Importance of subchondral bone in the pathogenesis and management of osteoarthritis from bench to bed

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Introduction

Osteoarthritis (OA) is the most prevalent degenerative joint disorder, mainly afflicting the weight-bearing joints such as knees and hips. The prevalence of knee OA is approximately 6% of the general population with a prediction of 67 million people in the USA and 100 million people in China by 2020 [1]. It is the leading cause of pain, physical disability, and poor quality of life in older adults. Patients at the end stage of OA have to receive arthroplasty surgery, a major operation with significant morbidities and complications [2]. The clinical demand on surgeries has increased rapidly in the past 10 years and the average waiting time for arthroplasty in Queen Mary Hospital of Hong Kong is now more than 3 years [2]. The direct cost of surgery is approximately 70,000 HKD/case per year [3]. OA has caused a heavy socioeconomic burden in Hong Kong [3,4]. At present, therapies for early OA are limited to symptom relief such as...
analgesic or joint lubricants [5]. An in-depth understanding of the pathomechanism and pathophysiology of OA is essential to develop disease-modifying therapies.

The aetiology of OA is multifactorial. A number of risk factors have been identified in the pathogenesis of OA such as ageing, being postmenopausal, obesity, genetic predisposition, and previous joint injuries [6]. Current therapies for OA do not target the cause of OA, but aim at preventing or reversing the structural deterioration of a joint. The hallmark of OA is loss of articular cartilage, which cushions the joint during movement. Yet its homeostasis and integrity relies on the biochemical and biomechanical interplay with subchondral bone and other joint tissues [7]. Subchondral bone provides the mechanical support and nutrition supply for overlying articular cartilage, and it undergoes constant adaptation in response to changes of the biomechanical environment in the weight-bearing knee joint such as excessive body weight, weakening muscles with ageing, or joint instability after previous ligament injury [8]. Disturbances of subchondral bone such as osteoporosis, subchondral bone cyst (SBC), and sclerotic changes are major radiological features of OA. There is mounting evidence recently suggesting the potential of some bone active agents such as alendronate and strontium in the treatment of OA not only in animal models but also in human models [9,10].

Hence, the aim of this review is to summarise the current understanding of and consensus on the role of subchondral bone disturbance in the pathogenesis of OA in both clinical and preclinical settings. The advantages and limitations of various animal models to mimic the clinical features of OA will be discussed to facilitate the knowledge transfer from bench to bedside.

Methods, applications, and limitations of animal models to mimic cartilage degeneration

Loss of articular cartilage is a primary concern in OA. It is macroscopically characterised as surface fibrillation, surface discontinuity, vertical fissures, erosion, denudation, or deformation of cartilage and dropout of articular chondrocytes. The collagen meshwork of articular cartilage becomes stiffened as a result of a gradual loss of water content and proteoglycan with the ageing process prior to the onset of OA [11,12]. Such collagen fibrils are susceptible to damage under physiological mechanical loading in daily activities. As a consequence, it will trigger the cascade of inflammatory reaction and activate the reparative process. A number of inflammatory mediators [complement component 5 (C5); interleukin-1 (IL-1) or IL-6] [13,14], downstream signalling [hypoxia-inducible factor-2α (HIF-2α)] [15–17] and proteinase [matrix metalloproteinase 13 (MMP13); and A disintegrin and metalloproteinase with thrombospondin motifs-5 (ADAMTS-5)] [18–21], have been identified in the destructive process of articular cartilage in OA. Other factors that govern the metabolism of articular chondrocytes such as parathyroid hormone-related protein (PTHrP), Indian hedgehog (IHH), and transforming growth factor-β1 (TGF-β1) signalling pathways have also been explored in the pathomechanism of OA [22–24]. PTHrP and IHH signalling are known as the key regulators in the control of proliferation and hypertrophic differentiation of chondrocytes in the growth plate during skeletal development [25–27]. TGF-β1 is another important signalling protein to play a part in the maintenance of the integrity of articular cartilage through repressing hypertrophic changes of articular chondrocytes [24]. The altered activin receptor-like kinase 1/5 (ALK1/ALK5) ratio with the activated TGF-β1 signalling pathway could induce the overexpression of type X collagen and MMP13 of articular chondrocytes, and trigger the initiation of OA [28].

