



Skeletal Changes Associated with Osteoarthritis

Neashan Mathavan¹ · Oran D. Kennedy¹

Published online: 19 July 2019

© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

Osteoarthritis (OA) is a degenerative joint disorder in which progressive articular cartilage loss occurs alongside pathological changes in subchondral bone and other joint tissues. The pathophysiological role of bone in OA has been a point of interest for many years and has resurfaced again in recent years as a potential target for new treatments. Articular cartilage and subchondral bone together form the osteochondral unit. Its homeostasis and integrity are reliant on biochemical crosstalk and biomechanical interplay between the two. Subchondral bone, with its relatively greater stiffness and strength, provides mechanical support to the overlying cartilage and absorbs much of the mechanical force transmitted through the joint. Mechanical instability in osteoarthritic joints is thought to be a significant risk-factor in joint disease, due to the mechano-sensitive nature of many of its native tissues. Although the progression of joint disease remains incompletely understood, significant changes in subchondral bone remodelling, structure, composition, and mechanical properties have been documented in animal and human studies of OA. The purpose of this review is to explore and discuss these bony changes associated with disease and, in particular, contextualise the basic science and clinical literature on the role of subchondral bone in OA.

Keywords Osteoarthritis · Subchondral Bone · Bone quality · Cartilage

Introduction

Osteoarthritis (OA), the most common degenerative joint disorder, affects more than 40 million people across Europe [1] with a 45% lifetime risk for developing symptomatic knee OA [2] and a 25% lifetime risk for developing symptomatic hip OA [3]. Although OA exhibits heterogeneous aetiologies, the radiographic loss of articular cartilage is its most common hallmark. Cartilage degeneration typically occurs in conjunction with other radiographic features, including sclerosis of subchondral bone and osteophyte formation—which are considered secondary hallmarks of the disease. OA presents clinically as joint pain and impaired function. Interventions at present are limited in their efficacy and are insufficient to stop or reverse disease progression. Thus, clinical management is limited to pain relief, conservative treatment, and, eventually, joint replacement.

The classical view of OA as a degenerative disease of cartilage alone has expanded in recent decades to recognise the

involvement of other joint tissues such as subchondral bone, ligaments, menisci, entheses, and synovial tissues [4, 5]. Many of the most pronounced pathological changes occur in cartilage and subchondral bone. Together, these two components form the osteochondral unit. Its mechanical role is to sustain and distribute forces across the joint. Subchondral bone, with its relatively greater stiffness and strength, provides support to the joint and absorbs/redistributes much of the mechanical force transmitted across it [6–8]. To successfully achieve this, bone must maintain a dynamic structure and adapt to its mechanical environment through modifications in composition and microstructure. Moreover, these changes in bone properties may also influence cartilage homeostasis via biochemical crosstalk and biomechanical interplay. Thus, these mechano-sensitive mechanisms in subchondral bone are integral to healthy joint homeostasis but may be disrupted in osteoarthritic bone environments.

Pre-clinical and clinical studies have demonstrated that pathological changes in subchondral bone are associated with OA. In animal models, multiple strategies have been used to induce OA. These broadly fall into two categories: (i) spontaneous models and (ii) induced models (surgical, chemical, and biomechanical). An assessment of the individual merits of these models is beyond the scope of this review but can be found in the literature [9]. Briefly, the Dunkin-Hartley guinea

✉ Oran D. Kennedy
orankennedy@rcsi.ie

¹ Department of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), 123 St Stephens Green, Dublin 2, Ireland

pig is a spontaneous model of OA. Chemically induced models of OA involve the introduction of reactive agents like monoiodoacetate (MIA) which, as an alkyl halide, reacts with cysteine residues in protein to degrade cartilage. Much of the literature features models where disease is induced by creating joint instability via surgical transection of specific joint structures (e.g. medial meniscus, cruciate ligament). A subset of these models replaces invasive surgical procedures with bio-mechanical loading protocols that induce damage to joint structures—without surgical incision. As with all model systems, each of these has advantages and disadvantages, and while most do generate responses in subchondral bone—it is not yet clear which is most representative of the clinical condition. In patients, the chief drawback is that recruitment criteria require radiological evidence of OA, which limits studies to patients with moderate/advanced OA. Nevertheless, these pre-clinical models and clinical studies have been, and will continue to be, indispensable in exploring the role of subchondral bone in OA.

The purpose of this review is to contextualise skeletal changes associated with OA based on current basic science and clinical evidence. With this objective in mind, we propose a hypothetical model of the chronological sequence of OA-associated changes in subchondral bone (Fig. 1). In this model, mechanical perturbation of the joint due to repetitive loading or joint trauma is the stimulus for OA initiation and the formation of microdamage. Two key events ensue (i) increased remodelling at the damaged sites thereby resulting in (ii) elevated levels of transforming growth factor $\beta 1$ (TGF- $\beta 1$) in subchondral bone (Fig. 1). These events set in motion changes to subchondral bone structure, composition, and mechanical properties and culminate in the well-recognised radiographic features of late-stage OA. The following sections in this review are subdivided to examine in detail these changes: “[Bone Remodelling](#),” “[Bone Structure](#),” “[Bone Composition](#),” “[Bone Mechanics](#),” and “[Bone–Cartilage Crosstalk](#)”.

Bone Remodelling

Bone Remodelling and Associated Morphological Changes in OA

Pathological changes in subchondral bone remodelling in the initiation and progression of OA are distinctly biphasic and corresponds to the early and late phases of the disease [10, 11]. Studies in both animal OA models and humans have observed increased subchondral resorption in early-stage OA with associated morphological changes characteristic of osteopenia (Fig. 2). Late-stage OA, contrastingly, is characterised by increased bone formation and subchondral sclerosis (Fig. 2). Of particular significance is the finding

that OA does not progress in the absence of increased remodelling in the early phases of the disease [12]. This provides a rationale for the use of anti-resorptive agents such as bisphosphonates as a potential treatment for OA, which will be discussed in more detail below.

Animal Studies

The biphasic remodelling response in OA has been demonstrated in a range of animal models [13–22]. Botter et al. reported increased osteoclast activity directly underneath the subchondral bone plate at 2 weeks following OA induction by collagenase injection in a murine model [22]. Subsequently, bone formation rate (BFR) almost doubled compared to controls in subchondral trabecular bone. Likewise, Benske et al. reported increases of 3–5-fold in the mineral apposition rate (MAR) in the knees of mice with spontaneous OA development [13]. Here, the phenomenon was localised to regions adjacent to degenerated cartilage. Subsequently, the maximal MAR decreased with OA progression [13]. In a murine anterior cruciate ligament transection (ACLT) model, Zhen et al. found subchondral bone remodelling was augmented as early as 1 week post-ACLT with large bone marrow cavities present at 4 weeks [14]. In contrast, total subchondral bone tissue volume was 20% higher at 8 weeks following surgery [14]. In a rat ACLT model, Hayami et al. observed subchondral bone resorption at 2 weeks post-surgery and then sclerosis and osteophyte formation at 10 weeks [15, 16]. Similarly, bone mineral density (BMD) was lower at 4 weeks and higher at 12 weeks in the knees of meniscectomised guinea pigs [17].

In general, the transient morphological changes accompanying the biphasic remodelling phases are similar in both subchondral cortical and cancellous sites. However, trabecular bone volumes have been found in some instances to remain osteopenic or unchanged in the late, sclerotic stages of the disease. This discrepancy may be explained by the regions of interest (ROI) considered in the analyses. Pathological changes in morphology are highly localised to sites adjacent to cartilage degradation. Both Hayami et al. and Dedrick et al. noted that these morphological changes were not present if the entirety of the epiphyseal region was included in their analyses instead of localised trabecular sites [16, 18, 19].

Biphasic subchondral bone remodelling may result from different responses by which bone and cartilage adapt to their changing mechanical environment. This differential capacity was demonstrated by Ko et al. in a non-invasive murine model [23], where knees were subjected to a single cyclic loading session, but no macroscopic joint damage. Loading induced a transient subchondral remodelling response where cancellous bone loss and thinning were observed at 1 week, and was consistent with a coincident increase in osteoclast numbers. Bone loss was reversed at 2 weeks with morphological parameters returning to

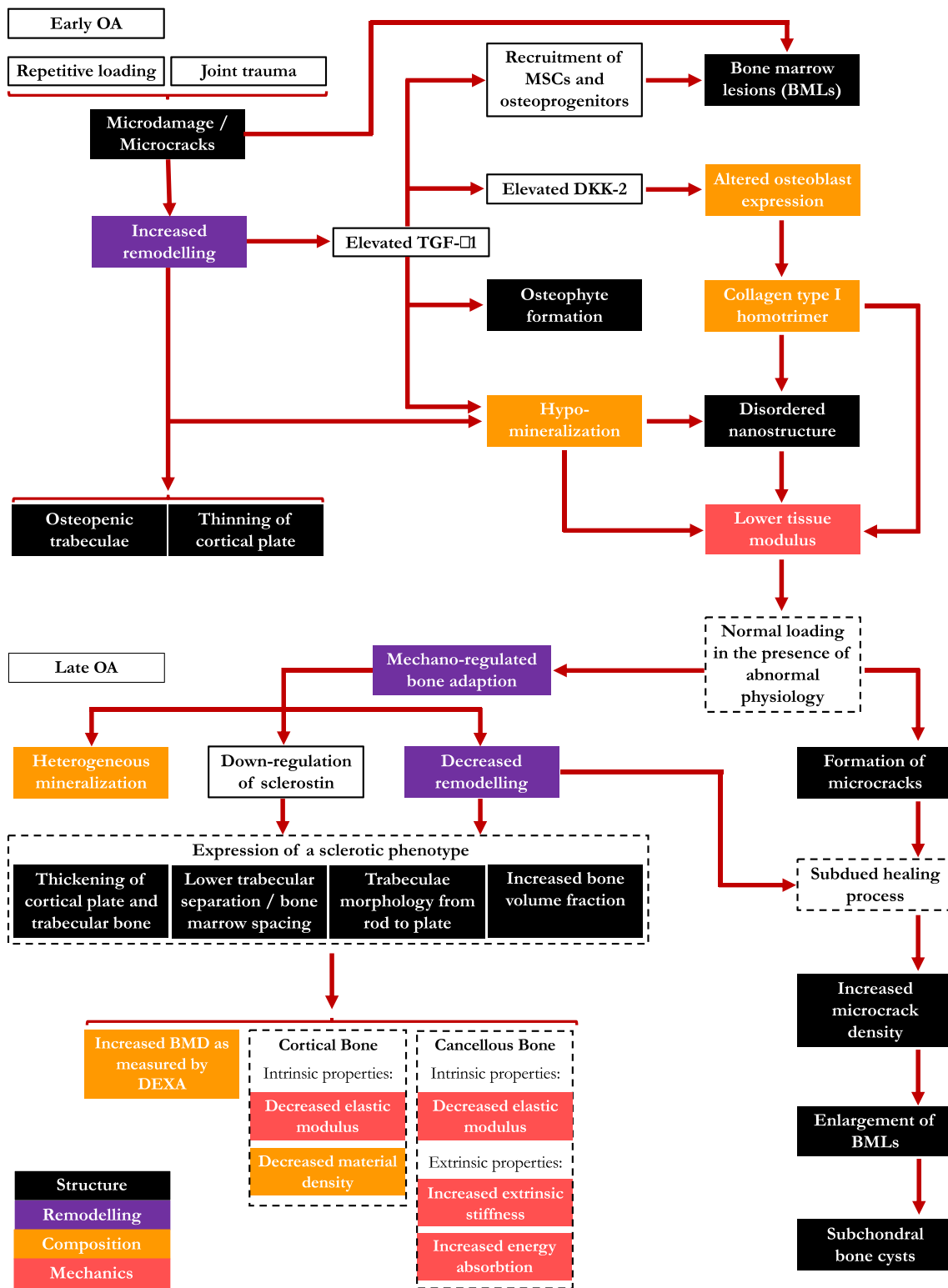


Fig. 1 Hypothetical model of the sequence of subchondral bone changes associated with osteoarthritis

