Chapter 1
Pathogenesis of Osteoarthritis

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Key Points

- Osteoarthritis (OA) is one of the most chronic health disorders in the western world and becomes particularly common with advanced age. The joints most commonly affected by OA include the knees, hips, ankle, elbow, shoulder, hand, wrist and spine.
- Risk factors that may increase the risk of developing OA are age, gender, joint injury or overuse caused by physical labour or sports, obesity, and joint alignment etc.
- Symptoms of OA may appear well after disease onset. Such symptoms include joint pain, limitation of motion, stiffness after inactivity, tenderness, crepitus, and joint enlargement.
- While previously characterized as a disease of progressive articular cartilage degradation, OA pathophysiology involves all of the tissues that form the synovial joint which are the subchondral and metaphyseal bone, synovium, ligaments, joint capsules, and the muscles acting across the joint. Subchondral bone remodelling, osteophyte formation, synovial inflammation, ligamentous laxity (loose ligaments), and the weakening of periarticular muscles exemplify several joint structure alterations observed.

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- Chondrocytes, the only cell types present in the articular cartilage, are responsible for maintaining an equilibrium between the anabolic and catabolic activities in the extracellular matrix (ECM).
- The trigger of OA is unclear; however, it may begin with tissue damage from mechanical injury, infiltration of inflammatory mediators from the synovium into the cartilage, or defects in cartilage metabolism/homeostasis. Chondrocytes attempt to repair cartilage damage/degradation by increasing the production of ECM macromolecules. As degeneration continues, catabolic mechanisms overpower the anabolic capabilities of chondrocytes, and the homeostatic balance is tipped resulting in progressed cartilage breakdown.

Introduction

Osteoarthritis (OA) is a debilitating disease that involves all structures of the affected joint. It is one of the most common chronic health disorders in the western world; with a higher prevalence among the ageing population [1, 2]. The National Arthritis Data Workshop reported a rise in OA prevalence with an estimated 27 million US adults in 2005 having clinical OA of their hand, knee, or hip joint, an increase from 21 million in 1995 [3]. For a disease with such a strong age-related association, such an increase is likely with the ageing population. The incidence of OA was also seen to rise hand in hand with the escalation of obesity in the population. Obese women have nearly four times the risk of knee OA as compared with nonobese women; for obese men, the risk is nearly five times greater [4]. Hence, obesity has been established as a major risk factor for the development and progression of OA. Other risk factors include sex, race and ethnicity, genetics, nutrition, smoking, and injuries/trauma to the joint [1, 5–13]. If an individual has the genetic predisposition to develop OA, they may not develop it unless they have experienced insult to the joint or are accompanied by one or more of the other risk factors. The relative significance of certain risk factors may differ from joint to joint, for early versus end-stage OA, for development as opposed to progression of disease, and for radiographic versus symptomatic disease. Before describing the pathogenesis of the joint structure during OA, it is important to understand the nature and function of the joint structure under normal conditions. In this chapter, we discuss the composition of the joint, the interplay of the joint components to maintain homeostasis, and the disruption of the homeostatic mechanisms that drive the development of OA.

Articular Cartilage: Structure, Function, and Composition

While OA is characterized as a progressive loss of articular cartilage, joint degeneration involves all of the tissues that form the synovial joint which are the subchondral and metaphyseal bone, synovium, ligaments, joint capsules, and the

muscles acting across the joint [14]. Subchondral bone remodelling, osteophyte formation, synovial inflammation, ligamentous laxity (loose ligaments), and the weakening of periarticular muscles occur as a result of an imbalance in the equilibrium between the breakdown and repair of joint tissue [15]. Consequently, the affected individual experiences joint pain, stiffness, and limitation of movement. Without treatment, these symptoms slowly evolve to whole joint failure with pain and disability.

The primary functions of articular cartilage are to lubricate the surface of synovial joints allowing for painless, low-friction movement of the opposing joint surfaces and to facilitate the distribution of loads, thereby minimizing stress on the underlying subchondral bone [14].

Articular cartilage lacks blood vessels, nerves, and lymphatic vessels. Instead, it consists primarily of extracellular matrix (ECM) with sparsely distributed, highly specialized cells called chondrocytes [16]. The chondrocyte is the only cell type residing in the articular cartilage. The articular cartilage ECM is composed of tissue fluid and a framework of structural macromolecules (collagens, proteoglycans, and non-collagenous proteins and glycoproteins) synthesized by chondrocytes. Each chondrocyte is responsible for the establishment and maintenance of a specialized microenvironment in its surrounding area [17]. The interaction between the tissue fluid and the macromolecular framework helps retain water within the ECM, which is crucial to maintain its unique mechanical properties of stiffness and flexibility. The tissue fluid is 80 % of the wet weight of articular cartilage. It is essentially water but also contains gases, small proteins, metabolites, and a high concentration of cations to balance the negatively charged proteoglycans. About 30 % of the water exists within the intrafibrillar space within the collagen and appears to exist as a gel, while a small percentage is contained in the intracellular space. The rest is contained in the pore space of the matrix. In addition to providing lubrication, the flow of water through the cartilage and across the articular surface helps to transport and distribute nutrients to the chondrocytes.

Among the structural macromolecules of the ECM, collagen is the most abundant, contributing to about 60 % of the dry weight of articular cartilage. Specifically, type II collagen represents 90–95 % of the collagen in the ECM. Additional distinct collagen types I, IV, V, VI, IX, X, and XI contribute a minor proportion and serve to form and stabilize the type II collagen fibril network that intertwines with proteoglycan aggregates. The organization of this tight meshwork that extends throughout the tissue provides the tensile stiffness, cohesiveness, and strength of articular cartilage [18, 19].

The second-largest group of macromolecules in the ECM are proteoglycans. There are two major classes of proteoglycans: large aggregating molecules (aggrecans) and smaller proteoglycans (decorin, biglycan, and fibromodulin) [20]. Aggrecans interact with hyaluronic acid (also known as hyaluronan or HA) and link proteins to form large proteoglycan aggregates. This aggregation helps anchor proteoglycans within the matrix and provides the cartilage with its osmotic properties, which is essential to its role in resisting compressive loads [21–23]. Unlike aggrecans, the small nonaggregating proteoglycans do not contribute directly to the mechanical behaviour of articular cartilage. Decorin and fibromodulin are involved

in fibrillogenesis and interfibril interactions via their interactions with type II collagen fibrils. Biglycan is localized in the immediate surroundings of chondrocytes and may interact with type VI collagen [16, 24, 25].