Currently, there are a few experimental approaches to mimic the destructive process of articular cartilage, including intra-articular injection of enzymes [17,29–33], physical immobilisation [34], surgical destabilisation [22,35], and transgenic modification [14,19,23,35,36]. The intra-articular injection of reagents for OA induction includes papain Refs. [33], idoacetate [29], and collagenase [17,30–32]. These are able to create an acute model to investigate acute cartilage degradation and significant joint pain in animals. However, they have obvious limitations as a model for OA. Intra-articular injection of chemicals usually induces significant inflammatory reactions and extensive and rapid chondrocyte death. It contradicts the features of human OA, such as the chronic course of disease, low level of inflammation, co-occurrence of chondrocyte death and cloning, and prominent subchondral bone changes.

Physical immobilisation is another approach to induce OA in animals, for example, rat, rabbit, and dog [34]. An understanding of the alterations in articular cartilage following short- and long-term immobilisation is useful for the optimisation of rehabilitation protocols for patients. It was reported that the overall thickness of articular cartilage in the knee decreases up to 9% after 11 weeks of immobilisation and the deformation rate under a test load increases up to 42%. However, the atrophy and necrosis of articular chondrocytes occurred instead of displaying chondrocyte cloning in OA cartilage. Studies investigating subchondral bone changes and its relation with overlying cartilage degradation in immobilised joints remain lacking.

Surgical destabilisation of joints is the most common procedure for OA induction [14,17,19,22,23,35,37]. Anterior cruciate ligament transection (ACLT), destabilisation of medial meniscus (DMM), or combined surgeries has been widely used. It has been demonstrated that the severity and progression of OA-like changes is dependent on the grade of joint instability induced by the types of surgeries [38]. For example, ACLT induces a relatively mild OA model showing only partial cartilage destruction at 8 weeks after surgery, following significant subchondral bone damage in a mouse model [35]. By contrast, the DMM-induced OA model is prone to develop significant cartilage destruction with subsequent osteophyte formation [15–17]. Studies on the degeneration of articular cartilage commonly employed the DMM approach rather than the ACLT model.

Transgenic mouse models have also been used in OA studies. It was reported that the knockout of these structural proteins in articular cartilage, for example, collagen type IX, results in the breakdown of the extracellular matrix of hyaline cartilage [39]. Similarly, defective collagen type I led to structural changes of subchondral bone and subsequent degradation of articular cartilage in mice [40].
In addition, the genetic depletion of signalling molecules or proteinases such as Smad3 [41], HIF-2α [15–17], ADAMTS-5, and MMP13 [18–21], altering the metabolism of articular cartilage, is not surprising. The aetiology of OA is multifactorial, not simply a genetically determinant disorder, although genetic contributions to the pathogenesis of OA do indeed exist, for example, mutation or genetic variation (e.g., SMA3) [42–44]. Abnormalities in transgenic mouse models include problems of growth, not of decay. Transgenic mouse models should not be used to recapitulate OA but can be employed to prove the concept about a certain cellular or molecular mechanism in the regulation of cartilage bone metabolism.

Although significant progress has been made in the understanding of the mechanism of cartilage degeneration, few structure-modifying therapies with real clinical impact are being developed to attenuate or reverse the progressive deterioration of articular cartilage in OA [22,37]. It has been recognised that OA is not solely a problem of cartilage damage but also a whole joint disorder [7,45]. In particular, articular cartilage and subchondral bone function as a unit to maintain the structural and functional integrity of the joint [7]. Thus, animal models to recapitulate the disturbance of subchondral bone as well as cartilage degeneration are needed to better understand the cartilage bone unit in the pathogenesis of OA. Here, we will continue to discuss the ACLT surgical-induced and spontaneously occurring OA animal models with prominent subchondral bone changes, respectively.

