Chapter 2
Basic Science Concepts in Musculoskeletal Regenerative Medicine

Allison C. Bean

Introduction

Injury and degeneration of musculoskeletal tissues of the spine and joints are common causes of pain and disability, creating a significant worldwide health and economic burden. These tissues are particularly at risk due to their limited intrinsic healing capacity in conjunction with repetitive exposure to high mechanical loads over a lifetime. Following injury, many musculoskeletal tissues are unable to fully recover, leading to persistent alterations in mechanical properties that may initiate a cascade of progressively worsening tissue degradation and functional impairment.

Regenerative medicine has been studied as a method to repair or replace damaged cells, tissues, and organs. Numerous strategies have been investigated, including but not limited to tissue engineering, autologous cell therapy, gene therapy, and administration of growth factors (Fig. 2.1) [1]. Tissue engineering strategies typically focus on combining cells, scaffolds, and biochemical factors to create a functional tissue in vitro that may subsequently be implanted. Other regenerative approaches may rely on altering the in vivo environment via injection or implantation of cells and biochemical factors in order to stimulate the body's innate healing mechanisms to repair or regenerate the damaged tissue.

Regardless of the approach, thorough knowledge of the biological structure and function of the tissue niche is essential to develop effective regenerative therapies. This chapter will focus on the basic science concepts that guide the development and application of regenerative medicine for treatment of spine and joint dysfunction. An overview of the developmental biology of the joints, spine, and associated

A. C. Bean (✉)
Department of Physical Medicine and Rehabilitation,
University of Pittsburgh Medical Center, Pittsburgh, PA, USA
e-mail: beanac2@upmc.edu

G. Cooper et al. (eds.), *Regenerative Medicine for Spine and Joint Pain*,
https://doi.org/10.1007/978-3-030-42771-9_2

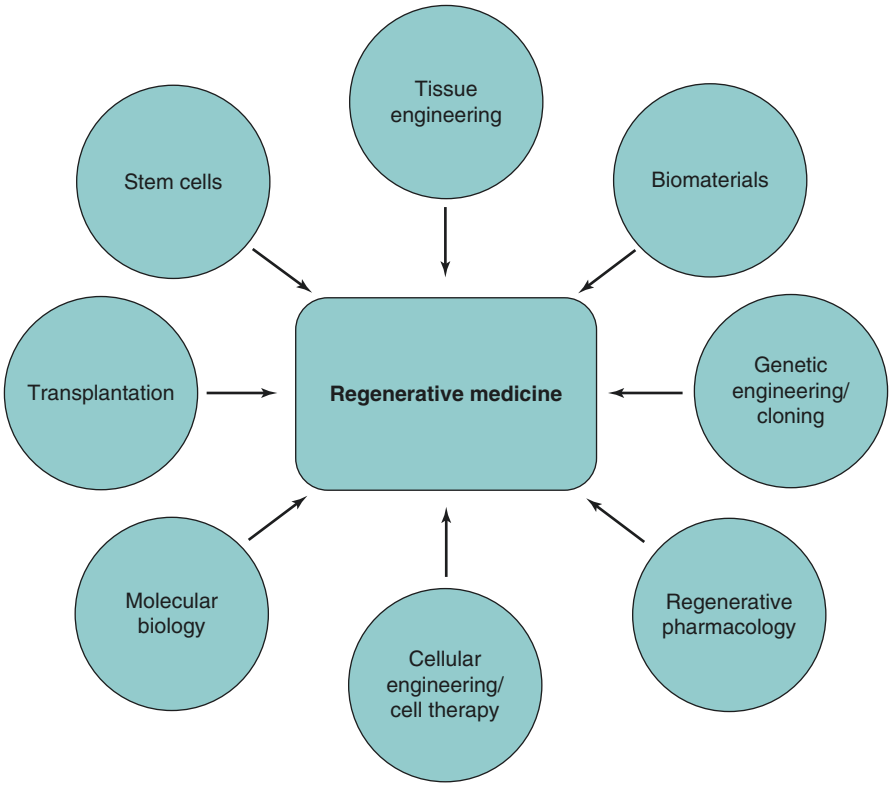

Fig. 2.1 Schematic representation of the various components of regenerative medicine. (Reprinted from Yalcinkaya et al. [1], with permission from Elsevier)

tissues from fertilization to maturity will be presented, followed by a summary of the current scientific understanding of the pathophysiology underlying degeneration of skeletal tissues.

