will be useful. The subchondral mineralized unit is a complex composite structure containing three distinct tissues: (1) Zone of Calcified Cartilage (ZCC), (2) Cortical Plate (CP), and (3) Subchondral Trabecular Bone (STB) [10-12]. Each of these tissues has unique physiological, morphological and mechanical characteristics. The ZCC likely has an important role both mechanically and physiologically in joint injury, but relatively little is known about its properties [13-15]. Traditionally thought to be mechanically intermediate between underlying cortical bone and the more compliant cartilage, some recent evidence suggests it may actually be

more highly mineralized than bone in certain regions [16]. In healthy joints, the ZCC represents a boundary between avascular cartilage and the vascular subchondral bone that is resistant to vascular invasion. There is also a physiological role for this tissue in OA *via* reactivation of the 'tidemark', which is often present in cases of advanced OA [17-19].

The cortical plate (CP) is located deep to the ZCC and the two are connected by interdigitation at the osteochondral junction. The cortical plate is relatively well vascularized and is populated by osteocytes, which extend their cellular processes through canalicular channels. The vasculature and canaliculi in the cortical plate of subchondral bone thus provide a network to facilitate molecular transport across the osteochondral junction. Subchondral trabecular bone (STB) has long been implicated in OA development, but no specific mechanism to describe its involvement has been clearly elucidated. STB represents a likely candidate for involvement in OA due the clinical prevalence of BMLs which are often seen there, and also due to the rich vascular and innervation of this compartment.

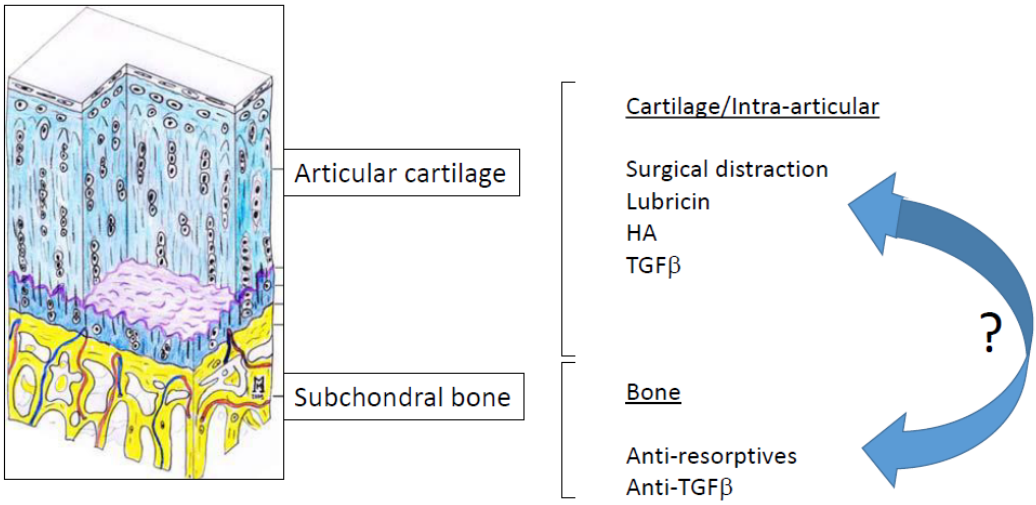

Figure 2: The image shows articular cartilage overlaying sequentially the calcified cartilage layer, the subchondral bone plate, and the subchondral trabecular bone. Potential treatments that might address the cartilage compartment, or the bone compartment, respectively, may also be protective of the other compartment, due to communication between the two. Thus, surgical distraction of the knee joint and HA treatment have been claimed to have efficacy for the cartilage clinically, and lubricin and TGF-b in animal models. Treatment of the bone compartment with anti-resorptives and anti-TGF-b at specific early time-points has been shown to have chondroprotective effects in animal models.

cortical plate are impenetrable barriers for solute transport. However, more recent data suggest there are numerous canals and porosities that connect the two [12, 20]. Interestingly these features were primarily located beneath the meniscal regions. Some of these porosities appeared to penetrate through to the marrow compartment [21]. Recent data from a study using a rat model of knee PTOA showed that subchondral porosity co-localizes with the point of load during ambulation - again suggesting a mechanical component in this relationship [22]. These findings are consistent with the evolving view that the junction region is a complex dynamic interface, and suggests a potential route for molecular communication between the adjacent compartments.