**Pros and cons of ACLT model to recapitulate subchondral bone disturbance**

The importance of subchondral bone disturbance in the pathomechanism and pathophysiology of OA has been recognised a long time ago when people named it as "osteo"-arthritis. There is mounting evidence showing correlations between radiological findings of subchondral bone disturbance and clinical symptoms of OA. For example, bone marrow lesions (BMLs) are a major radiological finding at the early stage of OA under magnetic resonance imaging (MRI) examination. BMLs are an oedema-like lesion, which was further characterised as sclerotic but less mineralised bone [46]. It was reported that SBCs develop in the same region as BMLs [47,48]. The presence of SBCs, in conjunction with BMLs, is associated with the severity of pain Refs. [49–51]. They are able to predict tibial cartilage volume loss and risk of joint replacement surgery in knee OA patients [52–55]. The occurrence of subchondral cysts may lead to the increase of osseous pressure [56,57], which in turn results in sclerotic changes of bone surrounding cystic lesions according to Wolff’s law. The exact mechanism underlying subchondral bone pathologies and its relation with the progression of OA remains largely unknown, although the aforementioned associations have been identified [58].

Attempts have been made to investigate the nature of BMLs in OA. Subchondral BMLs were initially linked with perfusion abnormalities such as increased vascular permeability and ischaemia [59,60]. Impaired vascular supplies also affect subchondral bone metabolism and subsequently OA [61,62]. BMLs from knee OA were once characterised by sclerotic bone tissue that was less mineralised [46], which indicates subchondral osteoblast dysfunction in OA with overproduction of collagen type I but poor mineralisation [63]. Defective subchondral osteoblasts produced more inflammatory cytokines and mediators, such as IL-1β, IL-6, IL-8, and TGF-β1 [64]. In addition, the defective subchondral osteoblasts from OA patients could alter the phenotype of normal articular chondrocytes towards hypertrophic differentiation by decreasing expression of PTHrP, reducing production of aggrecan, and upregulating MMP13, a major enzyme in decaying the cartilaginous matrix [64–66]. Factors that govern subchondral perfusion abnormalities such as BMLs could be candidate therapeutic targets to treat OA. To the best of our knowledge, there are few reports that have identified BMLs in OA animal models until recently, and this was done in an ACLT mouse model [35].

Rupture of the anterior cruciate ligament (ACL) increases the risk of knee OA, as approximately 20–35% of these patients are estimated to have had an incidental ACL tear [67,68]. The ACLT model is a clinical relevant model to mimic OA secondary to ligament injuries. In response to joint instability after ligament injury, subchondral bone, articular cartilage, and other joint tissues undergo a dramatic remodelling process and adaptive changes [8]. Subchondral bone remodelling occurred with angiogenesis prior to microscopic changes in articular cartilage on the posterior tibia in the ACLT mouse model (Fig. 1) [35]. Importantly, the ACLT model has been reported to present all types of radiological features similar to human OA, such as subchondral BMLs [35] and cystic formation [69], in addition to osteoarthropathy and sclerosis. Overall, the ACLT model will fulfill the needs for preclinical studies with subchondral bone disturbance as a target of structure-modifying therapies for OA. Yet several issues should be taken into consideration in establishing an ACLT model.

First, the skeletal maturation and anatomic characteristics of animals should be considered in creating the models [70]. The age for skeletal of mouse is 10–12 weeks, whereas it is 9 months in rabbits and 2 years for sheep, respectively. ACL injuries usually afflict young adults, and the comparable age in mouse equals 3–6 months old, which is recommended for model establishment. In addition, outcomes of the ACLT model also vary in different species, for example, mouse, rat, and rabbit. Damage of the articular cartilage usually develops on the posterior site in the medial compartment of the mouse or rat knee joint, yet it is present on the lateral side of the knee joints in rabbits after ACLT surgery. Hence, mouse and rat are preferred for the ACLT model compared with rabbit.