The structural macromolecules and chondrocytes are organized in a highly ordered structure to form the articular cartilage. The composition, organization, cell morphology, and mechanical properties of the matrix vary between zones of the cartilage. Within each zone, matrix composition, organization, and function also vary with the distance from the chondrocyte – giving rise to the pericellular region, the territorial region, and the interterritorial region. The four zones from the articular surface to the subchondral bone are defined as the superficial zone, the transitional zone, the middle (radial or deep) zone, and the calcified cartilage zone [14, 19].

The superficial zone is in contact with the synovial fluid and is the thinnest articular cartilage zone. It contains a relatively high number of flattened chondrocytes as well as mostly type II and type IX collagen tightly packed and aligned parallel to the articular surface. This zone is important for the protection and maintenance of the deeper zones. Additionally, the densely packed collagen fibrils lying parallel to the joint surface give the cartilage its tensile stiffness and enable the cartilage to resist the sheer, tensile, and compressive forces generated during joint use [26].

With 40–60 % of the total cartilage volume, proteoglycans, and thicker collagen fibrils, the transitional zone is the first line of resistance to compressive forces. The transitional zone also provides an anatomic bridge between the superficial and deep zones. The collagen fibrils have the largest diameter and are arranged in a perpendicular fashion. Also, the deep zone contains the highest proteoglycan content and the lowest water concentration. These properties render the deep zone responsible for providing the greatest resistance to compressive forces [14, 19].

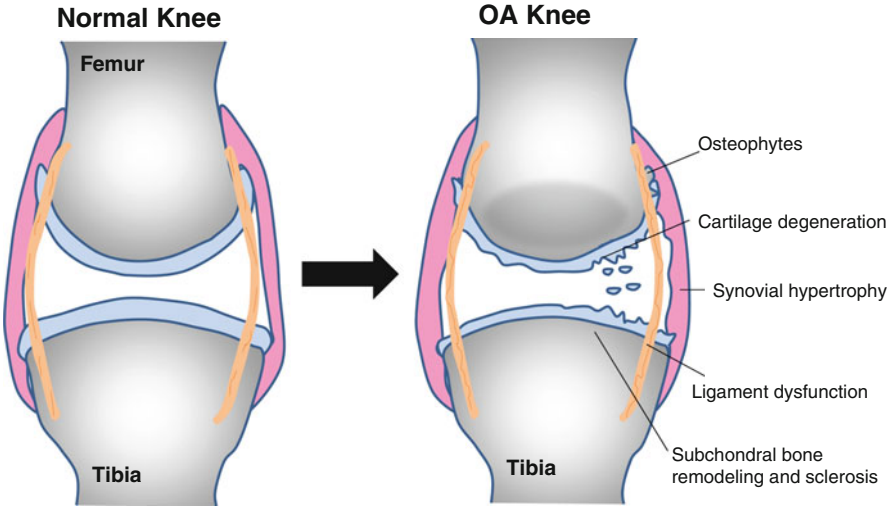

Fig. 1.1 Schematic of normal vs. osteoarthritic knee joint. OA is accompanied by considerable cartilage degradation, the generation of wear particles, thickening of synovium, subchondral bone alterations, and the growth of osteophytes at the margins of the joint

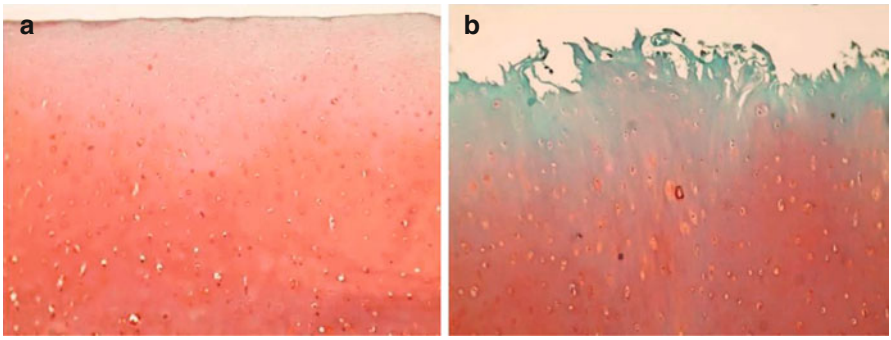

Fig. 1.2 Safranin-O staining of (**a**) normal and (**b**) OA human knee joint cartilage showing cartilage degradation and loss of proteoglycan

Finally, the 'tidemark', a dynamic structure that appears as a basophilic line in histological sections, separates the deep zone from the calcified cartilage. The calcified cartilage zone functions to secure the cartilage to the bone, by anchoring the collagen fibrils of the deep zone to the subchondral bone [27]. Additionally, calcified cartilage is permeable to small-molecule transport and plays an important role in the biochemical interaction between non-calcified cartilage and subchondral bone (Figs. 1.1 and 1.2) [28, 29].

Synovium

As mentioned in the beginning of this chapter, the characterization of OA not only involves the destruction of articular cartilage but also involves the integrity of multiple joint tissues [30]. Synovial joints include a joint cavity filled with synovial fluid, which is surrounded by articular cartilage and a fibrous capsule, including the inner lining synovium. The synovial fluid is in direct physical contact with the cartilage and synovium and exhibits biomechanical, metabolic, and regulatory functions [31, 32]. This physicality allows the synovial fluid to interact with and mediate interactions between synovial joint tissues. By providing boundary lubrication, the synovial fluid reduces friction and helps to protect and maintain the integrity of articular cartilage surfaces [32]. Two important molecules secreted by synovial lining cells and cells within the synovial joint space are the lubricant hyaluronan (HA) and proteoglycan 4 (PRG4, also known as lubricin and superficial zone protein (SZP)). HA contributes to the viscosity of synovial fluid and provides outflow buffering (the maintenance of synovial fluid volume by coupling between draining and input rates), while the mucinous glycoproteins, SZP and lubricin, mediate boundary lubrication of articular cartilage [33–36].