It has been shown that fluid flow across the interface, and thus the potential for molecular transport, increases in association with increasing cartilage erosion, subchondral bone plate thickness and vascularity [23]. Others have shown the dense subchondral vasculature in close proximity to the deep cartilage layer, and that micro-channels can penetrate the subchondral bone in disease, permitting communication between bone and cartilage [24]. Those studies also suggested that more than half of the glucose, oxygen, and water requirements of cartilage are provided by perfusion from these subchondral vessels. Consistent with this, experimentally induced hypoxia was found to cause cell death in both the epiphyseal and deep articular cartilage zones [25]. Arkill et al. (2008) carried out studies to establish ZCC permeability and the mechanism of molecular transport from the subchondral microcirculation, using an intact perfused joint to test the effects of static loading on transport. Using the equine metacarpophalangeal joint as a model, fluorescein and rhodamine (approximately 400 Da) were employed as tracers, introduced in the subchondral compartment, and then followed throughout the joint by quantitative fluorescence microscopy. The ZCC was indeed permeable to both solutes, from both the superficial and the subchondral sides. The effective diffusivity of each solute was of the order of 9×10^{-9} cm^2 s^{-1}, fivefold less than uncalcified cartilage. Employing a similar strategy, Pan et al. (2009) showed that comparable tracer molecules (sodium fluorescein, 376 Da) could diffuse readily between the subchondral and the joint compartments in mouse knees. These observations show the possibility of direct signaling between the subchondral and articulating compartments, at least for small molecules and at least in animal models. These data suggest that cartilage and bone form a functional unit, both mechanically and biochemically, which may have a role in joint homeostasis and disease.

Assuming that small molecules can move across the bone-cartilage interface, the next logical step is to determine whether specific factors of relevance, related to joint physiology, are present and can do so. Intra-articular delivery of recombinant lubricin was found to attenuate the onset of OA by positive feedback loop between articular cartilage and subchondral bone in a rat OVX model [26]. Treatment was found to be chondroprotective in the joint space, and also effective in normalizing subchondral bone remodeling. The authors also suggest that these changes in turn attenuated the articular cartilage degradation by inhibition of vascular invasion based on reduced numbers of CD31 positive cells in calcified cartilage and angiography in subchondral bone.

The growth factor TGF β is a central regulator of bone and cartilage homeostasis that likely plays a pivotal role in joint crosstalk. Deregulation of TGFβ signaling in either cartilage or subchondral bone can drive the progression of joint disease in a compartment-specific manner. For example, in work by the authors (TA), chondrocyte-intrinsic ablation of the canonical TGFβ effector Smad3 causes cartilage degeneration by suppressing collagen II and aggrecan synthesis while inducing MMP13 expression. Therefore, insufficient TGFβ signaling in articular cartilage exacerbates joint disease. On the other hand, excessive TGFβ signaling in subchondral bone also causes cartilage degeneration. Transgenic overexpression of TGFβ in mesenchymal progenitors propels the progression of OA [27]. Administration of TGFβ inhibitors to the subchondral bone compartment can mitigate the severity of cartilage degeneration. Beyond these mechanistic studies in mouse models, deregulation of TGFβ is apparent in humans, where TGFβ is present in increased amounts in OA bone [28] and synovial fluid [29]. In some of the authors' own work (DMF), elevated levels of TGFβ mRNA were found in bone from end-stage OA human hip joints [30]. Furthermore, osteoblasts that were isolated from similar samples showed increased TGFβ expression, and

altered relationships with the expression of other cell regulatory molecules [31]. Additional research is needed to better understand the compartment-specific effects of TGFβ in bone and cartilage, how its function is corrupted in joint disease, and how it can be targeted therapeutically.

Physical cues resulting from abnormal mechanical stresses in joint disease are also important biological regulators. Changes in the mechanical or material environment of the joint may directly influence behavior in one or other compartment, which in turn may alter the dynamic relationship between the two. TGFβ signaling is mechanoregulated at multiple levels of the signaling cascade, from extracellular ligands to transcriptional control by Smads. It is just one of many factors that may participate in aberrant mechanobiologic crosstalk following joint injury. Soluble mediators released by bone cells in response to mechanical loading were tested in terms of their ability to induce catabolic activities in chondrocytes. Mouse osteoblast lineage cells were subjected to cyclic compression, and then their conditioned medium (CM) was used to stimulate chondrocytes. Expression of matrix metalloproteinase 3 (MMP-3), MMP-13, type II collagen, and aggrecan was then assessed. Stimulation of chondrocytes with CM from mechanically stimulated bone cells strongly induced MMP-3 and MMP-13 and inhibited the collagen II and aggrecan expression. In parallel, differential secretome analysis showed that 10 proteins were up-regulated in stimulated bone cells. Among them, soluble 14-3-3ε (s14-3-3ε) dose-dependently induced the release of catabolic factors by chondrocytes. Addition of a 14-3-3ε blocking antibody greatly attenuated the CM-mediated induction of MMP activity. These results identify s14-3-3 ε as a novel soluble mediator critical in the communication between subchondral bone and cartilage in OA. Thus, this may be a potential target for future therapeutic or prognostic applications in OA. [32]