Second, the gender of animals should also be considered. It was reported that the severity of OA was markedly higher in males than females after destabilisation surgery [71]. Ovariectomy (OVX) mice experienced less OA than intact males, whereas females after OVX developed significantly more severe OA than control females [71]. It suggests that sex hormones play a critical role in the progression of OA. Ovarian hormones may decrease the severity of OA in female mice. Male hormones, such as testosterone, exacerbate OA in male mice. Female mice might be chosen to induce a relatively slower course of OA development.
The ACLT model has been widely used for biomarker hunting [72] and therapeutic efficacy studies for OA [73–78]. However, we still need to be aware of the major limitations of the ACLT model. First, the ACLT model should mimic the secondary OA with a previous history of joint injury. Yet the majority of human OA is multifactorial and occurs spontaneously. Moreover, joint instability is not a sole pathomechanism in the destruction of the joint. The inflammation post-surgery (injury) also plays an essential role in the pathogenesis of OA after ACL injury. We should bear this in mind when we interpret data collected from the ACLT model.

Values and drawbacks of spontaneous occurring OA models

OA is an age-related disease and develops spontaneously without an exact cause(s). Such naturally occurring OA has also been reported in some animals, for example, the Dunkin–Hartley strain guinea pig and STR/ORT strain mouse. For example, the natural history of Dunkin–Hartley guinea pigs has been documented in the literature (Fig. 2). The naturally occurring OA animal models indeed resemble the pathophysiological process of human OA in some aspects. First, it develops in a chronic course similar to human OA. Second, subchondral bone disturbance has been identified as a key element with the progression of OA as well as overlying cartilage degeneration [59,79–82]. Third, multiple risk factors, such as ageing, sex hormone, obesity, impaired blood supply, and ligament laxity have been found in association with the progression of disease in Dunkin–Hartley guinea pigs [59,79,81]. Last but not least, the relatively larger size of the animals favours the analysis of advanced imaging modalities such as MRI, which might be easily linked with radiological findings in human OA [59,60].

Guinea pigs are more closely related to porcupines and chinchillas rather than to mice and rats; unlike most other rodents they have no tail. “The laboratory guinea pig was derived from domesticated stocks of wild guinea pigs originated in Peru, South America. Many of the laboratory strains used today originated with the breeding work by Dunkin and Hartley since 1926. The Dunkin–Hartley and Hartley stocks remain the major outbred stocks of guinea pigs used in the laboratory today. Sewell Wright also developed a number of the inbred strains of guinea pig for genetic studies. Of the original 20 or so inbred strains of guinea pigs, only two remain today, Strains 2 and 13” (http://www.nus.edu.sg/iacuc/files/The%20Laboratory%20Guinea%20Pig.pdf). These inbred guinea pigs are now available in some research institutes in the UK and USA. A number of laboratory studies have confirmed the occurrence of spontaneous knee OA in albino Dunkin–Hartley guinea pigs [59,79–85]. There is no report, to date, on the development of knee OA in pigmented (two-colour or three-colour) guinea pigs, which are also available for laboratory use as the control group for albino pigs in mainland China [86]. In the literature, the inbred Strains 2 and 13 guinea pigs are regarded as the OA-resistant model, which were used as the control group for albino Dunkin–Hartley guinea pigs [79,80,82,85].

Compared with the ACLT model, OA-like changes progressed slowly in Dunkin–Hartley guinea pigs (Fig. 3). It was observed that subchondral trabecular bone underwent significant remodelling with a thickening of calcified cartilage in the 6-month-old guinea pigs in comparison with the 3-month-old pigs. Further, the number of articular chondrocytes firstly increased along with subchondral bone remodelling, followed by a dropout with the 12-month-old pigs with structural deterioration. Such temporal changes of articular chondrocytes closely resembled our observations in human OA specimens (Fig. 4).

As previously reported, damage was initially present in ~5-month-old guinea pigs [82,85,86]. Regarding their lifespan (5–8 years) and skeletal maturation (at 6 months old), it should be considered as an early onset of OA. Genetic and developmental factors should be prioritised as possible causes of OA in guinea pigs. However, the genome information of guinea pigs remains lacking and there are few molecular biology tools available for the in-depth understanding of its underlying pathomechanism.