Cytokines and growth factors present in synovial fluid are important regulatory factors for cells within the synovium as well as chondrocytes in the cartilage [31]. According to their predominant tissue-specific effects, cytokines can be classified as

either proinflammatory or anti-inflammatory. Proinflammatory cytokines in synovial fluid include interleukin (IL)-1α, IL-1β, tumour necrosis factor-α (TNF-α), leukaemia inhibitory factor (LIF), IL-6, IL-8, IL-17, and IL-18 [37–40]. Anti-inflammatory cytokines in synovial fluid include IL-4, IL-10, and IL-13 [38]. Growth factors found in synovial fluid include transforming growth factor beta 1 (TGF-β1) and insulin growth factor (IGF-1) and have anabolic effects [41]. Most cytokines and growth factors are at relatively low concentrations in normal synovial fluid and are significantly elevated in joint injury and disease [31, 42]. Later in this chapter, we will discuss the role played by these cytokines in OA pathogenesis and acceleration of joint destruction.

Proteolytic enzymes mediate degradative processes in the synovial joint and are carefully regulated [43]. Matrix-degrading enzymes, such as matrix metalloproteinases (MMPs), are a group of Zn^{2+}-dependent extracellular enzymes that function in normal and pathological tissue remodelling [44]. MMPs are capable of degrading all of the components of the ECM. Depending on their substrate and domain structure, MMPs are classified into collagenases (MMP-1, MMP-8, MMP-13), gelatinases, stromelysins (MMP-3), and membrane-type MMPs [45]. MMPs are present in normal synovial fluid; however, their levels are elevated in joint injury and disease as evidenced by increased mRNA levels in tissue and elevated levels of proMMPs in synovial fluid [46–48]. MMPs are secreted primarily from chondrocytes as zymogens, or proMMPs, with propeptide domains that are cleaved during extracellular activation [49]. Similarly, requiring subsequent activation are disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) proteinases that degrade aggrecan [50–54]. Tissue inhibitors of metalloproteinases (TIMPs) and inhibitors of proteinases that activate proMMPs are also present. Thus, changes in the levels and activities of matrix-degrading enzymes, and their corresponding inhibitors and activators, alter anabolic and catabolic homeostasis in joint injury and disease [55, 56].

Synovial fluid is an ultrafiltrate of blood plasma and is relatively acellular compared to whole blood, containing less than 200 leukocytes per mm^3 compared to 3,540–9,060 per mm^3 in whole blood [57, 58]. Also present are lymphocytes, macrophages, and shed lining cells [59–61]. The synovium, or synovial membrane, is a vascularized, thin sheet of connective tissue with fibroblast-like (type B) cells and macrophage-like (type A) cells within an ECM composed predominantly of HA, collagen, and proteoglycans [31]. Molecular sieving by the synovial membrane matrix is size dependent, with lubricant molecules HA and PRG4 retained within the synovial joint, while low-molecular-weight species, such as metabolic substrates and by-products, cytokines, and growth factors, are not [62–64].

Subchondral Bone

For many years, OA was characterized as a primary disorder of articular cartilage; however, the discovery of the contribution of other joint tissues to the pathophysiology of OA has changed the definition of OA. Subchondral bone remodelling is

commonly associated with articular cartilage defects and subchondral sclerosis, along with progressive cartilage degradation, that are heavily involved in the pathogenesis of the disease [65, 66].

Subchondral bone refers to the bony lamella lying distal to calcified cartilage [67, 68]. The subchondral bone can be separated into the subchondral bone plate and subchondral trabecular bone [69]. The subchondral bone plate is rather porous and lies immediately beneath the calcified cartilage. It contains channels that provide a direct link between articular cartilage and subchondral trabecular bone [70]. Arterial and venous vessels penetrate through the channels and send tiny branches into calcified cartilage [67, 71]. Supporting trabeculae arise from the subchondral bone plate and make up the subchondral trabecular bone together with deeper bone structure [72]. Subchondral trabecular bone is more porous and metabolically more active than the subchondral bone plate, containing blood vessels, sensory nerves, and bone marrow. It has shock-absorbing as well as supportive functions and may also be important for cartilage nutrient supply and metabolism [68]. Subchondral bone is a very dynamic structure and is uniquely adapted to the mechanical forces imposed across the joint [68, 72]. Accordingly, mechanical stress modifies the contour and shape of subchondral bone by means of bone modelling and remodelling [73–75]. Similar to the 'tidemark', which separates the two dissimilar cartilage regions, there is also a sharp borderline between calcified cartilage and subchondral bone, called the 'cement line' [68]. Evidently, close contact exists between the deeper layer of non-calcified cartilage, the tidemark, calcified cartilage, the cement line, and subchondral bone – forming a closely composited functional unit called the 'osteochondral junction' [76]. The biomechanical and biochemical cross-talk across this region seems to play a role in maintenance and degeneration of the joint [77]. As we shall see, alterations of any of these components will modulate the properties and functions of other parts of the osteochondral junction.

Infrapatellar Fat Pad

One of the most commonly affected joints by OA is the knee [78, 79]. The presence of the infrapatellar fat pad (IFP), or Hoffa's fat pad, differentiates the knee joint from other articular joints [80]. The IFP is composed of a fibrous scaffold, on which fat tissue is embedded. Located underneath the patella, between the patellar tendon, femoral condyle, and tibial plateau, this intracapsular and extrasynovial adipose structure is in close contact with the articular cartilage, bone, and synovium [81–83]. Besides its role in facilitating the distribution of synovial fluid and absorbing forces through the knee joint, not much is known about how the IFP contributes to knee function [84]. Notably, earlier studies have shown that the IFP is preserved under extreme starvation conditions despite subcutaneous adipose tissue elimination [85, 86]. This suggests critical physiological importance for the presence of this fat depot in the knee.