baseline values. In contrast, loading induced a progressive catabolic chondrocyte response in cartilage with concomitant proteoglycan loss and localised cartilage thinning. Thus, adaptation to acute mechanical loading in bone is remarkably rapid and indeed

reversible once a physiological loading state is restored. Whether there is a link between the limited capacity of chondrocytes to restore cartilage integrity and the subsequent sclerosis of subchondral bone remains unknown.

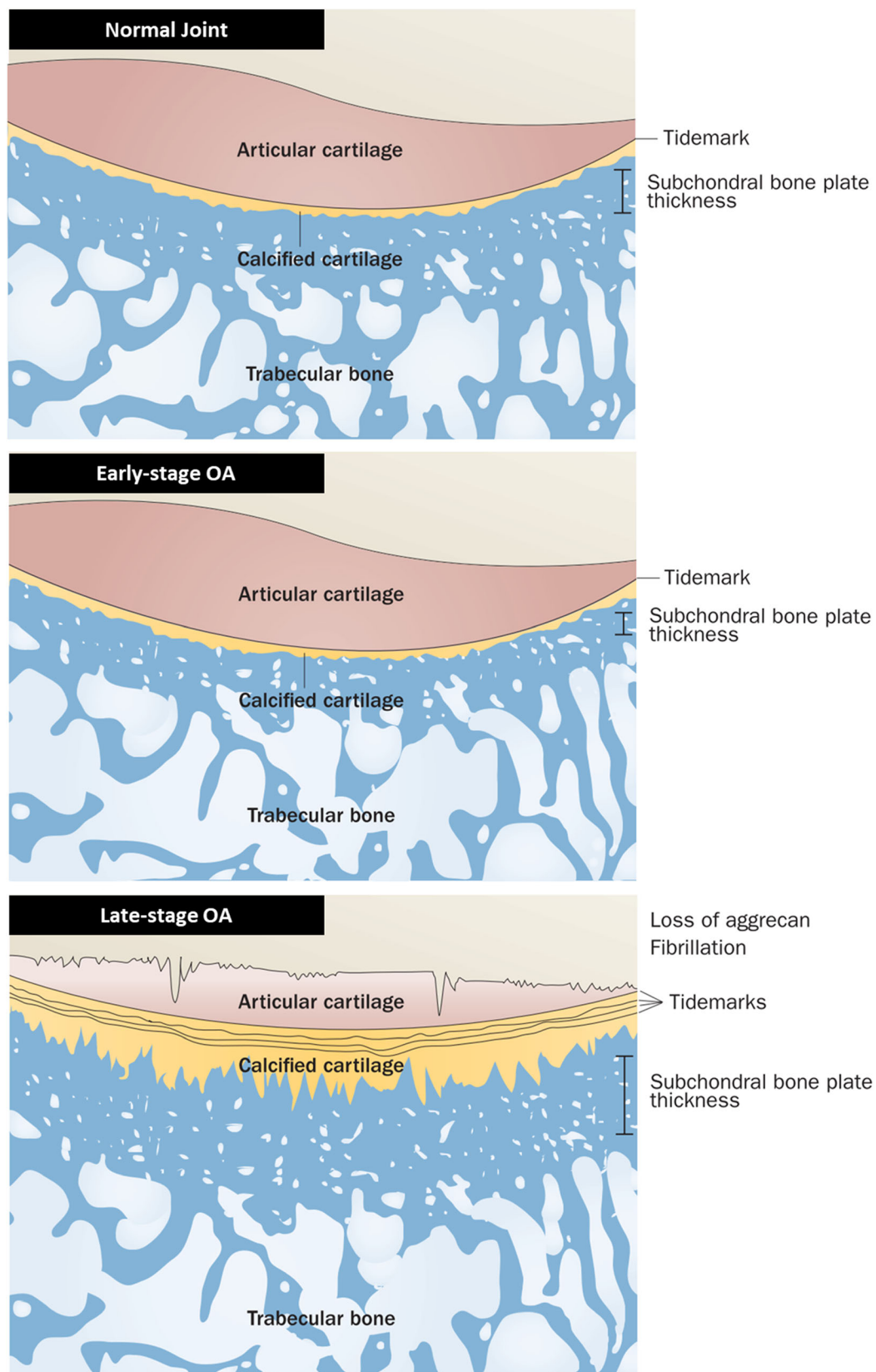


Fig. 2 Morphological changes in the osteochondral unit at different stages of disease progression. Early-stage OA is characterised by osteopenia with decreased subchondral cancellous bone mass and a thinner, more porous subchondral cortical plate. Late-stage OA is

characterised by sclerosis with increased cancellous bone volume and a thicker cortical plate. Reprinted by permission from Springer Nature: Springer Nature, Nature Reviews Rheumatology [10], © 2012

Human Studies

Several key findings relating to bone remodelling and OA have been observed in clinical studies. In a biochemical study, Mansell et al. reported bone remodelling to be increased 20-fold in retrieved OA femoral heads relative to controls [24, 25]. The rate of type I collagen synthesis was determined via C-terminal propeptide of type I collagen (PICP), while degradation was determined by matrix metalloproteinase 2 (MMP-2) activity. The increase in type I collagen synthesis was reflected by an increase in alkaline phosphatase, a bone formation marker. In a complementary study, Bettica et al. reported increased levels of bone resorption markers, namely type I collagen C-terminal and N-terminal telopeptides, in postmenopausal women with progressive OA in a longitudinal study of women aged 45–64 [12]. Importantly, this increase in bone resorption was not observed in patients with non-progressive OA.

Scintigraphy studies in humans have also demonstrated increased subchondral bone turnover with scintigraphic detection preceding radiographic evidence [26–29]. Most notably, in a study using technetium-labelled bisphosphonate, Dieppe et al. identified increased subchondral bone turnover in patients with established OA using this method [27]. Furthermore, the authors found that cartilage degeneration does not significantly progress in the absence of a concomitant change in subchondral bone turnover.

Multiple MRI studies have also highlighted the role of subchondral bone in OA progression. Reichenbach et al. reported the presence of bone attrition (i.e. altered bone contours) in the knees of early OA patients before joint space narrowing was visible radiographically [30]. Furthermore, Neogi et al. demonstrated a strong association between subchondral bone attrition and localised cartilage loss within the same sub-region in a cohort of individuals who had, or were at high risk for developing, knee OA [31]. The risk of localised cartilage loss was increased 7-fold in regions with subchondral bone attrition. Similarly, Bolbos et al. reported a correlation between loss of subchondral trabecular bone and cartilage degradation in a study of young and middle-aged OA patients (aged 29–72 years) [32].

Aberrant Activation of TGF- β

At the molecular level, the uncoupling of bone resorption from formation in OA joints is indicative of aberrations in the signalling pathways governing bone remodelling. Most notable is the transforming growth factor-beta (TGF- β) signalling pathway which is a key regulator of homeostasis for both bone and cartilage [33]. It has been identified as a key signalling pathway in the pathogenesis of OA (as illustrated in Fig. 1) [14, 33]. Unlike other cytokines, TGF- β is secreted into the extracellular matrix (ECM) of

different tissues in an inactive or latent form. Its activation is achieved by precise spatiotemporal regulation and occurs in response to tissue remodelling/injury or alterations in mechanical loading. Activation of latent TGF- β in the matrix orchestrates the coupling of osteoclast and osteoblast activity. Specifically, active TGF- β 1 is released during bone resorption and induces migration of mesenchymal stem cells (MSCs) to the resorption site [34]. TGF- β , in coordination with other signalling molecules and the physico-chemical properties of the exposed bone site, then stimulate the differentiation of MSCs into osteoblast lineage cells [35–37]. Disruption of this pathway thus has the potential to uncouple bone formation from resorption.

In OA joints, deregulation of TGF- β signalling impairs the structural and mechanical integrity of both subchondral bone [14, 33] and articular cartilage [38–41] and may contribute to the progression of OA. Indeed, OA progression has been linked with high levels of active TGF- β in subchondral bone in both human and animal studies [14]. Constitutive expression of active TGF- β 1 by osteoblastic cells in transgenic mice was found to induce an osteoarthritic phenotype with abnormal subchondral bone structure and significant degeneration of articular cartilage [14, 34]. Specifically, as illustrated in Fig. 1, the elevation in TGF- β 1 levels in subchondral bone has been linked to osteophyte formation (discussed in the “[Bone Structure](#)” section) and alterations in osteoblast expression and hypomineralisation (discussed in the “[Bone Composition](#)” section). Further underscoring its critical role, Zhen et al. demonstrated localised inhibition of TGF- β in subchondral bone attenuated cartilage degeneration in different murine models of osteoarthritis [14]. This intervention strategy is not without its challenges as TGF- β has distinct roles in subchondral bone and articular cartilage (as described in the “[Bone–Cartilage Crosstalk](#)” section). Thus, high concentrations of TGF- β in subchondral bone seem to initiate the cascade of pathological changes in subchondral bone, and interventions to restore TGF- β levels following acute joint injury may prevent OA development.