Musculoskeletal Development

Developmental biology focuses on understanding the physical and chemical cues that lead to tissue and organ formation. Regenerative medicine seeks to create or heal tissues through manipulation of cells and the diseased tissue environment. Applying knowledge of developmental processes to regenerative medicine strategies can allow for improved control over cell behavior and potentially result in more effective therapies.

Early Musculoskeletal Embryogenesis

Most scientific knowledge of musculoskeletal development is derived from experiments performed in chick and mouse embryos. The majority of musculoskeletal tissues, except for craniofacial tissues that arise from the neural crest, are derived from the mesodermal layer of the embryo. The axial skeleton arises from the paraxial mesoderm while the limbs are derived from the lateral plate mesoderm [2]. Skeletogenesis is regulated through several signaling pathways. In particular, members of the transforming growth factor-beta (TGF-β) superfamily, which include TGF-β as well as bone morphogenic proteins (BMPs), fibroblast growth factors (FGFs), and growth differentiation factors (GDFs) play important roles throughout bone and cartilage development and in maintaining tissue homeostasis during adulthood [3].

Bone Embryogenesis

Bone formation occurs through two different mechanisms. The flat bones of the skull form through a process known as intramembranous ossification, during which mesenchymal cells directly differentiate into osteoblasts, laying down osteoid matrix that is then mineralized. The process of intramembranous ossification will not be covered in this chapter, but has been described in detail elsewhere [4, 5]. In contrast, long bones and vertebrae develop through a process known as endochondral ossification, where tissues proceed through a cartilaginous phase prior to mineralization (Fig. 2.2).

The initial step in limb bone and joint formation begins with clustering of mesenchymal cells within the limb bud in a process known as mesenchymal condensation. Following condensation, under regulation by the transcription factor Sox9, the mesenchymal cells begin to differentiate into two separate populations of cells – an avascular core containing rounded chondrocytes and an outer layer of flattened perichondrial cells closely associated with the surrounding vasculature [7, 8]. The chondrocytes proliferate, producing an initial cartilaginous extracellular matrix template, or anlage, which segments to form early the individual skeletal elements. Chondrocytes at the center of the anlage eventually stop proliferating and undergo hypertrophy, shifting from secretion of type II to X collagen and inducing matrix mineralization. Hypertrophic chondrocytes also secrete paracrine factors including Indian hedgehog (IHH), signaling perichondrial cells to undergo differentiation, and vascular endothelial growth factor (VEGF), triggering blood vessel invasion.

Hypertrophic chondrocytes eventually undergo apoptosis as mineralization limits nutrient delivery to the interior of the tissue [9–12]. Perichondrial cells adjacent to the hypertrophic zone differentiate into osteoblasts, which create a mineralized