The IFP contains large numbers of adipocytes, fibroblasts, macrophages, leukocytes, and other immune cells capable of producing inflammatory cytokines [84, 87, 88]. The presence of these cells indicates possible protective and/or damaging roles of adipose tissue in the inflammatory reactions in OA. Nociceptive nerve fibres are also present in the IFP. Substance P-positive nerves innervating the IFP, indicating that they are peptidergic C-fibres, are increased in the IFP of patients with chronic anterior knee pain [89, 90]. Hence, anterior knee pain, which is the most common symptom experienced by patients with knee OA, is thought to be associated with pathology of the IFP.

Cellular changes in the IFP during knee OA involves the infiltration of immune cells in the IFP, which contributes to disease progression by stimulating the production of numerous inflammatory mediators [85, 91]. Inflammatory cytokines may act to alter the sensitivity of the nerve fibres, lowering the threshold of the joint nociceptors, thus inducing and worsening pain [92]. The numbers of neutrophils, eosinophils, basophils, and monocytes were seen to be elevated in the IFPs from patients with knee OA [93]. Neutrophils produce cytokines such as IL-1, IL-8, and MMP-8, which contribute to cartilage breakdown and necrosis of adipose tissue [84, 93, 94]. Eosinophils and basophils release histamine, which increases the production of matrix-degrading enzymes and pro-inflammatory mediators in synovial fibroblasts and cartilage [95]. Lymphocytes have also been found in the IFP expressing Th1 cytokines, which can either degrade cartilage directly or activate macrophages through cell-cell interaction, to produce cartilage degrading mediators [96, 97]. Thus, inflammatory cells within the IFP may influence the inflammatory and destructive responses in knee OA.

While immune cells in the adipose tissue are responsible for the production and release of most inflammatory mediators, adipocytes are responsible for the secretion of the adipokines, such as leptin and adiponectin [87, 98, 99]. In OA cartilage, leptin stimulates IL-1β production, increases the effect of pro-inflammatory cytokines, and induces the expression of MMPs [100–103]. Leptin also contributes to inflammatory responses by facilitating the activation of macrophages, neutrophils, dendritic cells, natural killer cells, and T helper 1 (Th1) cells [104]. While adiponectin is known to act as a protective adipokine against obesity and vascular diseases [105], it is suggested to act as a pro-inflammatory agent in joint diseases, such as knee OA [106, 107]. Adiponectin induces MMP-1 and IL-6 production in synovial fibroblasts, which have adiponectin receptors [108]. These receptors are also present in normal or OA chondrocytes, since adiponectin-treated chondrocytes produce IL-6, MMP-3, MMP-9, and monocyte chemoattractant protein 1 (MCP1) [99, 107].

Alteration of Joint Homeostasis During OA

Chondrocytes are responsible for the development, maintenance, and repair of the ECM via degradative enzymes, MMPs (collagenase, gelatinase, and stromelysin), and cathepsins B and D [14]. As post-mitotic cells, chondrocytes have a low rate of replication resulting in a limited ability for articular cartilage to maintain and repair itself

[109]. Maintenance of the articular surface requires turnover of the matrix macromolecules, as well as alteration in the matrix macromolecular framework in response to joint use [14]. Although chondrocytes have low mitotic activity, they are still metabolically active. Their metabolic activity can be altered by changes in their surrounding mechanical as well as chemical environment [19]. While ECM protects chondrocytes from the potentially damaging biomechanical forces, it is the job of chondrocytes to sustain a homeostasis of ECM metabolism by sensing changes in matrix composition and then responding by degrading or synthesizing appropriate types and amounts of macromolecules. With age, the capacity of chondrocytes to synthesize certain proteoglycans, their proliferative capacity, and their response to anabolic stimuli including growth factors decrease [109]. As a result, the ability of chondrocytes to maintain and restore articular cartilage decreases, resulting in an increase in the risk of development and progression of articular cartilage degradation.

It is well established that the risk of developing OA increases dramatically with age; however, age is not the sole determinant of developing the disease [2, 5]. Genetic, environmental, metabolic, and biochemical factors or a combination of the above may lead to more severe outcomes [5]. Furthermore, inactivity of the joint may lead to accelerated cartilage degradation [110]. The progressive loss of articular cartilage is accompanied by alterations of the underlying subchondral bone, which include bone remodelling, sclerosis, and in many cases the presence of subchondral bone cysts and osteophytes [111]. The concomitant, albeit moderate inflammation observed in the synovial tissue introduces a clinical impact of synovitis to the initiation and/or progression of OA [112]. It is this inflammatory response that puts the '-itis' in osteoarthritis, previously known as osteoarthrosis [113]. Together, these structural changes combine forces to result in the symptoms: joint pain, restriction of motion, crepitus with motion, joint effusions, and deformity – as experienced by the affected individual [114].

The pathophysiological process of OA can be divided into three overlapping stages [115, 116]:

1. ECM network damage/alteration at a molecular level
2. Chondrocyte response to tissue damage
3. Failure to restore cartilage and progressive loss of tissue due to a decline of chondrocyte synthetic response

The early changes in joint degeneration are seen microscopically as localized fibrillation or disruption of the articular cartilage superficial zone [117, 118]. As the degeneration continues, the roughened and irregular articular surface forms clefts, and the fibrillation extends deeper throughout the cartilage zones until the fissures reach subchondral bone [119]. The superficial tips of the fibrillated cartilage eventually tear, decreasing the cartilage thickness and releasing free fragments into the joint space. When the products of cartilage breakdown come in contact with the synovium, synovial cells are activated and produce catabolic and pro-inflammatory mediators that can activate chondrocytes to produce MMPs, which result in further cartilage breakdown and an unforgiving vicious cycle ensues [120, 121].