Bisphosphonate Therapy for OA

Therapeutic interventions to regulate bone remodelling in OA have been proposed as a treatment in the past. The rationale is based on the significance of increased remodelling in the early phase of disease progression (refer to Fig. 1) and the finding that OA does not progress in its absence [12]. Recent experimental studies have sought to test whether bisphosphonate therapy could be useful as an OA therapy. Bisphosphonates exhibit a high specificity for active sites in bone and can potently suppress remodelling activity. In pre-clinical studies, bisphosphonates have demonstrated potential in preventing or slowing the

progression of bone pathology [15, 42–47]. Indeed, some studies found bisphosphonates exerted a chondroprotective effect despite there being no known direct effect on chondrocytes [15, 43–45]. It is also notable that efficacy in pre-clinical studies has not been consistent [48–50]. In patients, the translation of bisphosphonate therapy to the clinic has had limited success in randomised controlled trials (RCTs) [51–56]. These discrepancies between pre-clinical and clinical studies are multifactorial. Foremost is the recruitment of patients with radiographic or functional evidence of OA and thus may present stages too advanced for bisphosphonate therapy to be effective. Identification of patients in the early stages of OA is challenging though and may only be feasible in subjects with secondary, post-traumatic OA (PTOA).

Bone Structure

Morphological changes in subchondral bone are well documented at the microscale (Fig. 2). These changes reflect the biphasic remodelling stages of OA progression discussed above. Early OA is characterised by increased resorption with decreased subchondral bone mass and a thinner, more porous cortical plate [10, 15, 16, 20, 22, 32, 57–61]. Late phase OA is characterised by subchondral sclerosis which manifests as densification and thickening of the cortical plate [10, 15–17, 19, 22, 57, 62–66]. Jia et al. proposed a mechanism by which subchondral sclerosis is stimulated in murine models of severe, advanced OA [67, 68]. Using a novel μ CT protocol to generate colour maps of subchondral plate thickness, the authors established that site-specific plate thickening is correlated to localised loading and to degradation of the overlying articular cartilage. Reduced levels of sclerostin, an inhibitor of bone formation, were also observed in conjunction with cortical plate thickening. Expression of sclerostin by osteocytes is mechano-regulated, and this study demonstrated that sclerostin was downregulated by abnormal loading. Here, the authors suggest that subchondral bone sclerosis is a secondary consequence of cartilage degeneration and is induced by mechanically mediated downregulation of sclerostin. Wu et al. further corroborated this finding in their observation that sclerostin levels within the subchondral bone plate in OA patients negatively correlated with OA severity [69]. Coincident with their observation of reduced sclerostin expression was the activation of the Wnt/ β -catenin signalling pathway, thus increasing osteogenesis and inhibiting bone resorption.

In addition to these remodelling-induced morphological changes, osteoarthritic subchondral bone shows evidence of pathological features including (i) microdamage, (ii) bone marrow lesions (BMLs), and (iii) osteophytes.

Microdamage

Physiological loading of bone is known to routinely cause the formation of microdamage. In healthy tissue, repair of microdamage is orchestrated by osteocytes and carried out by basic multicellular units (BMUs) [70]. With ageing, disease, and treatment, this process may be compromised and microdamage can accumulate [71]. The consequences of microdamage accumulation are both mechanical and biological. Mechanically, structural integrity is compromised and fracture risk increased. In fact, microdamage accumulation in the range of 1–2% (volume fraction) can reduce strength by 50–60% [72–77]. Biologically, the accumulation of microdamage elicits damage responses. Osteocytes in the vicinity of microdamage undergo apoptosis which, in turn, initiates osteoclast-mediated remodelling via increased production of RANKL which stimulates osteoclastogenesis [78].

Much of the current understanding of microdamage-associated remodelling is derived from experimental studies of diaphyseal cortical or trabecular bone sites [79–81]. Microdamage in subchondral bone appears to produce similar responses (Fig. 1) but its significance in joint disease and failure remains unknown [82–85]. Ramme et al. demonstrated the co-localisation of microdamage with subchondral remodelling following ACL rupture in a rodent model [86]. Clinically, the presence of subchondral microdamage following acute joint injury has not yet been demonstrated. However, BMLs, which have been associated with subchondral microdamage, are regularly observed on MRIs following clinical knee injury [87, 88]. Subchondral microdamage may thus have a significant role in OA (and particularly PTOA) initiation but this remains to be explored and fully characterised.

Bone Marrow Lesions

In recent years, BMLs have been recognised as an integral feature in acute joint injury [72]. It has been proposed that they are involved in the development of PTOA. However, this potential relationship is not well understood. BMLs are ill-defined regions of hyperintense signal in fluid-sensitive, fat-suppressed MR images [89]. Histological analyses of BML biopsies from patients undergoing joint arthroplasty have revealed the accumulation of microfractures at different stages of healing, bone marrow fibrosis, bleeding, oedema, and necrosis [90–93]. BMLs are likely induced by mechanical overload during joint trauma (Fig. 1). Observations in support of this include their presence following an acute ACL injury in 80–98% of patients [94–96] and the localisation of BMLs within a joint to sites of mechanical damage [93, 97]. Thus, microdamage accumulation may constitute a mechanism by which BMLs form

(Fig. 1). Intriguingly, it has been proposed that microdamage accumulation may initiate a reparative response with localised inflammatory events, and BMLs may represent an impaired fracture repair process [98]. Immune cells have significant roles in fracture repair and there may exist a distinct osteoimmunological link, potentially via the OPG/RANKL system [98–100]. In addition, TGF- β 1 may also have a role in BML formation based on the observation of osteoid islets forming in the subchondral bone marrow in response to aberrant activation of TGF- β 1 in mice (Fig. 1) [14].

BMLs are clinically relevant as their presence increases the risk for structural degeneration of articular cartilage [93, 101], development of knee pain [53, 102–104], and disease progression [93, 105–109]. Furthermore, they are linked with the development of cysts and have been proposed as early pre-cystic lesions (Fig. 1) [110, 111]. Intriguingly, BMLs have been found to be strongly indicative of disease severity [93, 108] and have been proposed as potent predictors of disease progression. Libicher et al. reported that BMLs preceded cartilage degeneration in an MRI study using an ACLT canine model [112]. Clinically, the presence of BMLs has been documented in asymptomatic patients and may be predictive of an increased risk for OA [106, 113–115]. BMLs have also been documented in patients with symptomatic early-stage OA and in patients with severe late-stage OA [93, 101, 103, 105, 113, 116]. Observations in asymptomatic or early OA subjects found that BMLs may decrease in size or resolve completely [101, 109, 117]. Conversely, BMLs were likely to persist and enlarge in size in subjects with progressive OA [93]. Indeed, Laslett et al. reported that decreased BML size was concomitant with reduced knee pain in patients treated with Zoledronate [53].

Formation of Osteophytes

Osteophytes are another pathological feature of OA and are outgrowths of cartilage which subsequently undergo ossification [118, 119]. Their presence in radiographs is a significant criterion in the diagnosis of disease. Osteophyte formation normally occurs at the joint margins and outgrowths in central regions of the articular space present in approximately 15% of patients [120]. Osteophyte formation follows the prenatal bone development pathway with chondrogenic differentiation of MSCs resulting in cartilaginous outgrowths [118, 121]. Subsequently, these ossify via a combination of endochondral and intramembranous ossification [119, 122]. Their formation culminates in structures extending from the joint margins which are integrated with the native bone and covered by cartilage [118, 119].

Osteophytes are regarded as pathological adaptations in response to joint instability. The role of the mechanical

environment has been demonstrated in animal studies where osteophyte growth was stimulated with exercise and inhibited by immobilisation [123–127]. Hsia et al. found osteophyte formation following ACL rupture in a murine model coincided with joint restabilisation and reduced range of motion [128]. Likewise, Pottenger et al. found that the removal of marginal osteophytes in patients with primary knee OA increased joint motion and concluded that osteophytes act as mechanical stabilisers in osteoarthritic knees [129]. In agreement, Murata et al. reported that restoration of physiological joint kinematics following ACL transection in a rat model inhibited osteophyte growth [130]. Noteworthy also is the role of aberrant TGF- β /BMP signalling in osteophyte formation with TGF- β 1 and BMP-2 identified as potent inducers (Fig. 1) [131–133].

Bone Composition

Homotrimeric Collagen

Type I collagen is the principal organic constituent of bone and confers the tissue with tensile strength and toughness [134, 135]. In its dominant isoform, type I collagen is a heterotrimeric triple helix composed of two α 1 chains and one α 2 chain. However, its normal phenotypic expression by osteoblasts is altered in OA bone with increased secretion of a homotrimeric isoform of type I collagen composed of three α 1 chains [136]. The homotrimeric isoform of type I collagen has previously been found in foetal tissues, fibrosis, and cancer in humans albeit with genetically distinct α 1 chains [137]. In contrast, the homotrimeric isoform present in OA bone is characterised by an excess of α 1 chains substituting the α 2 chains [136]. This homotrimeric phenotype is also naturally expressed in the murine model of osteogenesis imperfecta (oim), a genetic brittle bone disease, resulting in spontaneous skeletal fractures [138]. However, the mechanical consequences of homotrimeric type I collagen specific to OA are, as yet, unclear. Indeed, Couchourel et al. observed collagen to be hypomineralised in an *in vitro* characterisation study of OA osteoblasts but this does not appear to translate to an increased susceptibility to fracture at the whole bone level [139].

The mechanisms responsible for this abnormal osteoblast phenotype in OA have been partly discerned but much remains unknown. *In vitro* studies of OA osteoblasts found increased levels of TGF- β 1 expression [139–141]. Couchourel et al. proposed that elevated TGF- β 1 levels were responsible, in part, for increased expression of α 1 chains in OA subchondral bone (Fig. 1) [139]. TGF- β 1 is known to inhibit mineralisation *in vitro* either directly or via BMP-2 stimulated

mineralisation [139]. The significant role of TGF- β 1 in altered OA osteoblastic function was substantiated when its inhibition in osteoblasts was found to reduce the α 1-to- α 2 ratio [139]. Subsequently, Chan et al. found elevated expression of TGF- β 1 stimulated expression of the Dickkopf-2 protein (DKK2) in OA osteoblasts (Fig. 1) [142]. DKK2 is an antagonist of the Wnt signalling pathway which has a critical role in osteogenesis and the regulation of terminal osteoblast differentiation. Indeed, inhibition of either TGF- β or DKK2 expression restored phenotypic expression of OA osteoblasts (Fig. 1) [142].