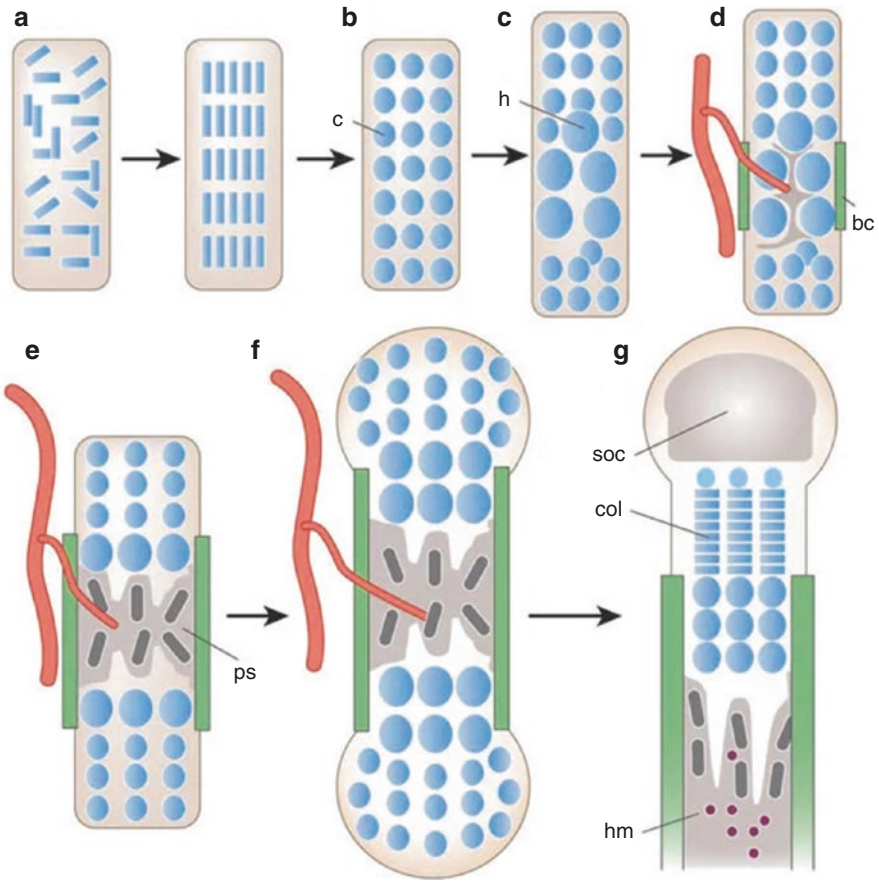

Fig. 2.2 Endochondral bone formation. (**a**) Mesenchymal cells condense. (**b**) Cells of condensations become chondrocytes (c). (**c**) Chondrocytes at the center of condensation stop proliferating and become hypertrophic (h). (**d**) Perichondrial cells adjacent to hypertrophic chondrocytes become osteoblasts, forming the bone collar (bc). Hypertrophic chondrocytes direct the formation of mineralized matrix, attract blood vessels, and undergo apoptosis. (**e**) Osteoblasts of primary spongiosa accompany vascular invasion, forming the primary spongiosa (ps). (**f**) Chondrocytes continue to proliferate, lengthening the bone. Osteoblasts of primary spongiosa are precursors of eventual trabecular bone; osteoblasts of the bone collar become the cortical bone. (**g**) At the end of the bone, the secondary ossification center (soc) forms through cycles of chondrocyte hypertrophy, vascular invasion, and osteoblast activity. The growth plate below the secondary center of ossification forms orderly columns of proliferating chondrocytes (col). Hematopoietic marrow (hm) expands in the marrow space along with stromal cells. (Reprinted from Kronenberg [6] with permission from Springer Nature)

bone collar, forming early the cortical bone, and endothelial cells, which initiate vascular invasion into the tissue [7]. As blood vessels invade, they bring chondroclasts and hemopoietic stem cells. The chondroclasts resorb the cartilaginous matrix and osteoblast precursors use the remnants as a scaffold for bone matrix deposition. This tissue is known as the primary spongiosa and is later remodeled into a

mature trabecular bone. Hemopoietic stem cells migrate to the center of the eventual diaphysis, where they reside within the bone marrow postnatally [13]. This region is called the primary ossification center (POC). Chondrocytes at the epiphyseal ends continue to proliferate, elongating the bone, while progressive chondrocyte hypertrophy and subsequent ossification continue from the POC toward the epiphysis. Postnatally, a secondary ossification center (SOC) forms at the epiphysis in a process similar to the POC. Chondrocyte proliferation is then limited to the epiphyseal or growth plate, which closes at the end of puberty [9–12, 14, 15].