In order to relate these findings back to the issue of BMLs in joint disease – there is a need to link cases of BMLs with specific tissue characteristics. In a recent study from the authors' group (DMF) BMLs were identified in tissues from human total knee arthroplasty using two different MRI sequences termed 'PDFS only' or 'PDFS + T1'[33]. After scanning, multi-modal tissue analyses of the osteochondral interface was carried out. BMLs were detected in 74 % of tibiae, of which 59 % were designated BML 1 (detected only by PDFS) and 41 % were designated BML 2 (detected by both PDFS + T1). The presence of a BML was related to degeneration of the joint interface, particularly within the BML 2 category. When compared to controls (no BML) BML 2-containing joints showed impaired outcomes in cartilage and subchondral bone. For most measures, BML 1 was intermediate between No BML and BML 2. Thus, this suggests that MRI characteristics of BMLs may enable identification of different BML phenotypes and help target novel approaches to treatment and prevention of OA.

Mechanical factors that may underlie BMLs and drive their communication with other joint tissues.

It has been proposed that the MRI signal that defines the presence of BMLs represents physical damage (microdamage), or a response to such damage, in the subchondral bone compartment [34-36]. While this is quite a common assertion, there are relatively few studies which directly report specific data. This is largely because detection of bone microdamage is not a trivial task, particular in the clinical setting, thus confirmatory data is limited. Much of the bone microdamage characterization work carried out over the last 4 decades was done in preclinical models. That work provided reproducible ways to stain for, and identify, various types of microdamage. Important mechanical and biological differences were shown among linear-microcracks, diffuse damage, and trabecular microfracture [37].

Microdamage can form in both cortical and cancellous bone, and evokes measureable mechanical and biological consequences in each. From a mechanical perspective, microdamage can be generated by a single (monotonic) significant loading event, or by multiple (up to millions) lower level loading cycles. Using engineering tools and calculations, it is possible to assess the amount of microdamage in bone tissue, and then to calculate the overall effect of that damage on the structural tissue properties. Various studies have addressed this question, and each arrived at a broadly similar conclusion, which is that a small amount of microdamage can significantly affect the structural performance of bone [38-43]. From a biological perspective microdamage also has profound consequences on bone tissue. Early studies from one of the authors (ODK), using the *in vivo* rat ulnar fatigue loading model, showed that this approach creates regions of microdamage in the mid-diaphysis, which in turn result in targeted intra-cortical remodeling to remove and repair the damage [44]. This work was later expanded to show that a critical cellular mechanism behind this response is that microdamage causes apoptosis in osteocytes. Apoptosis is the central event which initiates the osteoclast-mediated reparative response [45]. The same group went on to determine that pharmacological inhibition of osteocyte apoptosis completely prevented the remodeling response [46], and that the underlying molecular signaling was mediated by osteocyte derived RANKL expression [47, 48]. These studies were carried out using the ulnar loading model, which generates microdamage at a purely cortical bone skeletal site. An important point to note in any discussion about microdamage is that its location, or more specifically the type of tissue it resides in (cortical, cancellous or subchondral bone), is likely to be a crucial consideration in understanding its implication.

In relation to the role of bone in OA, the most relevant location/tissue-type is the subchondral compartment. It should be noted that subchondral bone microdamage in OA has been investigated previously, and was found to play a limited role. However – a simple reading of this can be misleading. The underlying mechanism that was proposed in that work was purely mechanical. Specifically, the hypothesis was that trabecular microfractures heal *via* the formation of a microcallus, and that the accumulation of multiple microcallus' would result in stiffening of the subchondral compartment, which would in turn increase the shear stress levels in overlying cartilage – leading to its degeneration. This purely mechanical theory proved to have limited applicability. More recent theories have a similar starting point, which is that microdamage occurs in the subchondral compartment. However, the consequences of its presence are now thought to be strongly linked to biological injury and repair responses like those described above, rather than directly mechanical *per se*.