Hypomineralisation

Hypomineralisation of subchondral bone has been consistently reported in in vitro and ex vivo studies of OA joints. Mansell et al. conducted biochemical characterisation of OA femoral heads and showed collagen I synthesis to be augmented but with significantly reduced calcium-to-collagen ratios [24]. Similarly, Li et al. presented comparisons of material density, which is a reflection of tissue mineralisation, in cortical and cancellous subchondral bone from OA femoral heads [143, 144]. OA bone exhibited significantly reduced material density indicative of hypomineralisation at both cortical and cancellous sites, and this finding was further corroborated by a significant decrease in mineral content as measured by gravimetric analyses. Density fractionation measurements of cortical and cancellous samples from the same site by Grynepas et al. also found hypomineralisation in osteoarthritic bone [62]. Similarly, Ferguson et al. found mineralisation, as determined by scanning electron microscopy using backscattered electrons (qBSE), to be significantly lower at the same site [145]. Moreover, in a complementary study, Li et al. found this state of hypomineralisation in OA hip joints extended to the trabeculae of the femoral neck [146].

Notably, the cumulative data from these studies suggest that proximity to the cartilage surface influences the degree of hypomineralisation with the effect most pronounced at skeletal sites adjacent to the joint [24, 62, 143, 144, 146]. Cox et al. quantified the depth-dependency of mineralisation in osteochondral plugs harvested from the proximal tibiae of OA patients presenting different stages of the disease [147]. Mineralisation was found to be lowest in samples with the highest severity of cartilage degeneration. Hypomineralisation varied from 6% at subchondral sites at a depth of 1 mm from the cartilage to 4% at subchondral sites at a depth of 3 mm from the cartilage. Thus, there exists a relationship at a highly localised level between bone mineralisation and cartilage degeneration.

Moreover, Zuo et al. presented comparisons of mineral distribution in the subchondral cortical and cancellous regions of the proximal tibia in samples classified as either Grade I or Grade IV [148]. Mineralisation, as characterised by BSE, was observed to progress from homogeneous distribution in

cortical/cancellous regions in grade I samples to a heterogeneous distribution with concentrated areas of high mineralisation in grade 4 samples (Fig. 1).

Alterations in mineralisation are primarily a consequence of alterations in bone remodelling. Specifically, increased remodelling inhibits mineral accretion in newly formed bone and yields hypomineralised bone. Moreover, hypomineralisation was also observed by Couchourel et al. in their in vitro characterisation study of OA osteoblasts [139]. Thus, this deficit in mineralisation is potentially exacerbated by the presence of both (i) the homotrimeric collagen phenotype and (ii) elevated production of both TGF- β 1 and Dickkopf related protein 2 (DKK2) in OA subchondral bone (Fig. 1). Indeed, mineralisation by BMP-2, an anabolic agent, is less potent in subchondral bone due to elevations in either TGF- β 1 or DKK2 expression [139, 142]. Samples in all the above studies were harvested prior to arthroplasty and therefore are representative of moderate or late phase OA. Thus, it is conceivable that subchondral bone in OA attains a hypomineralised state early in OA progression and this state is not remedied by the reduced bone turnover in late phase OA.

Noteworthy also, is the frequently reported association between OA and an *increase* in BMD at both the affected joint and the appendicular skeleton in clinical literature [149–154]. However, it is important to note that BMD, as measured by dual energy x-ray absorptiometry (DEXA), reflects bone quantity and is not a measure of the material density of the bone itself. Therefore, an increase in BMD can be consistent with the sclerotic response of bone in the advanced stages of OA.

Bone Mechanics

OA bone is generally characterised by a decrease in intrinsic tissue properties and an ensuing adaptive response that results in an increase in extrinsic bone tissue mechanical properties. Literature on the mechanical properties of subchondral bone presents either (i) *intrinsic or material properties*, constituting a measure of the mechanical properties independent of bone size and structure, or (ii) *extrinsic or apparent properties*, representing the properties of the bone structure in its entirety. This is an important distinction in interpreting the literature and an in-depth review can be found elsewhere [155].

Intrinsic Properties: Elastic Modulus

Both cortical and cancellous sites in OA bone are reported to exhibit decreased elastic moduli across different length scales. Day et al. reported a 60% decrease in the elastic modulus of subchondral trabeculae in the proximal tibia of individuals with mild cartilage degeneration [156]. The authors used a

combination of finite element (FE) modelling, μ CT imaging, and compression mechanical testing in determining the elastic moduli. Similarly, the elastic moduli of subchondral cortical bone, as determined by ultrasound measurements, were found to be decreased in the femoral heads of OA subjects [143]. In contrast, Hargrave-Thomas et al. found no significant differences in the elastic moduli of the cortical plate of bovine patellae at the macroscale and microscale using three-point bending and microindentation respectively [157].

More recent studies have reported elastic moduli obtained at the nanoscale using nanoindentation techniques. Pragnère et al. found a 16% decrease in the elastic moduli of the cortical plate at 6 weeks following OA induction in a PTOA rabbit model [158]. Of considerable interest in their results was the existence of a more pronounced stiffness gradient between cartilage and subchondral bone as a result of OA induction. Stiffness values decreased by 42% and 37% in hyaline cartilage and calcified cartilage respectively compared with only 16% in the cortical plate.

Also noteworthy in the literature is the finding that the elastic modulus at the nanoscale initially decreases with the onset of OA and subsequently appears to regain baseline values at later stages. Hargrave-Thomas et al. found elastic moduli in the cortical plate of bovine patellae to significantly decrease in samples with mild cartilage degeneration only to then recover in samples presenting moderate cartilage degeneration [157]. Zuo et al. also reported similar results in comparisons of Grade I and Grade IV human proximal tibial trabeculae with increases in the reduced modulus (E_r , a measure of both sample and indenter compliance) of 19% and 25% at trabecular osteon and lamellar sites respectively [148]. Moreover, Ferguson et al. found no significant differences in elastic moduli in the cortical plate of human femoral heads between end-stage OA and normal post mortem samples [145].

The stiffness of mineralised tissues is strongly influenced by their degree of mineralisation [159, 160]. Thus, lower stiffness values in these samples are consistent with the hypomineralised nature of OA bone. Moreover, the apparent recovery of bone stiffness at the nanoscale with late phase OA progression is likely a consequence of the sensitivity of nanoindentation to the heterogeneous distribution or aggregation of mineral crystals with late phase disease progression [148].

Much has been written and postulated on the significance of subchondral bone stiffness in the initiation and progression of OA. Notably, in the 1970s and 1980s, Radin et al. proposed the hypothesis that initiation and progression of cartilage degradation were induced by an *increase* in the elastic modulus of the subchondral bone and resulting in a stiffness gradient between the bone and the overlying articular cartilage [161, 162]. Although prescient for its time, the absence of substantive evidence in the intervening decades has necessitated re-evaluation of this premise. Furthermore, subchondral bone

plate stiffness has been reported to be lower in the explanted femoral heads of individuals with either osteoporosis (OP) or OA [143]. This suggests that lower stiffness of the subchondral bone plate cannot, in itself, account for the preservation of the overlying cartilage in the OP samples nor explain its destruction in the OA samples.

Extrinsic Properties

In general, measures of extrinsic or apparent bone mechanical properties are augmented with disease progression. Li et al. conducted unconfined compression testing of subchondral trabeculae samples from OA femoral heads and reported increased stiffness and energy absorbed to yield parameters [144, 163]. However, there are also exceptions to this in the literature wherein no demonstrable change in extrinsic mechanical properties is present [164, 165].

Increased mechanical strength/stiffness might seem paradoxical in light of the documented hypomineralisation of subchondral OA bone but it can be understood in the context of coincident pathological changes. For example, Li et al. showed that subchondral trabecular bone volume was 60% greater in OA subjects [144]. This increase in bone volume fraction characteristic of late phase OA could be an adaptive response to the increased bone strain that is a consequence of hypomineralisation in bone. Day et al. constructed models of subchondral trabecular bone using high-resolution μ CT imaging to simulate mechano-regulated bone adaptation in response to the degeneration of intrinsic bone mechanical properties [155]. Using their strain-driven model, the authors demonstrated that a decrease in intrinsic bone stiffness necessitated an increase in bone volume fraction to compensate. Namely, the authors reported that a reduction of 20% in intrinsic bone stiffness resulted in an increase of 25% in the extrinsic stiffness. Thus, their model is supportive of the idea that subchondral stiffening is a necessary compensatory mechanism to counteract the greater compliance of OA bone.

Bone–Cartilage Crosstalk

Pathological changes associated with OA are evident in both cartilage and subchondral bone, and it has stimulated interest in the potential signalling or crosstalk mechanisms between the two tissues. A brief commentary is presented here, but comprehensive reviews on the subject can be found elsewhere [166–168].

Several studies have documented evidence for the feasibility of bone–cartilage crosstalk across the osteochondral junction [22, 169–171]. Pan et al. demonstrated the diffusion of small molecules between the bone marrow and articular space using fluorescent tracer molecules in mouse joints [169]. Histological analyses of the osteochondral interface by

Imhof et al. detailed the dense subchondral vasculature which permits communication between the tissues [171]. Furthermore, studies have suggested that crosstalk is enhanced with OA progression. Hwang et al. reported increased fluid flow conductance in human osteochondral plugs in association with OA severity [172]. Increased porosity of the subchondral plate has also been documented with OA progression and has been attributed to increased osteoclast activity [22, 172, 173]. Moreover, these increases in plate porosity and remodelling activity seem to subsequently permit a vascular invasion of the cartilage [22, 172, 174]. Formation of microcracks/fissures in response to abnormal loading in OA joints may also function as transport conduits between cartilage and bone [167].

Accumulating evidence also supports the existence of molecular interactions between the two compartments [166, 167, 175, 176]. The TGF- β and Wnt/ β -catenin signalling pathways have critical regulatory roles in maintaining the integrity of the osteochondral unit. We have previously described OA-induced aberrations in TGF- β (as discussed in the “Bone Remodelling” section) and Wnt/ β -catenin (as discussed in the “Bone Structure” section) signalling in subchondral bone. In cartilage, TGF- β -mediated signalling has an important regulatory role in maintaining its structural integrity and mechanical function [38, 41, 177–179]. Suppression of TGF- β signalling in cartilage has been found to aggravate cartilage degeneration and impair cartilage repair in osteoarthritic murine models [40, 133, 180]. Indeed, TGF- β has been found to be almost absent in OA cartilage compared with the high levels at which it is expressed in healthy cartilage [39]. This is in contrast to the high levels of TGF- β present in OA subchondral bone. Thus, aberrations in TGF- β levels are present in both tissues with OA progression. Notably, inhibition of TGF- β activity in subchondral bone attenuates OA progression in both tissues and further underscores their interdependence.

Interventions in the articular cartilage can similarly rescue both tissues of the osteochondral unit. Wnt/ β -catenin signalling molecules have important roles in regulating the function of articular chondrocytes. Wnt antagonists such as DKK1 and sclerostin have been suggested as potential therapeutic targets as the pathway is strongly associated with OA [166, 167, 181, 182]. Oh et al. demonstrated overexpression of DKK1 in the articular cartilage of a murine OA model significantly inhibited cartilage degradation, osteophyte formation, and sclerosis in subchondral bone [181]. Thus, bone-cartilage crosstalk presents opportunities for therapeutic interventions to arrest or slow disease progression.