Once cartilage degradation has initiated, synovial cells phagocytose the breakdown products released into the synovial fluid resulting in hypertrophy and hyper-

plasia of synoviocytes, accompanied by inflammatory cell infiltration of the tissue by mononuclear cells such as lymphocytes and macrophages [122]. On account of its association with an increased degree of inflammatory cell infiltration of the synovial tissue, an increased concentration of systemic high-sensitivity C-reactive protein (hsCRP) can be used as a predictor of rapid disease progression in early knee OA. hsCRP levels are also associated with level of pain, clinical severity, and disability [123–126]. Another molecule that shows distinct alterations in the initial stages of OA is cartilage oligomeric matrix protein (COMP) [127, 128]. In normal adult cartilage, COMP is primarily found some distance from articular cartilage chondrocytes, i.e. the interterritorial region. This protein plays a role in early stages of fibril formation to promote fibrillogenesis of collagens I and II, as well as cross bridging of the matrix collagen fibre network [129, 130]. However, during early OA, there is a characteristic change in the distribution pattern. A severe loss of COMP is observed from the interterritorial matrix through degradation accompanied by a new accumulation of the protein close to the cells, as a result of new synthesis [131]. Hence, altered distribution of COMP provides a distinct and characteristic hallmark of impaired cartilage during the early osteoarthritic process.

The involvement of the synovium in early OA can be seen histologically by changes that occur in the osteoarthritic synovial membrane in areas adjacent to sites of chondropathy [122]. However, the underlying molecular mechanisms during early OA are almost impossible to examine, since the disease is usually not diagnosed until the pronounced alterations lead to pain and radiographically detectable changes. For this reason, animal models of OA have been developed to help us examine the underlying biochemical and molecular processes leading to the histologically visible alterations [132–134].

ECM fragments, such as fibronectin and collagen type II fragments, may activate the innate immune response via pattern recognition receptors, which include membrane-associated Toll- like receptors (TLRs) [135, 136]. This is the first level of nonspecific immune system activation. TLRs are typically activated by microbial ligands during an infection, activating the immune system to elicit an appropriate response [136]. However, they can also be activated by pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs) occurring during cellular stress and ECM damage [137]. Therefore, this innate immune response has been regarded as a predominant feature in various non-infectious diseases where tissue injury and/or defective repair takes place. In this context, the disruption of matrix homeostasis that occurs in an osteoarthritic joint mirrors a chronic injury. There are ten functional mammalian TLR homologues (TLR-1 to TLR-10). Some are constitutively expressed by many cells including macrophages and can be induced or up-regulated on other cell types [136]. Previous studies have shown that there is up-regulated expression of TLR-2 and TLR-4 in articular chondrocytes of OA lesional cartilage compared to non-OA and nonlesional cartilage [138]. Furthermore, TLR-2 and TLR-4 ligands such as small-molecular-weight species of HA [139–141], fibronectin isoforms [142], tenascin C [143, 144], and biglycan [24, 145, 146] were found in high concentration in OA synovial fluid. TLR-2 and TLR-4 signals then mediate catabolic responses by

increasing MMP-3 and MMP-13 production, which result in cartilage degradation and the release of matrix components, which again activate TLRs to elicit further catabolic responses and hence, a self-perpetuating loop of cell activation [146, 147]. In the synovial membrane, TLR activation stimulates NF-κB activation and the subsequent production of chemokines (e.g. IL-8) and cytokines (e.g. IL-1β, IL-6, and TNF-α), which activate and promote cellular infiltration of macrophages, granulocytes, and lymphocytes [148]. As a result, the tightly regulated anabolic and catabolic processes responsible for the maintenance of cartilage homeostasis are disturbed due to the stimulation of inflammatory mechanisms and the release of cytokines. Therefore, TLR activation has been shown to have serious implications in promoting synovitis in OA [112].

In addition to the appearance of cartilage fibrillation microscopically, the matrix macromolecular framework is destabilized at the molecular and macromolecular level. Proteolytic degradation of proteoglycans, most pronounced in the superficial region, during early OA decreases the chain length of the proteoglycan, thus inhibiting the formation of macromolecular complexes and decreasing proteoglycan aggregation [149]. The breakdown of proteoglycan architecture, along with an increase in water content, leads to a more permeable matrix and reduces the compressive stiffness of the tissue [150, 151]. Taken together, these alterations may increase the vulnerability of the tissue to further mechanical damage.

Alterations of the subchondral bone accompany the degeneration of articular cartilage; however, whether these changes are a driving force or a consequence of articular cartilage breakdown still remains unclear [152, 153]. At early stages of OA, there is elevated bone remodelling, particularly in the areas underlying the regions of articular cartilage damage. Bone loss is also observed, notably in the subchondral bone plate resulting in reduced thickness of the subchondral bone plate and increased porosity [69, 154]. Further down in the subchondral trabecular bone, increased trabecular separation and deterioration and decreased bone volume fraction and trabecular thickness are detected in animal models of OA [68]. These subchondral bone changes cause alterations in joint shape and load transmission that may propagate further cartilage loss. Microdamage of calcified cartilage and subchondral bone is widely detected in osteoarthritic joints in the form of short interstitial cracks or microcracks [155]. Microcracks act as an initiator of the bone remodelling process, as well as provide a means of communication of catabolic agents across the osteochondral junction, i.e. between cartilage and subchondral bone [65, 68].

Depending on the type and location of joint affected, the growth of osteophytes is observed as another alteration that changes the structure of the subchondral bone during OA. These fibrous, cartilaginous, and bony protrusions may be marginal, capsular, or central with characteristic patterns of formation. Intraosseous lesions, termed subchondral bone cysts (SBCs), are also reported in patients with OA [156]. SBCs are composed of fibroconnective tissue that initially contain fluid but ossify with time; they present as well-defined lucent areas with sclerotic rims on radiographic images. The presence of osteophytes and SBCs can restrict motion and contribute to pain with joint movement [156].

As ECM degeneration continues and the chondrocytes' biomechanical environment is altered, mediators are released that stimulate the chondrocytes to elicit a

cellular repair response. This response consists of a boost of anabolic and proliferative activity, primarily in the upper cartilage zones, in an attempt to restore the homeostatic matrix environment [149]. Suggestive of a tissue repair response, type II collagen deposition increases in the deeper cartilage zones [157]. The mechanism of chondrocyte stimulation is unclear; however, it may be that the chondrocytes in these areas have better access to the anabolic and mitogenic growth factors from the synovial fluid due to fissuring or loosening of the macromolecular framework [158]. Anabolic cytokines such as TGF-β, IGF-I, fibroblast growth factors (FGF-2, FGF-4, and FGF-8), and bone morphogenetic proteins (BMPs) have an important role in stimulating the synthesis of ECM macromolecules (e.g. type II, VI, IX, XI collagen) [38, 159–161]. In addition, an increased expression of type I collagen, a main component of fibrous cartilage, is seen, which modifies the composition of the ECM and accordingly its properties [157]. Unlike normal chondrocytes, OA chondrocytes have up-regulated proliferative activity in response to cartilage damage [162]. In fact, the presence of clones of proliferating cells, or clusters, surrounding newly synthesized matrix molecules is one of the characteristic hallmarks of the chondrocytic repair response to cartilage degeneration [14].