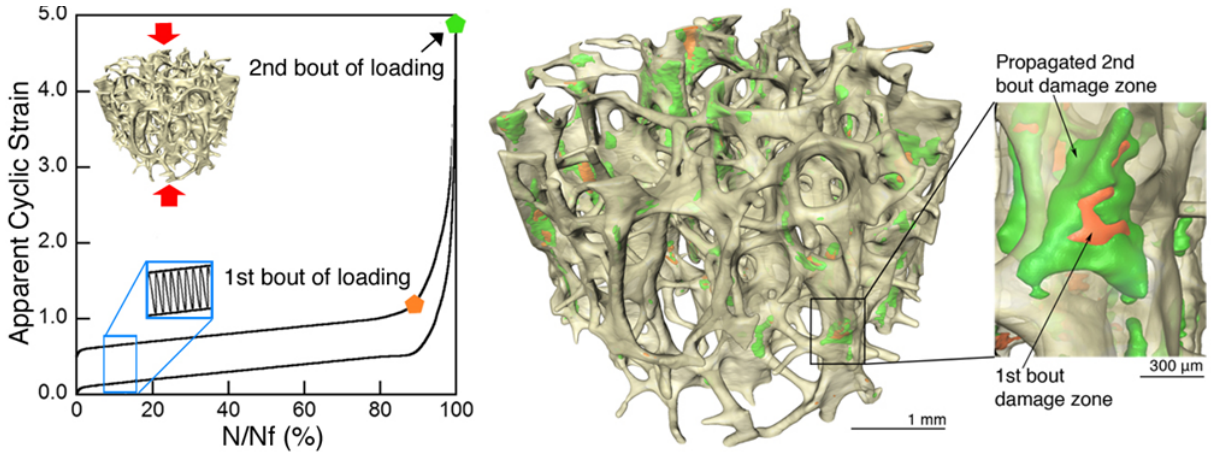

Figure 3: Cyclic loading causes the initiation (orange) and propagation (green) of tissue microdamage in cancellous bone. Tissue material properties were associated with damage propagation. Microdamage and associated bone remodeling within a BML

Since many of the BMLs that are seen clinically are located in the subchondral trabecular bone compartment, it is instructive to discuss what is currently known about microdamage behavior in this tissue type, and also the biological response to its presence. In a study from one of the authors' labs (CJH) showed that loading mode and microstructure influence microdamage behaviour in cancellous bone following a single overload and that the proportion of damaged tissue was greater for tension than for compression loading. Microdamage tended to occur in regions of greater trabecular thickness but not near observable resorption cavities suggesting that accumulation of microdamage in cancellous bone is influenced more by tissue material properties than traditional measures of trabecular microarchitecture [38, 49]. However, in a related study from the same group, microdamage was found to correlate with the number of rod-like trabeculae [50].

The authors then went on to test a commonly held hypothesis, which holds that resorption cavities on trabecular surface, resulting from osteoclast activity, act as stress risers and promote microdamage initiation and propagation. The data show that this is not the case, and microdamage is actually associated with tissue material properties as opposed to tissue stresses [51]. In cancellous bone, microdamage propagation during cyclic loading is dictated by heterogeneity of material properties associated with increased ductility at trabecular surfaces. This is somewhat unexpected because the opposite pattern occurs with surface treatment to increase fatigue life in man-made materials, which reduce surface ductility. In trabecular bone the more ductile surfaces are a result of reduced accumulation of advanced glycation end products compared with the interior, where damage is more likely to accumulate. Thus, cancellous bone becomes more tolerant of stress concentrations at strut surfaces. This also allows the structure to recover more deformation after failure and return to a closer approximation of its original shape. These intriguing findings

suggest the presence of a biomimetic design strategy in which tissue level material heterogeneity is used to improve recovery after failure or damage. More research will be required to determine whether there is a link between these material level phenomena, at the micron scale in subchondral trabecular bone, to BMLs and disease related changes in articular cartilage.

Conclusion

Mounting evidence supports the role of subchondral bone changes in OA development. In the early stages of disease, prior to any radiographic changes, subchondral bone remodeling is increased. This may be a response to subchondral microdamage, which likely forms as a result of malalignment or injury. As the disease progresses and remodeling abates, an uncoupling of bone resorption from formation leads to a net increase in bone volume. It may be that the relationship between these two opposing processes, early net-resorption followed by later net-formation, plays an integral role in joint degeneration. The use of animal models will be key to developing our understanding of these complex processes. While no one model provides an exact recapitulation of the human condition, each provides a useful aspect, which can be exploited to tackle a specific question relating to injury/disease development. Use of anti-resorptives to suppress osteoclast-mediated subchondral bone remodeling may yet be a promising line of investigation for new therapeutics against OA. However, it is likely that timing is critical in their use, and early intervention before the appearance of radiographic/clinical OA, may be required to observe this potentially beneficial effect. The findings reviewed here provide an excellent platform to continue this important area of research in the future, where novel interventions and therapies will be crucial for improving musculoskeletal healthcare.

Authors contributions: All authors contributed equally to this paper including concept, content, and editing. Each author has read and approved the final submitted version of this manuscript.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 799283 (ODK).

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