Synovial Joint Development

Development of synovial joints begins at the time of cartilage anlagen segmentation as mentioned previously. The first step is condensation of cells into a densely packed region called the interzone. The interzone layer gradually thickens and then separates to form early the joint space. Cells in the interzone express Gdf5 and eventually give rise to the articular cartilage covering the joint surface, as well as other joint tissues including the joint capsule, synovium, ligaments, and menisci. They also contribute to chondrocyte proliferation and bone maturation at the SOC [16–18].

Articular cartilage maturation continues postnatally, with chondrocytes continuing to proliferate and produce matrix proteins. Eventually, the tissue is organized into four zones: superficial, middle, deep, and calcified. Articular cartilage ECM is primarily composed of type II collagen and proteoglycans, the most prevalent being aggrecan. The superficial zone contains flattened chondrocytes expressing lubricin and hyaluronic acid, creating a smooth, low-friction surface and preventing overgrowth of synovial cells [19]. The collagen matrix in the superficial zone runs parallel to the tissue surface. In the middle/intermediate zone, chondrocytes have a more rounded morphology while collagen fibers are thicker and loosely organized into radial bundles. Chondrocytes in the deep zone are organized into columns and secrete less collagen and more aggrecan. Lastly, chondrocytes in the deep calcified zone located adjacent to the subchondral bone are hypertrophic and terminally differentiated, expressing type X collagen and alkaline phosphatase [20]. This tissue organization enables cartilage to effectively absorb and dissipate the forces generated during loading.

Spine Joint Development

At each spinal level, three joints link adjacent vertebrae and stabilize the spine. Zygapophysial or facet joints are located posteriorly on each side of the vertebral column and are articular joints that form between superior and inferior processes of adjacent vertebrae [21]. Between each bony vertebra lies an intervertebral disc (IVD) which functions to stabilize the spine, acts as a shock absorber during

loading, and allows for multidirectional movement of the spinal column [22]. The IVD is bound rostrally and caudally by the endplate (EP), a thin layer of articular cartilage less than 1 mm thick, that separates the IVD from the vertebral bodies and aids in mechanical load distribution [23]. During embryogenesis, blood vessels transverse through the EP and into the IVD, supplying nutrients to the AF and NP. As development progresses, the vessels regress, and the IVD becomes avascular by adulthood, relying on diffusion of nutrients through the endplate from vessels terminating within the subchondral bone [23, 24].

The NP is derived from cells originating from the embryonic notochord [25, 26]. The notochord initially begins as a rod-like structure oriented along the rostrocaudal access of the embryo and acts as a signaling center, directing patterning of the neural tube and other tissues. The mechanisms driving the transformation of the notochord into the NP are not fully understood; however, notochordal cells eventually differentiate into chondrocytic NP cells and secrete a gelatinous ECM composed primarily of aggrecan along with sparse, randomly oriented type II collagen fibers. The glycosaminoglycan (GAG) chains of aggrecan proteoglycans are negatively charged and hydrophilic, creating high osmotic pressures within the NP and giving it the ability to withstand and distribute compressive loads [22].

The AF is composed of fibrochondrocytes that secrete an ECM predominately composed of aligned collagen with small amounts of proteoglycans organized into 15–25 lamellar sheets. The collagen fibers of consecutive layers are obliquely oriented and alternate in direction with each layer, creating an angle-ply structure. This arrangement gives the AF the ability to withstand the high tensile forces during compressive loading [22, 27]. The outer AF has a more fibrous structure containing more type I collagen, while the inner zone is more cartilaginous with higher aggrecan and type II collagen content [28].

Tendon and Ligament Development

While not part of the joint proper, tendons play an important role in joint motion, since they couple muscle to bone across joints. Ligaments also play an important role in joint stabilization as they form bone-to-bone connections. Research focused on ligament development is limited; however, there appears to be significant overlap with tendon, as these tissues have comparable composition and properties [29]. Given these similarities and lack of scientific literature specific to ligament development, this text will focus predominately on the formation of tendons.