Conclusion

Cumulatively, the discussions presented here underscore the role of subchondral bone in OA pathogenesis and progression.

To summarise these changes, we refer back to the hypothetical model of disease progression proposed in Fig. 1.

In this model, mechanical perturbation of the joint due to repetitive loading or joint trauma is the stimulus for the formation of microdamage. Increased remodelling ensues and induces resorptive changes in subchondral bone structures and elevated TGF- β 1 levels in subchondral bone. Subchondral bone becomes hypomineralised as increased remodelling and the elevation in TGF- β 1 hinders the accretion of mineral in the newly formed bone. Moreover, the elevation in TGF- β 1 stimulates the formation of osteophytes and alters osteoblast expression with the secretion of a homotrimeric isoform of type I collagen. Together, the changes in collagen structure and hypomineralisation contribute to a lower tissue modulus. Concomitant with these changes is the formation of BMLs. Continued normal loading of the joint subsequently downregulates sclerostin expression and stimulates the sclerotic phenotype characteristic of late-stage OA.

Collectively, the sequence of events presented in this model serves to underscore the extent and variety of changes that are possible in subchondral bone during disease progression. Furthermore, it suggests that a complete understanding of all aspects of this system will be necessary to develop interventions to cease or reverse disease progression.

Funding Science Foundation Ireland (17/CDA/4699) H2020-MCSA-IF-799 283.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. The burden of musculoskeletal conditions at the start of the new millennium. World Health Organ Tech Rep Ser. 2003.
2. Murphy L, Schwartz TA, Helmick CG, Renner JB, Tudor G, Koch G, et al. Lifetime risk of symptomatic knee osteoarthritis. *Arthritis Care Res.* 2008;59:1207–13.
3. Murphy LB, Helmick CG, Schwartz TA, Renner JB, Tudor G, Koch GG, et al. One in four people may develop symptomatic hip osteoarthritis in his or her lifetime. *Osteoarthr Cartil.* 2010;18:1372–9.
4. Aspden RM, Saunders FR. Osteoarthritis as an organ disease: from the cradle to the grave. *Eur Cells Mater.* 2019;37:74–87.
5. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum.* 2012;64:1697–707.
6. Radin EL, Paul IL, Lowy M. A comparison of the dynamic force transmitting properties of subchondral bone and articular cartilage. *J Bone Joint Surg Am.* 1970;52:444–56.

7. Radin EL, Paul IL. Does cartilage compliance reduce skeletal impact loads? The relative force-attenuating properties of articular cartilage, synovial fluid, periarticular soft tissues and bone. *Arthritis Rheum.* 1970;13:139–44.
8. Malekipour F, Whitton C, Oetomo D, Lee PVS. Shock absorbing ability of articular cartilage and subchondral bone under impact compression. *J Mech Behav Biomed Mater.* 2013;26:127–35.
9. Kuyinu EL, Narayanan G, Nair LS, Laurencin CT. Animal models of osteoarthritis: classification, update, and measurement of outcomes. *J Orthop Surg Res.* 2016;19.
10. Burr DB, Gallant MA. Bone remodelling in osteoarthritis. *Nat Rev Rheumatol.* 2012;8:665.
11. Kwan Tat S, Lajeunesse D, Pelletier JP, Martel-Pelletier J. Targeting subchondral bone for treating osteoarthritis: what is the evidence? *Best Pract Res Clin Rheumatol.* 2010;24:51–70.
12. Bettica P, Cline G, Hart DJ, Meyer J, Spector TD. Evidence for increased bone resorption in patients with progressive knee osteoarthritis: longitudinal results from the Chingford study. *Arthritis Rheum.* 2002;46(12):3178–84.
13. Benske J, Schünke M, Tillmann B. Subchondral bone formation in arthrosis: polychrome labeling studies in mice. *Acta Orthop.* 1988;59:536–41.
14. Zhen G, Wen C, Jia X, Li Y, Crane JL, Mears SC, et al. Inhibition of TGF- β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. *Nat Med.* 2013;19:704–12.
15. Hayami T, Pickarski M, Wesolowski GA, McLane J, Bone A, Destefano J, et al. The role of subchondral bone remodeling in osteoarthritis: reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. *Arthritis Rheum.* 2004;50:1193–206.
16. Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, Rodan GA, Duong LT. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. *Bone.* 2006;38:234–43.
17. Pastoureau PC, Chomel AC, Bonnet J. Evidence of early subchondral bone changes in the meniscectomized guinea pig. A densitometric study using dual-energy X-ray absorptiometry subregional analysis. *Osteoarthr Cartil.* 1999;7:466–73.
18. Dedrick DK, Goldstein SA, Brandt KD, O'Connor BL, Goulet RW, Albrecht M. A longitudinal study of subchondral plate and trabecular bone in cruciate-deficient dogs with osteoarthritis followed up for 54 months. *Arthritis Rheum.* 1993;36:1460–7.
19. Brandt KD, Myers SL, Burr D, Albrecht M. Osteoarthritic changes in canine articular cartilage, subchondral bone, and synovium fifty-four months after transection of the anterior cruciate ligament. *Arthritis Rheum.* 1991;34:1560–70.
20. Intema F, Sniekers YH, Weinans H, Vianen ME, Yocum SA, Zuurmond AMM, et al. Similarities and discrepancies in subchondral bone structure in two differently induced canine models of osteoarthritis. *J Bone Miner Res.* 2010;25:1650–7.
21. Pelletier JP, Boileau C, Brunet J, Boily M, Lajeunesse D, Reboul P, et al. The inhibition of subchondral bone resorption in the early phase of experimental dog osteoarthritis by licofelone is associated with a reduction in the synthesis of MMP-13 and cathepsin K. *Bone.* 2004;34:527–38.
22. Botter SM, Van Osch GJVM, Clockaerts S, Waarsing JH, Weinans H, Van Leeuwen JPTM. Osteoarthritis induction leads to early and temporal subchondral plate porosity in the tibial plateau of mice: an in vivo microfocal computed tomography study. *Arthritis Rheum.* 2011;63(9):2690–9.
23. Ko FC, Dragomir CL, Plumb DA, Hsia AW, Adebayo OO, Goldring SR, et al. Progressive cell-mediated changes in articular cartilage and bone in mice are initiated by a single session of controlled cyclic compressive loading. *J Orthop Res.* 2016;34:1941–9.
24. Mansell JP, Bailey AJ. Abnormal cancellous bone collagen metabolism in osteoarthritis. *J Clin Invest.* 1998;101:1596–603.
25. Bailey AJ, Mansell JP, Sims TJ, Banse X. Biochemical and mechanical properties of subchondral bone in osteoarthritis. *Biorheology.* 2004;41:349–58.
26. Danielsson LG, Dymling J-F, Heripret G. Coxarthrosis in man studied with external counting of Sr85 and Ca47*. *Clin Orthop Relat Res.* 1963;31.
27. Dieppe P, Cushnaghan J, Young P, Kirwan J. Prediction of the progression of joint space narrowing in osteoarthritis of the knee by bone scintigraphy. *Ann Rheum Dis.* 1993;52:557–63.
28. Hutton CW, Higgs ER, Jackson PC. 99m Tc HMDP bone scanning in generalised nodal osteoarthritis. II. The four hour bone scan image predicts radiographic change. *Ann Rheum Dis.* 1986;45:622–6.
29. McCarthy C, Cushnaghan J, Dieppe P. The predictive role of scintigraphy in radiographic osteoarthritis of the hand. *Osteoarthr Cartil.* 1994;2:25–8.
30. Reichenbach S, Guermazi A, Niu J, Neogi T, Hunter DJ, Roemer FW, et al. Prevalence of bone attrition on knee radiographs and MRI in a community-based cohort. *Osteoarthr Cartil.* 2008;16:1005–10.
31. Neogi T, Felson D, Niu J, Lynch J, Nevitt M, Guermazi A, et al. Cartilage loss occurs in the same subregions as subchondral bone attrition: a within-knee subregion-matched approach from the multicenter osteoarthritis study. *Arthritis Care Res.* 2009;61:1539–44.
32. Bolbos RI, Zuo J, Banerjee S, Link TM, Benjamin Ma C, Li X, et al. Relationship between trabecular bone structure and articular cartilage morphology and relaxation times in early OA of the knee joint using parallel MRI at 3 T. *Osteoarthr Cartil.* 2008;16:1150–9.
33. Zhen G, Cao X. Targeting TGF β signaling in subchondral bone and articular cartilage homeostasis. *Trends Pharmacol Sci.* 2014;227–36.
34. Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z, et al. TGF-B1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. *Nat Med.* 2009;15:757–65.
35. Watabe T, Miyazono K. Roles of TGF- β family signaling in stem cell renewal and differentiation. *Cell Res.* 2009;19:103–15.
36. Augello A, De Bari C. The regulation of differentiation in mesenchymal stem cells. *Hum Gene Ther.* 2010;21:1226–38.
37. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell.* 2006;126:677–89.
38. Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF- β and osteoarthritis. *Osteoarthr Cartil.* 2007;15:597–604.
39. Blaney Davidson EN, Vitters EL, Van Der Kraan PM, Van Den Berg WB. Expression of transforming growth factor- β (TGF β) and the TGF β signalling molecule SMAD-2P in spontaneous and instability-induced osteoarthritis: role in cartilage degradation, chondrogenesis and osteophyte formation. *Ann Rheum Dis.* 2006;65:1414–21.
40. Shen J, Li J, Wang B, Jin H, Wang M, Zhang Y, et al. Deletion of the transforming growth factor β receptor type II gene in articular chondrocytes leads to a progressive osteoarthritis-like phenotype in mice. *Arthritis Rheum.* 2013;65:3107–19.
41. Yang X, Chen L, Xu X, Li C, Huang C, Deng CX. TGF- β /Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. *J Cell Biol.* 2001;153:35–46.
42. Mohan G, Perilli E, Parkinson IH, Humphries JM, Fazzalari NL, Kuliwaba JS. Pre-emptive, early, and delayed alendronate treatment in a rat model of knee osteoarthritis: effect on subchondral trabecular bone microarchitecture and cartilage degradation of the tibia, bone/cartilage turnover, and joint discomfort. *Osteoarthr Cartil.* 2013;21:1595–604.