Chondrocytes in such clusters have been shown to produce alkaline phosphatase, annexin II, annexin V, and type X collagen [163]. These molecules are normally expressed in hypertrophic and mineralizing growth plate cartilage, suggesting that the osteoarthritic chondrocytes are undergoing terminal differentiation [164, 165]. Particularly, they express transcription factors Sox9 and Runx2, which play a role in differentiation and hypertrophy, respectively. Sox9 controls the differentiation of mesenchymal stem cells (MSCs) into chondrocytes, whereas hypertrophic differentiation of chondrocytes depends on the expression of Runx2 and the inhibition of Sox9 expression [166–169]. Hence, during OA, chondrocytes are believed to re-establish the process of endochondral ossification, a physiological process during embryonic development whereby cartilage is replaced by bone to form long bones [170]. The hypertrophic chondrocytes produce type X collagen (typically found in the calcified cartilage zone and the hypertrophic zone of growth plate), which is involved in cartilage mineralization [171, 172]. Thus, mineralization followed by chondrocyte replacement with bone tissue and ossification takes place. As a result, subchondral bone architecture is altered and cartilage thickness is decreased. Thinning of the cartilage adds insult to injury since it is now even more prone to damage. This process could explain the duplication and advancement of the tidemark, which is reflective of progressive calcification of the cartilage [173, 174]. Furthermore, an increased expression of vascular endothelial growth factor (VEGF) is associated with an increase in cartilage damage. This may contribute to the characteristically higher vasculature within the subchondral bone. The vascular channels containing blood vessels, sensory nerves, osteoblasts, and osteoclasts reach the non-calcified cartilage and enable molecular interactions between cartilage and bone leading to cartilage degradation [164, 175–177]. Hence, subchondral bone plate vascularity is associated with the severity of OA cartilage damage, as well as pain [178].

Characteristic microarchitectural subchondral bone changes can be detected in the late stage of OA. Thickening of the subchondral bone plate is observed, as well

as increased trabecular thickness, decreased trabecular separation and bone marrow spacing, and transformation of the trabeculae from rod-like to platelike [65, 179]. By this stage, the subchondral bone is described as sclerotic. Subchondral sclerosis is considered a characterizing feature of progressive OA [65].

Moving on to the third stage of cartilage degeneration, the biosynthetic anabolic activity of the chondrocytes is unable to keep pace with the degradative catabolic activity and homeostasis is lost [180]. At this point, the chondrocytic repair response cannot reverse the damage made to the cartilage. With increasing age and progression of disease, catabolic mechanisms continue to degrade articular cartilage; however, there is a decline in the chondrocytic anabolic and proliferative response [109]. An increase in type II collagen synthesis is insufficient to compensate its proteolysis. Furthermore, this increase in anabolic activity tends to occur in areas distinct from those of proteolysis [180]. Expression levels of inhibitors such as tissue inhibitor of metalloproteinases (TIMP)-1 are reduced and chondrocytes tend to exhibit an age-related decline in their response to anabolic cytokines, which shifts cartilage tissue homeostasis toward tissue destruction and eventual cell death [109]. Reduced cellularity, whether by apoptosis, autophagy-associated cell death, or senescence, correlates strongly with age and severity of OA [181].

Cell Death

It is difficult to establish the exact cause of cell death in OA due to the fact that primary OA seemingly develops over many years, with cells dying with advancing age and progressiveness of disease [182, 183]. As you may know by now, chondrocytes are responsible for mediating cartilage homeostasis. As degeneration continues, changes in the chondrocyte biomechanical environment alter the physical and biochemical signals that regulate cell response propagating cell death and tissue degeneration [184]. Cell death in the form of apoptosis is highly controlled and distinct from pathologic cell death or necrosis, which occurs as a result of cellular damage, hypoxia, or exposure to toxins [185]. Apoptosis can be initiated by intrinsic signals (e.g. mitochondria dependent) or extrinsic signals through cell surface death receptors followed by the sequential activation of a proteolytic cascade of enzymes called caspases [183, 186–188]. Effector caspases (e.g. caspases 1, 3, 6, and 7) then cleave target proteins such as poly adenosine diphosphate ribose polymerase (important for DNA repair), I-CAD (inhibitor of caspase-activated DNAse), and cytoskeletal proteins [189]. As a result, the apoptotic cell displays the characteristic morphological features including chromatin condensation, membrane blebbing, and the formation of rigid apoptotic bodies, which prevent leakage of intracellular contents into the local microenvironment [190].

Extracellular death ligands, Fas ligand (FasL/CD95L) and TNF-α, initiate extrinsic pathways through their respective cell surface death receptors, Fas and TNF-α receptor [187]. Fas (CD95) is expressed on the cell surface of cultured chondrocytes from normal and OA donors [191]. When activated by agonistic antibody, it leads to apoptotic cell death in cultured chondrocytes. However, in tissue where chondro-

cytes reside in their ECM, antibody to Fas fails to induce cell death. This may be due to the barrier created by the ECM that prevents antibody interaction with the chondrocytes. Moreover, chondrocytes in the ECM may be protected from Fas-dependent apoptosis through survival signals generated by the interaction of cell membrane receptors (e.g. integrins) with their respective ECM ligands (e.g. laminin, fibronectin, and collagen types II and IV) [192]. However, in the case of OA, a loosened, if not degraded, ECM may expose Fas receptors and activate the Fas/FasL system to induce apoptosis [182, 193]. Due to the lack of macrophages in cartilage tissue, apoptotic bodies cannot be phagocytosed [194]. Additionally, chondrocytes do not make cell-cell contacts; therefore, neighbouring cells are unable to phagocytose apoptotic bodies either. As a result, apoptotic bodies in cartilage tend to release their contents, which include proteases, into the ECM causing serious damage [195].