Cells that will differentiate into mature tendon cells are known as tenocytes. Axial tenocytes originate from a dorsolateral strip of the sclerotome in a region known as the syndetome. Syndetome formation is dependent on FGF signaling from the myotome, which induces expression of the transcription factor scleraxis (Scx), a key regulator of tendon development. Tendon progenitors are initially loosely organized between the developing bone and muscle. Then, under regulation by TGF-β

secreted by the bone and muscle, additional tendon precursors are recruited and the cells become organized, begin to differentiate, and integrate with bone and muscle at the enthesis and myotendinous junction [30, 31].

Limb tenocytes arise from the lateral plate mesoderm and in the early limb bud consist of ventral and dorsal blastema, from which the flexor and extensor tendons arise, respectively. Unlike the axial skeleton, muscle is not required for initial induction of tendon progenitors in the limbs, though it does appear to be required in later stages of differentiation. Instead, the blastemas are located under the ectodermal layer, from which they receive signals required for induction of Scx expression, which mediates expression of BMP4 [32]. As the limb bud lengthens, the tendon progenitor cells of the proximal limb realign between the differentiating muscle and bone, while distal tendon cells are already near their eventual position prior to induction.

Mature tendon ECM is predominately composed of aligned type I collagen fibers assembled in a hierarchical pattern, with small amounts of other collagens and proteoglycans. Initial tendon matrix synthesis begins with formation of thin collagen fibrils, which assemble together, gradually increasing in length and width, eventually forming collagen fibers. Fibers are bundled together into fascicles, which are separated by loose connective tissue composed of small collagen fibers and elastin called the endotenon, which is contiguous with the surrounding epitenon. Some tendons also have an outer sheath known as the paratenon, which allows tendons, such as at the Achilles, to slide more easily over bony protuberances [33].

The underlying mechanisms of the juncture of tendon with bone at the enthesis and tendon with muscle at the myotendinous junction (MTJ) are incompletely understood. Muscle cells, or myocytes, originate from the somite myotome. Cells in the dorsomedial portion of the myotome give rise to the axial muscles while those in the ventrolateral portion migrate toward the lateral plate mesoderm to eventually form the limb muscles [34]. As tendon and muscle precursors become closely approximated, a disorganized ECM including integrin ligands and thrombospondin 4 (Tsp4) is secreted by myoblasts, forming early the basement membrane. These proteins facilitate integrin binding, stabilizing myofibers and tendon collagen fibers at the MTJ. As myotubes begin to contract, the tension generated at the MTJ interface stimulates increased production and alignment of tendon collagen and parallel assembly of sarcomeres. Persistent mechanical forces promote maturation of collagen fibers and formation of the finger-like processes characteristic of the MTJ as noted above [35–37]. Unlike much of the musculoskeletal system, MTJ formation is complete by the time of birth [36].

Mature fibrocartilaginous entheses, which typically occur near joints, consist of four zones, gradually transitioning from tendinous to cartilaginous to mineralized tissue [38, 39]. After establishment of the primary cartilage anlagen, eminences appear at the site of the future enthesis and are composed of a separate pool of progenitor cells that initially co-express Scx and Sox9, as well as Gdf5 and later Gli1 [40, 41]. Gli1 is a downstream target of Hedgehog, and its expression is essential for enthesis development, where it may play a role in mineralization and widening

of the enthesis [42, 43]. Mechanical loading of the enthesis during early post-natal development has been shown to be essential for enthesis maturation, likely through modulation of Hedgehog expression, as reduction of loading results in impaired mineralization [42, 44].