43. Siebelt M, Waarsing JH, Groen HC, Müller C, Koelewijn SJ, de Blois E, et al. Inhibited osteoclastic bone resorption through alendronate treatment in rats reduces severe osteoarthritis progression. *Bone*. 2014;66:163–70.
44. Zhang L, Hu H, Tian F, Song H, Zhang Y. Enhancement of subchondral bone quality by alendronate administration for the reduction of cartilage degeneration in the early phase of experimental osteoarthritis. *Clin Exp Med*. 2011;11:235–43.
45. Shirai T, Kobayashi M, Nishitani K, Satake T, Kuroki H, Nakagawa Y, et al. Chondroprotective effect of alendronate in a rabbit model of osteoarthritis. *J Orthop Res*. 2011;29:1572–7.
46. Moreau M, Rialland P, Pelletier JP, Martel-Pelletier J, Lajeunesse D, Boileau C, et al. Tiludronate treatment improves structural changes and symptoms of osteoarthritis in the canine anterior cruciate ligament model. *Arthritis Res Ther*. 2011;13.
47. Strassle BW, Mark L, Leventhal L, Piesla MJ, Jian Li X, Kennedy JD, et al. Inhibition of osteoclasts prevents cartilage loss and pain in a rat model of degenerative joint disease. *Osteoarthr Cartil*. 2010;18:1319–28.
48. Ding M, Danielsen CC, Hvid I. The effects of bone remodeling inhibition by alendronate on three-dimensional microarchitecture of subchondral bone tissues in guinea pig primary osteoarthrosis. *Calcif Tissue Int*. 2008;82:77–86.
49. Bagi CM, Berryman E, Zakur DE, Wilkie D, Andresen CJ. Effect of antiresorptive and anabolic bone therapy on development of osteoarthritis in a posttraumatic rat model of OA. *Arthritis Res Ther*. 2015;17.
50. Myers SL, Brandt KD, Burr DB, O'Connor BL, Albrecht M. Effects of a bisphosphonate on bone histomorphometry and dynamics in the canine cruciate deficiency model of osteoarthritis. *J Rheumatol*. 1999;26:2645–53.
51. Bingham CO, Buckland-Wright JC, Garner P, Cohen SB, Dougados M, Adami S, et al. Risedronate decreases biochemical markers of cartilage degradation but does not decrease symptoms or slow radiographic progression in patients with medial compartment osteoarthritis of the knee: results of the two-year multinational knee osteoarthritis st. *Arthritis Rheum*. 2006;54:3494–507.
52. Jokar M, Mirfeizi Z, Keyvanpajoh K. The effect of alendronate on symptoms of knee osteoarthritis: a randomized controlled trial. *Iran J Med Sci*. 2010;35:9–15.
53. Laslett LL, Doré DA, Quinn SJ, Boon P, Ryan E, Winzenberg TM, et al. Zoledronic acid reduces knee pain and bone marrow lesions over 1 year: a randomised controlled trial. *Ann Rheum Dis*. 2012;71:1322–8.
54. Rossini M, Adami S, Fracassi E, Viapiana O, Orsolini G, Povino MR, et al. Effects of intra-articular clodronate in the treatment of knee osteoarthritis: results of a double-blind, randomized placebo-controlled trial. *Rheumatol Int*. 2015;35:255–63.
55. Spector TD, Conaghan PG, Buckland-Wright JC, Garner P, Cline GA, Beary JF, et al. Effect of risedronate on joint structure and symptoms of knee osteoarthritis: results of the BRISK randomized, controlled trial [ISRCTN01928173]. *Arthritis Res Ther*. 2005;7:R625.
56. Varena M, Zucchi F, Failoni S, Becciolini A, Berruto M. Intravenous neridronate in the treatment of acute painful knee osteoarthritis: a randomized controlled study. *Rheumatol (United Kingdom)*. 2015;54:1826–32.
57. Batiste DL, Kirkley A, Laverty S, Thain LMF, Spouge AR, Holdsworth DW. Ex vivo characterization of articular cartilage and bone lesions in a rabbit ACL transection model of osteoarthritis using MRI and micro-CT. *Osteoarthr Cartil*. 2004;12:986–96.
58. Sniekers YH, Intema F, Lafeber FPJG, Van Osch GJVM, Van Leeuwen JPTM, Weinans H, et al. A role for subchondral bone changes in the process of osteoarthritis; a micro-CT study of two canine models. *BMC Musculoskelet Disord*. 2008;9.
59. Botter SM, van Osch GJ, Waarsing JH, Day JS, Verhaar JA, Pols HA, et al. Quantification of subchondral bone changes in a murine osteoarthritis model using micro-CT. *Biorheology*. 2006;43:379–88.
60. Botter SM, van Osch GJVM, Waarsing JH, van der Linden JC, Verhaar JAN, Pols HAP, et al. Cartilage damage pattern in relation to subchondral plate thickness in a collagenase-induced model of osteoarthritis. *Osteoarthr Cartil*. 2008;16:506–14.
61. Botter SM, Glasson SS, Hopkins B, Clockaerts S, Weinans H, van Leeuwen JPTM, et al. ADAMTS5^{-/-} mice have less subchondral bone changes after induction of osteoarthritis through surgical instability: implications for a link between cartilage and subchondral bone changes. *Osteoarthr Cartil*. 2009;17:636–45.
62. Grynblas MD, Alpert B, Katz I, Lieberman I, Pritzker KPH. Subchondral bone in osteoarthritis. *Calcif Tissue Int*. Springer. 1991;49(1):20–6.
63. Hannan MT, Anderson JJ, Zhang Y, Levy D, Felson DT. Bone mineral density and knee osteoarthritis in elderly men and women. The Framingham study. *Arthritis Rheum*. 1993;36:1671–80.
64. Buckland-Wright C. Subchondral bone changes in hand and knee osteoarthritis detected by radiography. *Osteoarthr Cartil*. 2004;12:10–9.
65. Fazzalari NL, Parkinson IH. Fractal properties of subchondral cancellous bone in severe osteoarthritis of the hip. *J Bone Miner Res*. 1997;12:632–40.
66. Arden NK, Griffiths GO, Hart DJ, Doyle DV, Spector TD. The association between osteoarthritis and osteoporotic fracture: the Chingford study. *Rheumatology*. Oxford University Press. 1996;35:1299–304.
67. Jia H, Ma X, Wei Y, Tong W, Tower RJ, Chandra A, et al. Loading-induced reduction in sclerostin as a mechanism of subchondral bone plate sclerosis in mouse knee joints during late-stage osteoarthritis. *Arthritis Rheumatol*. 2018;70:230–41.
68. Burr DB, Utreja A. Editorial: Wnt signaling related to subchondral bone density and cartilage degradation in osteoarthritis. *Arthritis Rheumatol*. 2018;70:157–61.
69. Wu L, Guo H, Sun K, Zhao X, Ma T, Jin Q. Sclerostin expression in the subchondral bone of patients with knee osteoarthritis. *Int J Mol Med*. 2016;38:1395–402.
70. Schaffler MB. Role of bone turnover in microdamage. *Osteoporos Int*. 2003;14:73–80.
71. Schaffler MB, Choi K, Milgrom C. Aging and matrix microdamage accumulation in human compact bone. *Bone*. 1995;17:521–5.
72. Alliston T, Hernandez CJ, Findlay DM, Felson DT, Kennedy OD. Bone marrow lesions in osteoarthritis: what lies beneath. *J Orthop Res*. 2018;36:1818–25.
73. Lambers FM, Bouman AR, Rinnac CM, Hernandez CJ. Microdamage caused by fatigue loading in human cancellous bone: relationship to reductions in bone biomechanical performance. *PLoS One*. 2013;8.
74. Hernandez CJ, Lambers FM, Widjaja J, Chapa C, Rinnac CM. Quantitative relationships between microdamage and cancellous bone strength and stiffness. *Bone*. 2014;66:205–13.
75. Follet H, Viguet-Carrin S, Burt-Pichat B, Dépalle B, Bala Y, Gineys E, et al. Effects of preexisting microdamage, collagen cross-links, degree of mineralization, age, and architecture on compressive mechanical properties of elderly human vertebral trabecular bone. *J Orthop Res*. 2011;29:481–8.
76. Vashishth D, Koontz J, Qiu SJ, Lundin-Cannon D, Yeni YN, Schaffler MB, et al. In vivo diffuse damage in human vertebral trabecular bone. *Bone*. 2000;26:147–52.
77. Burr DB, Forwood MR, Fyhrie DP, Martin RB, Schaffler MB, Turner CH. Bone microdamage and skeletal fragility in osteoporotic and stress fractures. *J Bone Miner Res*. 1997;12:6–15.