**Figure 1** Subchondral bone changes in the anterior cruciate ligament transection (ACLT) mouse model. In comparison to pre-operation (A–D), significant bone loss (E–H) was present with increased angiogenesis (G) prior to loss of proteoglycan in articular cartilage at 1 month after surgery. Uncoupled bone resorption with formation led to abnormalities at the subchondral area in the ACLT model (H). (A,B,E,F: Safranin O staining; C,G: Goldner’s trichrome staining; and D,H: micro-computed tomography images of tibial plateau of mice joints.)
Integrated approaches for functional and structural assessments of OA animal models

The Mankin score is the classic histopathological evaluation tool for grading the severity of OA. Recently, more detailed grading systems have been proposed and recommended by the Osteoarthritis Research Society International (OARSI) for different species of animal models [87–93]. As aforementioned, some radiological features of subchondral bone disturbance in human OA can be reproduced in animal models, for example, the ACLT model or guinea pig model. For example, MRI is a powerful tool to detect the problem in subchondral perfusion and cystic lesion formation [59,69,85]. Imaging subchondral bone disturbance in small animals such as mouse is technically challenging. The 7-T or 11-T MRI modalities and longer scanning time are often necessary to achieve better single-to-noise ratio with higher image resolution of the mouse joint. Micro-computed tomography is another imaging modality to visualise and quantify subchondral bone mass and micro-structure ex vivo or in vivo in OA animal models [10]. It was once used for cartilage imaging with contrast enhancement [94]. In addition to routine bone parameters, the porosity of the subchondral plate is an emerging indicator in the early stage of OA in the ACLT mouse model [10].

Functional assessments are also challenging issues in OA animal models. Pain assessment in animal models usually relies on analysis of gait behaviour. However, the sensitivity and specificity of gait analyses are questionable in the laboratory setting and their results also need more

Figure 2  Natural history of spontaneously occurring osteoarthritis in Dunkin–Hartley guinea pigs.

Figure 3  Temporal changes of knee osteoarthritis in the Dunkin–Hartley guinea pig model (A,A’: 3-month-old; B,B’: 6-month-old; and C,C’: 12-month-old). The subchondral trabecular bone underwent significant loss with a thickening of calcified cartilage in the 6-month-old guinea pigs. Meanwhile, the chondrocyte number dramatically increased along with subchondral bone remodelling. Later, the dropout of chondrocytes was observed in the 12-month-old guinea pigs with structural deterioration, characterised by articular surface disruption.
validation. Atomic force microscopy and nanoindentation is an emerging nanomechanical tool for early detection of nanoscopic changes of articular cartilage at the onset of OA [11,12].

Potential use of bone drugs as OA therapies

Subchondral bone undergoes constant remodelling in response to mechanical loading of weight-bearing joints during life. A complete cycle of bone remodelling comprises osteoclast-mediated bone resorption and osteoblast-mediated bone formation [95]. In brief, bone resorption will release and reactivate a number of cytokines embedded in the bone matrix such as TGF-β1 and insulin-like growth factor 1 [73,96]; it will be followed by the recruitment of mesenchymal stem cells (MSCs) from bone marrow to resorption pits and then on to differentiate into osteoblasts for bone formation [97].

The key radiological features of OA such as BMLs (sclerotic bone but less mineralised) [46] and cyst formation [98] indicate the presence of uncoupled subchondral bone remodelling. The previously assumed primary osteoblastic dysfunction [63,64,99] is highly likely a result of uncoupled bone remodelling. Therefore, factors that govern the cycle of bone remodelling could be candidates of OA therapies in order to break the vicious cycle of uncoupled bone remodelling. Inhibition of osteoclastogenesis and osteoclast-mediated bone resorption has been proven to be an effective approach to alleviate subchondral bone disturbance and the severity of OA [9,100–106]. Alendronate, having two phosphonate (PO₃) groups and a similar structure to pyrophosphate [107], can inhibit subchondral bone loss by encouraging osteoclasts to undergo apoptosis, at the early stage of OA [10,104–106]. Moreover, cathepsin K is responsible for the degradation of type I collagen in osteoclast-mediated bone resorption. Inhibitors of cathepsin K were also able to treat OA in a preclinical setting [108,109]. The OPG/RANKL system plays a pivotal role in osteoclastogenesis, which has also been implicated in the pathogenesis of OA [110]. Strontium can modulate the OPG/RANKL ratio and potentially serve as a disease-modifying drug for OA [9,100–102]. These drugs were usually used for osteoporosis treatment in an attempt to correct uncoupled bone remodelling and to restore the balance between bone resorption and new bone formation. Both osteoporosis and osteoarthritis share the common feature of uncoupled bone remodelling. However, osteoporosis shows progressive bone loss, whereas osteoarthritis presents transient bone loss followed by uncontrolled bone formation. Thus, anti-osteoporosis drugs could be used to treat OA at the phase of bone loss during disease progression.