The cytotoxic free radical nitric oxide (NO) mediates apoptosis through a mitochondria-dependent mechanism [196]. NO is present in normal and young cartilage, but it is produced in higher levels by the synovium and cartilage during OA [197]. Studies have shown that enhanced NO and reactive oxygen species (ROS) expression in OA chondrocytes is induced by up-regulated pro-inflammatory cytokine production (i.e. IL-1β and TNF-α) in osteoarthritic cartilage [198, 199]. These cytokines, through the production of NO, have been demonstrated to cause mitochondrial dysfunction by inducing mitochondrial DNA (mtDNA) damage, decreasing energy production, and decreasing mitochondrial transcription [188]. The mitochondria is a prime target for oxidative damage, since it is the predominant site for intracellular ROS production [200]. ROS production in the chondrocyte not only damages mitochondrial lipids, proteins, and nucleic acids but also leads to mitochondrial permeability transition (MPT) [188]. A combination of these events results in the mitochondrial pathway of apoptosis. Since chondrocytes are the only source of ECM component synthesis in articular cartilage, and there is no renewal of chondrocyte population, apoptotic cell death has been demonstrated to play a major role in the degeneration of osteoarthritic cartilage. In contrast, it has also been shown that apoptosis occurs at a very low rate in osteoarthritic cartilage [201]. According to this study, the low population of apoptotic cells has a lesser impact than previously described on the pathology of OA. The highest numbers of apoptotic chondrocytes as evidenced by empty lacunae were located in the calcified cartilage layer [202]. The greatly reduced number of living chondrocytes in this cartilage zone may have significance in the later stages of OA, when this zone becomes considerably larger and represents a higher proportion of the articular cartilage [160]. Since apoptotic cells are not efficiently removed from the cartilage, the products of cell death such as pyrophosphate and precipitated calcium may contribute to cartilage degradation.

Autophagy

In order for articular cartilage to function normally, it is important for the joint tissue to maintain its structure, which is governed by the presence of an appropriate number of cells with normal biosynthetic function. Post-mitotic tissue such as

cartilage has a very minimal rate of cell replication, and cellular constituents cannot be continuously renewed [203]. Instead, cells such as chondrocytes depend on autophagy as a principal mechanism to remove damaged and dysfunctional organelles and macromolecules [204].

Autophagy is a lysosomal degradation pathway that is essential for survival, differentiation, development, and, of particular importance, homeostasis [205]. Inducers of autophagy include nutrient and energy deprivation, ROS, or hypoxia [204]. In response to a particular cue, an isolation membrane is formed around the contents to be degraded, which combines with a lysosome to form an autophagosome, the characteristic hallmark of autophagy [205]. The autophagy machinery is orchestrated by the Atg genes, first identified in yeast, with corresponding homologues identified in higher eukaryotes. Among the Atg genes, Atg1, Atg6, Atg8 (ULK1, Beclin 1, and LC3 in mammals, respectively), and Atg5 are four major regulators of the autophagy pathway [206]. ULK1 is a serine/threonine kinase that functions as an intermediate in the transduction of proautophagic signals to autophagosome formation [207]. Beclin 1 forms a complex with type II phosphatidylinositol 3-kinase (PI3K) and Vps34 allowing nucleation of the autophagic vesicle [208]. LC3 is present in two forms: LC3-I is located in the cytoplasm, while LC3-II is bound to the autophagosome membrane. During autophagy, LC3-I undergoes lipidation to be converted to LC3-II, resulting in the association of LC3-II with autophagy vesicles [209]. The enclosed contents are degraded when the autophagosome fuses with the lysosome and the constituents are released and reused.

Autophagy is constitutively active and maintains homeostatic functions in articular cartilage. It does so by removing aggregate-prone or misfolded proteins and dysfunctional organelles, including mitochondria, peroxisomes, and ribosomes [204]. As mentioned previously, the up-regulated expression of proinflammatory cytokines in osteoarthritic tissue results in mitochondrial dysfunction and excessive ROS production [186]. By preventing the accumulation of defective mitochondria, autophagy protects the tissue from a loss of homeostasis and cartilage damage and dysfunction [204].

The correlation between the loss of autophagy and ageing has been well established and believed to be mainly related to the failure of lysosomal hydrolases, resulting in an increase of toxic protein products and slow clearance of autophagosomes in the ageing tissues [210]. ULK1, Beclin 1, and LC3 have been shown to be expressed in normal human articular cartilage, suggesting activation of autophagy [211]. Moreover, the presence of LC3-II is a direct indication of autophagosome formation. However, the expression of these autophagy markers is significantly decreased in OA cartilage and chondrocytes [211]. Defective or reduced autophagy is apparent from the reduction of LC3-II expression. These observations are consistent in the context of ageing-related OA. Just the same, a reduction of and loss of expression of autophagy markers and, hence, a decrease of autophagy activity have also been reported in surgically induced mouse OA models, as well as OA following exposure to mechanical injury in porcine cartilage [211]. Furthermore, a reduction of these key regulators of autophagy is accompanied by increased cell death due to apoptosis [212, 213]. These observations underline the importance of autophagy in physiological and pathological (e.g. osteoarthritic) events and demonstrate that autophagy is not solely associated with ageing-related mechanisms.

Chondrocyte Senescence

Cellular senescence typically refers to the loss of the ability of mitotic cells to further divide in culture after reaching 30–40 population doublings, also known as the 'Hayflick limit' [214]. This form of replicative senescence, resulting from arrest in cell cycle progression, has been established as a protective mechanism to avoid tumour formation by preventing cells with damaged DNA from being replicated [215]. In actively dividing cells, telomeres, which are found at the ends of chromosomes are incompletely replicated during mitosis and shorten with each round of cell division [216–218]. This 'end-replication problem' is not encountered in post-mitotic or quiescent cells such as neurons or chondrocytes [219]. It is much more likely that chondrocyte senescence is a result of extrinsic factors giving rise to 'stress-induced senescence'. Stress-induced senescence can occur from various stimuli including ultraviolet radiation, oxidative damage, activated oncogenes, and chronic inflammation. Oxidative damage can, in fact, result in telomere shortening similar to that seen with replicative senescence, since chromosome ends are particularly sensitive to oxidative damage [216, 220–222].