78. Verborgt O, Gibson GJ, Schaffler MB. Loss of osteocyte integrity in association with microdamage and bone remodeling after fatigue in vivo. *J Bone Miner Res.* 2000;15:60–7.
79. Seref-Ferlengez Z, Kennedy OD, Schaffler MB. Bone microdamage, remodeling and bone fragility: how much damage is too much damage? *Bonekey Rep.* 2015;4.
80. Torres AM, Matheny JB, Keaveny TM, Taylor D, Rinnac CM, Hernandez CJ. Material heterogeneity in cancellous bone promotes deformation recovery after mechanical failure. *Proc Natl Acad Sci.* 2016;113:2892–7.
81. Bentolila V, Boyce TM, Fyhrie DP, Drumb R, Skerry TM, Schaffler MB. Intracortical remodeling in adult rat long bones after fatigue loading. *Bone.* 1998;23:275–81.
82. Mori S, Harruff R, Burr DB. Microcracks in articular calcified cartilage of human femoral heads. *Arch Pathol Lab Med.* 1993;117:196–8.
83. Sokoloff L. Microcracks in the calcified layer of articular cartilage. *Arch Pathol Lab Med.* 1993;117:191–5.
84. Radin EL, Parker HG, Pugh JW, Steinberg RS, Paul IL, Rose RM. Response of joints to impact loading - III. Relationship between trabecular microfractures and cartilage degeneration. *J Biomech.* 1973;6.
85. Muratovic D, Findlay DM, Cicuttini FM, Wluka AE, Lee YR, Kuliwaba JS. Bone matrix microdamage and vascular changes characterize bone marrow lesions in the subchondral bone of knee osteoarthritis. *Bone.* 2018;108:193–201.
86. Ramme AJ, Lendhey M, Raya JG, Kirsch T, Kennedy OD. A novel rat model for subchondral microdamage in acute knee injury: a potential mechanism in post-traumatic osteoarthritis. *Osteoarthr Cartil.* 2016;24:1776–85.
87. Selvarajah L, Curtis AM, Kennedy OD. Bone microdamage in acute knee injury. *Curr Rheumatol Rep.* 2018;20:89.
88. Coughlin TR, Kennedy OD. The role of subchondral bone damage in post-traumatic osteoarthritis. *Ann N Y Acad Sci.* 2016;1383:58–66.
89. Bonadio MB, Ormond Filho AG, Helito CP, Stump XM, Demange MK. Bone marrow lesion: image, clinical presentation, and treatment. *Magn Reson Insights.* 2017;10.
90. Taljanovic MS, Graham AR, Benjamin JB, Gmitro AF, Krupinski EA, Schwartz SA, et al. Bone marrow edema pattern in advanced hip osteoarthritis: quantitative assessment with magnetic resonance imaging and correlation with clinical examination, radiographic findings, and histopathology. *Skelet Radiol.* 2008;37:423–31.
91. Zanetti M, Bruder E, Romero J, Hodler J. Bone marrow edema pattern in osteoarthritic knees: correlation between MR imaging and histologic findings. *Radiology.* 2013;215:835–40.
92. Leydet-Quilic H, Le Corroller T, Bouvier C, Giorgi R, Argenson JN, Champsaur P, et al. Advanced hip osteoarthritis: magnetic resonance imaging aspects and histopathology correlations. *Osteoarthr Cartil.* 2010;18:1429–35.
93. Hunter DJ, Zhang Y, Niu J, Goggins J, Amin S, LaValley MP, et al. Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. *Arthritis Rheum.* 2006;54:1529–35.
94. Frobell RB, Roos HP, Roos EM, Hellio Le Graverand MP, Buck R, Tamez-Pena J, et al. The acutely ACL injured knee assessed by MRI: are large volume traumatic bone marrow lesions a sign of severe compression injury? *Osteoarthr Cartil.* 2008;16:829–36.
95. Gong J, Padoia V, Facchetti L, Link TM, Ma CB, Li X. Bone marrow edema-like lesions (BMELs) are associated with higher T1 ρ and T2 values of cartilage in anterior cruciate ligament (ACL)-reconstructed knees: a longitudinal study. *Quant Imaging Med Surg.* 2016;6:661–70.
96. Filardo G, Andriolo L, di Laura FG, Napoli F, Zaffagnini S, Candrian C. Bone bruise in anterior cruciate ligament rupture entails a more severe joint damage affecting joint degenerative progression. *Knee Surge Sport Traumatol Arthrosc.* 2019;27:44–59.
97. Boileau C, Martel-Pelletier J, Abram F, Raynauld JP, Troncy É, D'Anjou MA, et al. Magnetic resonance imaging can accurately assess the long-term progression of knee structural changes in experimental dog osteoarthritis. *Ann Rheum Dis.* 2008;67:926–32.
98. Weber A, Chan PMB, Wen C. Do immune cells lead the way in subchondral bone disturbance in osteoarthritis? *Prog Biophys Mol Biol.* 2018.
99. Manilay JO, Zouali M. Tight relationships between B lymphocytes and the skeletal system. *Trends Mol Med.* 2014;20:405–12.
100. Huang R, Wang X, Zhou Y, Xiao Y. RANKL-induced M1 macrophages are involved in bone formation. *Bone Res.* 2017;5.
101. Roemer FW, Guermazi A, Javaid MK, Lynch JA, Niu J, Zhang Y, et al. Change in MRI-detected subchondral bone marrow lesions is associated with cartilage loss: the MOST study. A longitudinal multicentre study of knee osteoarthritis. *Ann Rheum Dis.* 2009;68:1461–5.
102. Felson DT, Chaisson CE, Hill CL, Totterman SMS, Gale ME, Skinner KM, et al. The association of bone marrow lesions with pain in knee osteoarthritis. *Ann Intern Med.* 2001;134:541–9.
103. Sower MF, Hayes C, Jamadar D, Capul D, Lachance L, Jannausch M, et al. Magnetic resonance-detected subchondral bone marrow and cartilage defect characteristics associated with pain and X-ray-defined knee osteoarthritis. *Osteoarthr Cartil.* 2003;11:387–93.
104. Zhang Y, Nevitt M, Niu J, Lewis C, Torner J, Guermazi A, et al. Fluctuation of knee pain and changes in bone marrow lesions, effusions, and synovitis on magnetic resonance imaging. *Arthritis Rheum.* 2011;63(3):691–9.
105. Link TM, Steinbach LS, Ghosh S, Ries M, Lu Y, Lane N, et al. Osteoarthritis: MR imaging findings in different stages of disease and correlation with clinical findings. *Radiology.* 2003;226:373–81.
106. Wluka AE, Hanna F, Davies-Tuck M, Wang Y, Bell RJ, Davis SR, et al. Bone marrow lesions predict increase in knee cartilage defects and loss of cartilage volume in middle-aged women without knee pain over 2 years. *Ann Rheum Dis.* 2009;68:850–5.
107. Felson DT, Niu J, Guermazi A, Roemer F, Aliabadi P, Clancy M, et al. Correlation of the development of knee pain with enlarging bone marrow lesions on magnetic resonance imaging. *Arthritis Rheum.* 2007;56:2986–92.
108. Muratovic D, Cicuttini F, Wluka A, Findlay D, Wang Y, Otto S, et al. Bone marrow lesions detected by specific combination of MRI sequences are associated with severity of osteochondral degeneration. *Arthritis Res Ther.* 2016;18.
109. Davies-Tuck ML, Wluka AE, Wang Y, English DR, Giles GG, Cicuttini F. The natural history of bone marrow lesions in community-based adults with no clinical knee osteoarthritis. *Ann Rheum Dis.* 2009;68(6):904–8.
110. Carrino JA, Blum J, Parellada JA, Schweitzer ME, Morrison WB. MRI of bone marrow edema-like signal in the pathogenesis of subchondral cysts. *Osteoarthr Cartil.* 2006;14:1081–5.
111. Crema MD, Roemer FW, Zhu Y, Marra MD, Niu J, Zhang Y, et al. Subchondral cystlike lesions develop longitudinally in areas of bone marrow edema-like lesions in patients with or at risk for knee osteoarthritis: detection with MR imaging—the MOST study. *Radiology.* 2010;256:855–62.
112. Libicher M, Ivancic M, Hoffmann V, Wenz W. Early changes in experimental osteoarthritis using the Pond-Nuki dog model: technical procedure and initial results of in vivo MR imaging. *Eur Radiol.* 2005;15:390–4.
113. Zubler V, Mengiardi B, Pfirrmann CWA, Duc SR, Schmid MR, Hodler J, et al. Bone marrow changes on STIR MR images of asymptomatic feet and ankles. *Eur Radiol.* 2007;17:3066–72.

114. Baranyay FJ, Wang Y, Wluka AE, English DR, Giles GG, Sullivan RO, et al. Association of bone marrow lesions with knee structures and risk factors for bone marrow lesions in the knees of clinically healthy, Community-Based Adults. *Semin Arthritis Rheum.* 2007;37:112–8.
115. Guymer E, Baranyay F, Wluka AE, Hanna F, Bell RJ, Davis SR, et al. A study of the prevalence and associations of subchondral bone marrow lesions in the knees of healthy, middle-aged women. *Osteoarthr Cartil.* 2007;15(12):1437–42.
116. Roemer FW, Neogi T, Nevitt MC, Felson DT, Zhu Y, Zhang Y, et al. Subchondral bone marrow lesions are highly associated with, and predict subchondral bone attrition longitudinally: the MOST study. *Osteoarthr Cartil.* 2010;18:47–53.
117. Berry PA, Davies-Tuck ML, Wluka AE, Hanna FS, Bell RJ, Davis SR, et al. The natural history of bone marrow lesions in community-based middle-aged women without clinical knee osteoarthritis. *Semin Arthritis Rheum.* 2009;39:213–7.
118. van der Kraan PM, van den Berg WB. Osteophytes: relevance and biology. *Osteoarthr Cartil.* 2007;15:237–44.
119. Zoricic S, Maric I, Bobinac D, Vukicevic S. Expression of bone morphogenetic proteins and cartilage-derived morphogenetic proteins during osteophyte formation in humans. *J Anat.* 2003;202(3):269–77.
120. McCauley TR, Kornaat PR, Jee WH. Central osteophytes in the knee: prevalence and association with cartilage defects on MP imaging. *Am J Roentgenol.* 2001;176:359–64.
121. Arden N, Nevitt MC. Osteoarthritis: epidemiology. *Best Pract Res Clin Rheumatol.* 2006.
122. Gelse K, Söder S, Eger W, Diemtar T, Aigner T. Osteophyte development - molecular characterization of differentiation stages. *Osteoarthr Cartil.* 2003;11:141–8.
123. Williams JM, Brandt KD. Exercise increases osteophyte formation and diminishes fibrillation following chemically induced articular cartilage injury. *J Anat.* 1984;139:599.
124. Williams JM, Brandt KD. Temporary immobilisation facilitates repair of chemically induced articular cartilage injury. *J Anat.* 1984;138:435.
125. Williams JM, Brandt KD. Immobilization ameliorates chemically-induced articular cartilage damage. *Arthritis Rheum.* 1984;27(2):208–16.
126. Palmoski MJ, Brandt KD. Immobilization of the knee prevents osteoarthritis after anterior cruciate ligament transection. *Arthritis Rheum.* 1982;25:1201–8.
127. Hsia AW, Emami AJ, Tarke FD, Cunningham HC, Tjandra PM, Wong A, et al. Osteophytes and fracture calluses share developmental milestones and are diminished by unloading. *J Orthop Res.* 2018;36:699–710.
128. Hsia AW, Anderson MJ, Heffner MA, Lagmay EP, Zavadovskaya R, Christiansen BA. Osteophyte formation after ACL rupture in mice is associated with joint restabilization and loss of range of motion. *J Orthop Res.* 2017;35:466–73.
129. Pottenger LA, Phillips FM, Draganich LF. The effect of marginal osteophytes on reduction of varus-valgus instability in osteoarthritic knees. *Arthritis Rheum.* 1990;33(6):853–8.
130. Murata K, Kokubun T, Morishita Y, Onitsuka K, Fujiwara S, Nakajima A, et al. Controlling abnormal joint movement inhibits response of osteophyte formation. *Cartilage.* 2018;9:391–401.
131. Van Beuningen HM, Glansbeek HL, Van Der Kraan PM, Van Den Berg WB. Differential effects of local application of BMP-2 or TGF- β 1 on both articular cartilage composition and osteophyte formation. *Osteoarthr Cartil.* 1998;6:306–17.
132. Bakker AC, van de Loo FAJ, van Beuningen HM, Sime P, van Lent PLEM, van der Kraan PM, et al. Overexpression of active TGF- β 1 in the murine knee joint: evidence for synovial-layer-dependent chondro-osteophyte formation. *Osteoarthr Cartil.* 2001;9:128–36.
133. Scharstuhl A, Vitters EL, Van Der Kraan PM, Van Den Berg WB. Reduction of osteophyte formation and synovial thickening by adenoviral overexpression of transforming growth factor β /Bone morphogenetic protein inhibitors during experimental osteoarthritis. *Arthritis Rheum.* 2003;48:3442–51.
134. Seeman E, Delmas PD. Bone quality — the material and structural basis of bone strength and fragility. *N Engl J Med.* 2006;354:2250–61.
135. Wang X, Bank RA, Tekoppele JM, Agrawal CM. The role of collagen in determining bone mechanical properties. *J Orthop Res.* Wiley Subscription Services, Inc., A Wiley Company. 2001;19:1021–6.
136. Bailey AJ, Sims TJ, Knott L. Phenotypic expression of osteoblast collagen in osteoarthritic bone: production of type I homotrimer. *Int J Biochem Cell Biol.* 2002;34:176–82.
137. Chang SW, Shefelbine SJ, Buehler MJ. Structural and mechanical differences between collagen homo- and heterotrimers: relevance for the molecular origin of brittle bone disease. *Biophys J.* 2012;102:640–8.
138. Carriero A, Zimmermann EA, Paluszny A, Tang SY, Bale H, Busse B, et al. How tough is brittle Bone? Investigating osteogenesis imperfecta in mouse Bone. *J Bone Miner Res.* 2014;29:1392–401.
139. Couchourel D, Aubry I, Delalandre A, Lavigne M, Martel-Pelletier J, Pelletier JP, et al. Altered mineralization of human osteoarthritic osteoblasts is attributable to abnormal type I collagen production. *Arthritis Rheum.* 2009;60:1438–50.
140. Hopwood B, Tsykin A, Findlay DM, Fazzalari NL. Microarray gene expression profiling of osteoarthritic bone suggests altered bone remodelling, WNT and transforming growth factor- β /bone morphogenetic protein signalling. *Arthritis Res Ther.* 2007;9.
141. Massicotte F, Lajeunesse D, Benderdour M, Pelletier JP, Hilal G, Duval N, et al. Can altered production of interleukin-1 β , interleukin-6, transforming growth factor- β and prostaglandin E2 by isolated human subchondral osteoblasts identify two subgroups of osteoarthritic patients. *Osteoarthr Cartil.* 2002;10(6):491–500.
142. Chan TF, Couchourel D, Abed A, Delalandre A, Duval N, Lajeunesse D. Elevated Dickkopf-2 levels contribute to the abnormal phenotype of human osteoarthritic osteoblasts. *J Bone Miner Res.* 2011;26:1399–410.
143. Li B, Aspden RM. Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis. *Ann Rheum Dis.* 1997;56:247 LP–254.
144. Li B, Aspden RM. Composition and mechanical properties of cancellous bone from the femoral head of patients with osteoporosis or osteoarthritis. *J Bone Miner Res Wiley Online Library.* 1997;12:641–51.
145. Ferguson VL, Bushby AJ, Boyde A. Nanomechanical properties and mineral concentration in articular calcified cartilage and subchondral bone. *J Anat.* 2003;203:191–202.
146. Li B, Aspden RM. Material properties of bone from the femoral neck and calcar femorale of patients with osteoporosis or osteoarthritis. *Osteoporos Int.* 1997;7:450–6.
147. Cox LGE, van Donkelaar CC, van Rietbergen B, Emans PJ, Ito K. Decreased bone tissue mineralization can partly explain subchondral sclerosis observed in osteoarthritis. *Bone.* 2012;50:1152–61.
148. Zuo Q, Lu S, Du Z, Friis T, Yao J, Crawford R, et al. Characterization of nano-structural and nano-mechanical properties of osteoarthritic subchondral bone. *BMC Musculoskelet Disord.* 2016;17.
149. Hardcastle SA, Dieppe P, Gregson CL, Davey Smith G, Tobias JH. Osteoarthritis and bone mineral density: are strong bones bad for joints? *Bonekey Rep.* 2015;4.