The process of MSC recruitment and differentiation could be another target to maintain the balance of subchondral bone remodelling. It was reported that the large-scale CD271⁺ mesenchymal progenitors, having enhanced chondrogenic and osteogenic potentials [111,112], have been identified in subchondral bone in human OA [113]. Such progenitors from subchondral bone have also been isolated from OA cartilage [114,115]. TGF-β1 is a known growth factor to recruit MSCs to couple bone resorption with formation [73]. Pharmaceutical or genetic regulation of TGF-β1 signalling in nestin⁺ MSCs could attenuate the severity of OA in an ACLT mouse model [35].

Biological relation of subchondral bone disturbance in OA with systemic metabolic disorders

Metabolic syndrome including hypertension and type 2 diabetes mellitus (T2DM) are frequently encountered comorbidities in elderly patients with knee OA. It has been reported that approximately 55% of knee OA patients over 65 years old have hypertension and 13% are T2DM [116]. Hence, recently, there is growing research interest regarding the role of metabolic syndrome in the pathomechanism of knee OA among elderly persons [117]. The intimate relation between knee OA and metabolic syndrome significantly contributes to the mortality of patients compared with the general population [93]. The walking disabilities of people with OA knees have been identified as a major risk factor for the death of elderly patients with T2DM and cardiovascular diseases [93]. Although atherosomatous vascular disease and diabetes were once speculated

Figure 4 Histopathological features of human osteoarthritis tibial plateau collected during total knee replacement. On the lateral tibial plateau (A), the number of articular chondrocytes were randomly scattered and filled the whole thickness of OA cartilage. By contrast, the number of chondrocytes dramatically decreased with disruption of hyaline cartilage on the medial tibial plateau (B) in the same patient.
in the pathogenesis of OA [61,62,118], the exact impact of these comorbidities, such as hypertension and T2DM, on the severity of knee OA and the progression of disease remains to be elucidated [119]. As previously reported, high blood pressure was associated with low bone mass and high risk of fractures in elderly persons [120–122]. In T2DM patients, bone quality was also reduced with high risk of fracture [123], yet bone mineral density increased in the lumbar spine, hip, and radius in patients [124]. There is a research gap whether hypertension and T2DM as comorbid diseases would interfere with subchondral bone remodelling and aggravate the progression of OA [117,125].

Concluding remarks

The ideal animal model should recapitulate pathological features of human OA. The purpose of animal studies in a preclinical setting usually include: (1) exploring the structure–function relationship in the pathophysiology of OA, (2) searching for biomarkers at an early stage of disease for diagnosis of OA, and (3) testing the efficacy of therapies in prevention and treatment of OA. However, none of the animal models can reproduce all the pathological features of OA. The choice of cartilage-centred or bone-centred animal models will inevitably bring a bias in laboratory data interpretation and affect knowledge transfer from bench to bedside. The in-depth understanding of subchondral bone disturbance in OA will lead to a paradigm shift in therapies for OA. The ACLT model and the Dunkin–Hartley albino guinea pig model are recommended in preclinical studies on subchondral bone pathologies in the search for biomarkers and drug discovery. Integrated assessment approaches with advanced imaging modalities should be adopted to provide more comprehensive and convincing data for translational OA research. In the near future, contributions of metabolic risk factor(s) to subchondral bone disturbance in OA should be investigated further.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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References