Musculoskeletal Tissue Homeostasis and Response to Injury

Osteoarthritis (OA) is estimated to affect 10–15% of the population and is a leading cause of disability worldwide, particularly among older individuals [45]. OA most commonly affects the hips, knees, fingers, and spine but can occur in any joint. Development of OA is often multifactorial and is associated with systemic and biomechanical risk factors including but not limited to age, sex, genetics, weight, occupation, joint shape, joint alignment, and comorbid medical conditions [46]. OA is primarily characterized by cartilage deterioration, but surrounding joint tissues including the synovium, meniscus, ligaments, and subchondral bone are often involved [47]. In this section, we provide a brief summary of the pathophysiology of degenerative disease of joints, spine, and tendons, identifying potential mechanisms through which regenerative therapies may prevent or manage pain and disease progression. Proposed mechanisms of repair in currently used regenerative therapies such as platelet-rich plasma and stem cells will be covered in later chapters.

Osteoarthritis of Articular Cartilage

The normal cartilaginous tissues of articular joints are avascular and hypoxic, relying on diffusion for delivery of nutrients from the joint capsule, synovium, and underlying subchondral bone. As a result, chondrocyte metabolism and ECM turnover are limited under normal physiologic conditions, with the half-life of type II collagen and aggrecan estimated to be 120 years and 120 days, respectively [48]. Tissue homeostasis is maintained through a balance of anabolic and catabolic factors released by chondrocytes in response to environmental cues, carefully modulating the slow ECM turnover. Disruption of this balance leads to the complex cascade of changes seen in OA. Below, we briefly highlight some of the mechanisms that drive the development of OA. Additional comprehensive discussions of the important molecular pathways are found in other reviews [49–54]. It is important to note that much of the knowledge regarding these pathways has been obtained from animal studies and may not be completely translatable to the general human population.

The primary driver in development of osteoarthritis is thought to be abnormal mechanical loading of the joint. Chondrocytes are mechanoresponsive cells, altering their phenotype based on changing mechanical cues. Cyclic physiologic loading is important for maintaining cartilage health, and it has been suggested that in the absence of altered biomechanics or biology of the tissue, the cartilage

becomes conditioned to the physiologic loads generated during locomotion, maintaining homeostasis [55]. Previous studies have demonstrated that reduced loading due to immobilization has been shown to lead to decreased cartilage thickness [56, 57]. In several experiments using canine models, immobilization resulted in loss of proteoglycans in the cartilage superficial zone and reduced mechanical properties [58–60]. Mechanical overloading either through strenuous repetition or single high-magnitude loads can lead to increased catabolic activity and cartilage degradation. In in vitro and canine models, supramaximal repetitive loading causes tissue swelling, chondrocyte apoptosis, increased oxidative stress, reduced matrix protein production (including GAGs), increased matrix protein breakdown, and reduced mechanical properties, with the severity of findings often proportional to the magnitude of loading [61–68]. Furthermore, injuries to other joint tissues such as menisci or ligaments can lead to increased risk of developing post-traumatic osteoarthritis, which likely occurs secondary to long-term changes in joint kinematics as a result of the previous injuries [69]. Re-establishing healthy joint kinematics should be part of any OA treatment plan; however, this is a difficult task as small variations are difficult to detect.

The earliest change typically seen in OA is disruption of the collagen fibers in the superficial layer [70, 71]. In response to injury, chondrocytes proliferate and form clusters around the damaged area, releasing both anabolic and catabolic factors in an attempt to remodel the injured tissue [72]. However, the overall anabolic capabilities of chondrocytes are limited, and unless there is a full thickness injury penetrating the subchondral bone, progenitor cells with increased reparative abilities cannot be recruited due to the lack of vasculature. Even in full thickness injuries, the repair response is limited, with the repaired tissue lacking the organization and mechanical strength of the native tissue [73, 74]. In contrast, the catabolic processes initiated by chondrocytes following injury are robust and self-sustaining, shifting the balance toward progressive tissue degeneration and OA.