150. Stewart A, Black A, Robins SP, Reid DM. Bone density and bone turnover in patients with osteoarthritis and osteoporosis. *J Rheumatol*. 1999;26:622–6.
151. Dequeker J, Mokassa L, Aerssens J, Boonen S. Bone density and local growth factors in generalized osteoarthritis. *Microsc Res Tech*. 1997;37(4):358–71.
152. Burger H, Van Daele PLA, Odding E, Valkenburg HA, Hofman A, Grobbee DE, et al. Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. *Arthritis Rheum*. 1996;39(1):81–6.
153. Goker B, Sumner DR, Hurwitz DE, Block JA. Bone mineral density varies as a function of the rate of joint space narrowing in the hip. *J Rheumatol*. 2000;27:735–8.
154. Stewart A, Black AJ. Bone mineral density in osteoarthritis. *Curr Opin Rheumatol*. 2000;12(5):464–7.
155. Day JS, Van Der Linden JC, Bank RA, Ding M, Hvid I, Sumner DR, et al. Adaptation of subchondral bone in osteoarthritis. *Biorheology*. 2004;41:359–68.
156. Day JS, Ding M, Van Der Linden JC, Hvid I, Sumner DR, Weinans H. A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritis cartilage damage. *J Orthop Res*. 2001;19:914–8.
157. Hargrave-Thomas E, van Sloun F, Dickinson M, Broom N, Thambyah A. Multi-scalar mechanical testing of the calcified cartilage and subchondral bone comparing healthy vs early degenerative states. *Osteoarthr Cartil*. 2015;23:1755–62.
158. Pragnère S, Boulocher C, Pollet O, Bosser C, Levillain A, Cruel M, et al. Mechanical alterations of the bone-cartilage unit in a rabbit model of early osteoarthrosis. *J Mech Behav Biomed Mater*. 2018;83:1–8.
159. Currey JD. Effects of differences in mineralization on the mechanical properties of bone. *Philos Trans R Soc Lond Ser B Biol Sci*. 1984;304:509–18.
160. Currey JD. How well are bones designed to resist fracture? *J Bone Miner Res*. 2003;18:591–8.
161. Radin EL, Paul IL, Tolkoﬀ MJ. Subchondral bone changes in patients with early degenerative joint disease. *Arthritis Rheum*. 1970;13:400–5.
162. Radin EL, Rose RM. Role of subchondral bone in the initiation and progression of cartilage damage. *Clin Orthop Relat Res*. 1986;34–40.
163. Deligianni DD, Missirlis YF, Tanner KE, Bonfield W. Mechanical behaviour of trabecular bone of the human femoral head in females. *J Mater Sci Mater Med Springer*. 1991;2:168–75.
164. Brown SJ, Pollintine P, Powell DE, Davie MWJ, Sharp CA. Regional differences in mechanical and material properties of femoral head cancellous bone in health and osteoarthritis. *Calcif Tissue Int*. 2002;71:227–34.
165. Ding M, Danielsen CC, Hvid I. Bone density does not reflect mechanical properties in early-stage arthrosis. *Acta Orthop Scand*. 2001;72:181–5.
166. Findlay DM, Kuliwaba JS. Bone-cartilage crosstalk: a conversation for understanding osteoarthritis. *Bone Res*. 2016;4.
167. Yuan XL, Meng HY, Wang YC, Peng J, Guo QY, Wang AY, et al. Bone-cartilage interface crosstalk in osteoarthritis: potential pathways and future therapeutic strategies. *Osteoarthr Cartil*. 2014;22:1077–89.
168. Goldring SR, Goldring MB. Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage bone crosstalk. *Nat Rev Rheumatol*. 2016;12:632–44.
169. Pan J, Zhou X, Li W, Novotny JE, Doty SB, Wang L. In situ measurement of transport between subchondral bone and articular cartilage. *J Orthop Res*. 2009;27:1347–52.
170. Arkill KP, Winlove CP. Solute transport in the deep and calcified zones of articular cartilage. *Osteoarthr Cartil*. 2008;16:708–14.
171. Imhof H, Sulzbacher I, Grampp S, Czerny C, Youssefzadeh S, Kainberger F. Subchondral bone and cartilage disease: a rediscovered functional unit. *Investig Radiol*. 2000;35:581–8.
172. Hwang J, Bae WC, Shieu W, Lewis CW, Bugbee WD, Sah RL. Increased hydraulic conductance of human articular cartilage and subchondral bone plate with progression of osteoarthritis. *Arthritis Rheum*. 2008;58:3831–42.
173. Iijima H, Aoyama T, Tajino J, Ito A, Nagai M, Yamaguchi S, et al. Subchondral plate porosity colocalizes with the point of mechanical load during ambulation in a rat knee model of post-traumatic osteoarthritis. *Osteoarthr Cartil*. 2016;24:354–63.
174. Mapp PI, Walsh DA. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. *Nat Rev Rheumatol*. 2012;8:390–8.
175. Sanchez C, Deberg MA, Piccardi N, Msika P, Reginster JYL, Henrotin YE. Subchondral bone osteoblasts induce phenotypic changes in human osteoarthritic chondrocytes. *Osteoarthr Cartil*. 2005;13(11):988–97.
176. Priam S, Bougault C, Houard X, Gosset M, Salvat C, Berenbaum F, et al. Identification of soluble 14-3-3 ϵ as a novel subchondral bone mediator involved in cartilage degradation in osteoarthritis. *Arthritis Rheum*. 2013;65:1831–42.
177. van der Kraan PM, Blaney Davidson EN, Blom A, van den Berg WB. TGF- β signaling in chondrocyte terminal differentiation and osteoarthritis. Modulation and integration of signaling pathways through receptor-Smads. *Osteoarthr Cartil*. 2009;17:1539–45.
178. van der Kraan PM, Blaney Davidson EN, van den Berg WB. A role for age-related changes in TGF β signaling in aberrant chondrocyte differentiation and osteoarthritis. *Arthritis Res Ther*. 2010;12.
179. Van Der Kraan PM. The changing role of TGF β in healthy, ageing and osteoarthritic joints. *Nat. Rev. Rheumatol*. 2017;155–63.
180. Scharstuhl A, Glansbeek HL, van Beuningen HM, Vitters EL, van der Kraan PM, van den Berg WB. Inhibition of endogenous TGF- β during experimental osteoarthritis prevents osteophyte formation and impairs cartilage repair. *J Immunol*. 2002;169:507–14.
181. Oh H, Chun CH, Chun JS. Dkk-1 expression in chondrocytes inhibits experimental osteoarthritic cartilage destruction in mice. *Arthritis Rheum*. 2012;64:2568–78.
182. Chan BY, Fuller ES, Russell AK, Smith SM, Smith MM, Jackson MT, et al. Increased chondrocyte sclerostin may protect against cartilage degradation in osteoarthritis. *Osteoarthr Cartil*. 2011;19:874–85.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.