There is increasing evidence supporting the role that chondrocyte senescence plays in the initiation and progression of OA [222–226]. The lack of cell division and cellular turnover in normal adult articular cartilage means that the chondrocytes present in the cartilage of an older individual are decades old [219]. The long lifetime of chondrocytes allows them to accumulate the detrimental changes due to both ageing and extrinsic stress, and it is an accumulation of these dysfunctional senescent cells that contributes to loss of homeostasis and tissue damage.

An altered expression of regulatory proteins that function to control growth and proliferation is exhibited in senescent cells. These include p53 and the cyclin-dependent kinase inhibitors $p21^{CIP1}$ and $p16^{INK4A}$ [215]. These regulatory proteins are involved in two pathways, p53/p21 and $p16^{INK4A}$/retinoblastoma (Rb), that are essential for induction of senescence in response to external stimuli. DNA damage or telomere shortening activates p53, which inhibits cell-cycle progression. Activated p53 also contributes to senescence by increasing p21 expression. As p21 expression declines in senescent cells, p16 is increased leading to a more stable inhibition of cell-cycle progression by inhibiting Rb [215]. Besides increased p53 and p16 expression, altered chromatin structure can be used as a marker to signify a senescent cell. Altered chromatin structure in a senescent cell is presented as foci of heterochromatin or senescence-associated heterochromatin foci (SAHFs) [227].

Not only does senescence contribute to the pathology of OA by decreasing the number of functional chondrocytes, but also senescent chondrocytes have been shown to secrete factors that favour matrix degradation. Changes in gene expression that occur once a cell becomes senescent can lead to the increased production of cytokines (e.g. IL-1, IL-6, IL-8), MMPs, and growth factors (e.g. epidermal growth factor (EGF)) by the senescent cell [228–231]. Often referred to as the senescent secretory phenotype (SASP), this form of cellular senescence has significant implications in the development and progression of OA [219, 232].

As previously alluded to, with progression of disease, chondrocytes show a decline in the proliferative and anabolic response to growth factor stimulation [233]. Chondrocytes undergoing senescence exhibit an age-related loss in their mitogenic response to growth factors, such as TGF-β [234], basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and IGF-I [235, 236]. In vitro studies have shown an age-related decline in the ability of IGF-I and bone morphogenic protein-6 (BMP-6) to stimulate proteoglycan and collagen production [237, 238]. IGF-I is an important autocrine survival factor in cartilage [239]. Studies have shown that excess levels of a reactive nitrogen species, NO, reduce chondrocyte response to IGF-I [240]. Not only is there a decline in responsiveness to these growth factors, but there is also evidence for an age-related reduction in the levels of certain growth factors in cartilage [241–243]. While it is not clear why chondrocytes at this stage of disease have reduced growth factor responsiveness, it is evident that the repair capacity of senescent chondrocytes is compromised and an imbalance in anabolic and catabolic pathways favours matrix degradation.

In recent years, stress-induced senescence due to oxidative stress has been shown to play a major role in the pathogenesis and development of OA [219, 244]. A cell experiences oxidative stress when the amount of ROS exceeds the cell's antioxidant capacity. This can be a result of increased ROS production or reduced availability of antioxidants, such as glutathione and superoxide dismutase [245, 246]. An increased production of ROS may contribute to mutation in mitochondrial DNA, thus propagating mitochondrial dysfunction. Altered mitochondrial functions such as ATP production, modulation of calcium levels, and the redox state of the mitochondria increase oxidative stress in chondrocytes, which drives the cell to a senescent state [247]. ROS have been shown to be generated by chondrocytes as by-products of aerobic metabolism, as well as in response to stimulation by pro-inflammatory cytokines and growth factors, such as IL-1, TNF-α, FGF, and TGF-β [248, 249]. While in vitro studies show evidence that chondrocyte senescence is associated with oxidative stress, further studies would help to better describe the mechanism of oxidative-stress-induced chondrocyte senescence.

Conclusion

OA is a chronic degenerative joint disease that has long been considered an age-related disease of cartilage degeneration. Undeniably, age is one of the strongest predictors of OA development; however, risk factors such as genetics, gender, metabolic status, obesity, and trauma all contribute to the probability of disease development. Furthermore, it has now been established that OA is a whole joint disease. Maintenance of cartilage ECM homeostasis is the main function of chondrocytes, providing structural support and a reservoir for cytokines and growth factors – critical for cell survival and maintenance of normal joint function. A dysregulation of ECM homeostasis results in the degradation of cartilage, as well as remodelling of the subchondral bone

and synovial inflammation. Due to the close interactions between cartilage, bone, and synovium, alterations in one of these tissues do not seem to occur independently from the others. As cartilage degeneration continues, loss of ECM leads to the propagation of cell death and tissue degeneration. Matrix homeostasis relies on a balance between anabolic and catabolic activities, which are dependent on the number of viable chondrocytes. Hence, the contribution of cell death is an important factor in the progression and severity of disease. With increasing age, senescent chondrocytes are less able to maintain and repair articular cartilage tissue. In addition, the chondrocytes become less responsive to anabolic stimuli and show an age-related decline in response to anabolic cytokines and growth factors. Findings in animal models support the notion of the involvement of chondrocyte senescence with the progression of cartilage degeneration and advancement of disease.

Throughout the upcoming chapters of this book, the authors have attempted to provide a comprehensive and thorough understanding of distinct joints affected by OA including hip, knee, shoulder, elbow, spine, ankle, hand and wrist. This book also covers the current imaging practice in OA, joint conservation strategies, biomarkers, present and future drugs/agents for the treatment of OA as well as safety profile of current OA therapies. Finally, this book covers recent advances in regenerative and precision OA medicine.

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