In a process akin to endochondral ossification that occurs normally during development of long bones, following initial clustering and proliferation, chondrocytes in injured cartilage become hypertrophic, eventually initiating mineral deposition and thickening of the deep calcified zone. VEGF expression in the underlying subchondral bone increases concomitantly, inducing bone remodeling and vascular invasion into the cartilage layers, leading to impaired mechanical properties and progressive cartilage degradation and chondrocyte apoptosis. In later stages of OA, persistent activation of catabolic pathways may also stimulate other pathologic changes throughout the joint including meniscus and ligament degeneration, osteophyte formation, subchondral bone sclerosis, joint capsule hypertrophy, and synovial inflammation and fibrosis [75, 76]. Blood vessel ingrowth occurs with many of these changes and is typically accompanied by sensory nerves containing substance P and calcitonin gene-related peptide. These small unmyelinated nerves are thought to contribute to the development of pain typically seen in OA [77, 78].

Cartilage homeostasis is maintained by transcriptional control of the chondrocyte phenotype through several different interconnecting pathways. Following injury, activation of these pathways shift, driving chondrocytes toward a hypertrophic

phenotype and terminal differentiation, similar to that seen in endochondral ossification. Each of these pathways induces downregulation of Sox9 and upregulation of Runx2, and the cells begin to synthesize type X collagen while reducing production of type II collagen and aggrecan. Hypertrophic chondrocytes in injured articular cartilage also express high levels of proteases including metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), which degrade collagen and aggrecan, respectively. MMP-1, MMP-3, and MMP -13 and ADAMTS-4 and ADAMTS-5 have been shown to be particularly important in tissue degeneration in OA. Conversely, tissue inhibitors of metalloproteinases (TIMPs) are downregulated. Thus, inhibition of MMP and ADAMTS activity has been seen as a potential therapeutic target. While most of the specific inhibitors of these enzymes have not yet made it past pre-clinical testing [79, 80], an oral ADAMTS-5 inhibitor is currently being tested in a phase II clinical trial [81].

Inflammation plays an integral role in the progression of OA (Fig. 2.3). Molecules known as damage-associated breakdown products (DAMPs) are released by chondrocytes following injury and serve as ligands for pattern recognition receptors (PRRs) including toll-like receptors (TLRs) and receptor for advanced glycation end products (RAGE) expressed by chondrocytes and synovial cells. Interaction between DAMPs and PRRs induces release of pro-inflammatory cytokines including pro-inflammatory interleukins, IL-1β and IL-6, and tissue necrosis factor-alpha (TNF-α) from chondrocytes and macrophages. This signals chondrocytes to undergo hypertrophy and terminal differentiation and promotes tissue degradation through nuclear factor-kappaB (NF-κB) and MAPK pathways, resulting in upregulation of MMPs and ADAMTS, as well as other pro-inflammatory mediators including nitric oxide (NO), cyclooxygenase-2 (COX-2), and prostaglandin E2 (PGE2) [50, 52]. Activation of the complement system and infiltration of cell mediators of the adaptive immune system including T-cells, B-cells, and macrophages have also been found to be increased in the synovium of osteoarthritic joints [82–84]. In sum, the pro-inflammatory environment induced by cartilage injury leads to progressive synovitis and chondrocyte activation, promoting a cycle of inflammation and cell damage that results in progressive cartilage breakdown and changes in the other joint tissues as described above.

Medications commonly used in OA including corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), COX inhibitors, and hyaluronic acid are directed toward inhibition of inflammatory pathways; however, long-term use can result in significant side effects, including adverse gastrointestinal and cardiovascular events, and may even accelerate OA progression [85–87]. Anti-cytokine therapies have been demonstrated to be effective in the treatment of rheumatoid arthritis; however, their efficacy in OA thus far appears to be limited [88].

TGF-β is essential for chondrocyte maturation and differentiation during development and is present in low concentrations in young, healthy articular cartilage. TGF-β signaling through the Alk5-SMAD2/3 pathway has been shown to be essential for inhibiting chondrocyte hypertrophy and terminal differentiation [89, 90]. Following injury, TGF-β downstream signaling appears to shift from signaling through the Alk5-SMAD2/3 pathway to the Alk1-SMAD1/5/8 pathway, inducing

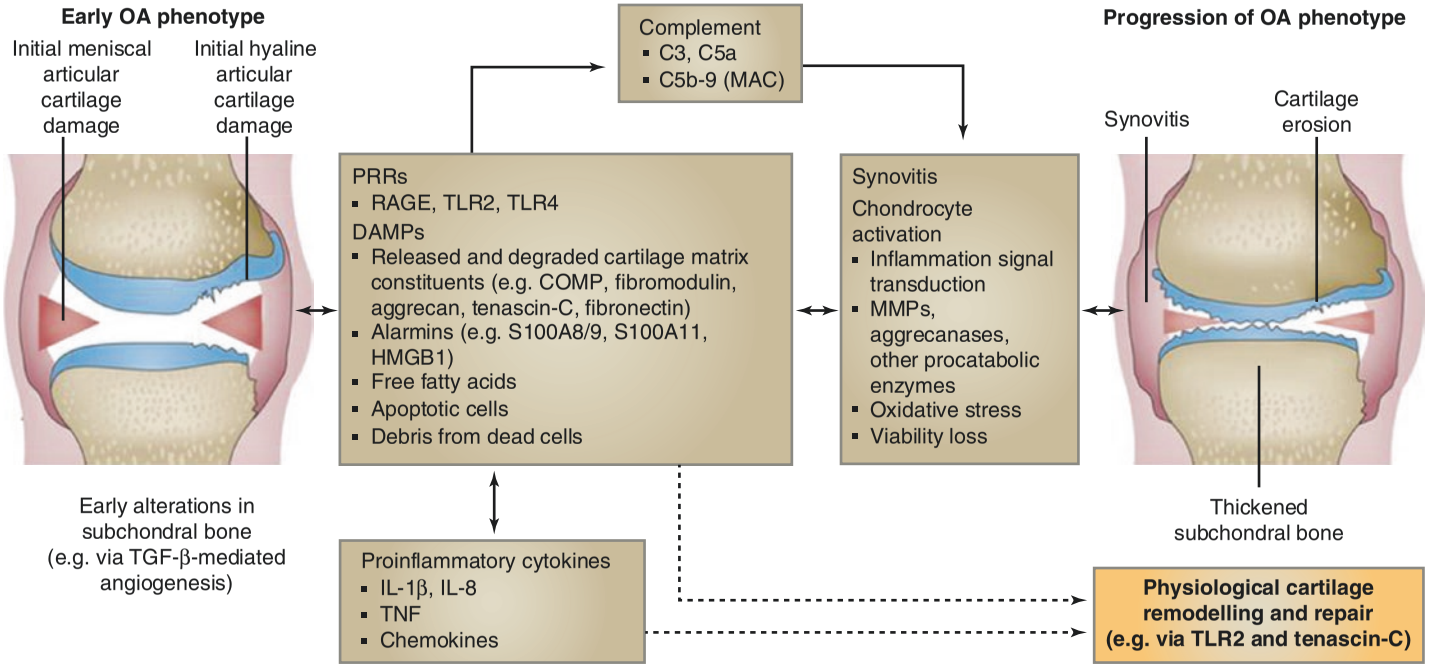

Fig. 2.3 Several of the different classes of inflammatory mediators, including PRRs and their DAMP ligands, conventional inflammatory cytokines, and activated complement proteins C5a and C5b-9 network to augment meniscal fibrocartilage and articular cartilage damage in early and progressive OA. These mediators promote macroscopic inflammation, including synovitis, and can drive cartilage matrix catabolism, but some also promote cartilage remodeling and repair. The number and diversity of inflammatory mediators in OA joints, the paradoxical roles of some of these mediators in tissue damage and repair, and the physiological roles of some mediators in host defense mean targeting individual mediators for OA therapy is difficult. Abbreviations: COMP, cartilage oligomeric matrix protein; DAMP, danger-associated molecular pattern; HMGB1, high mobility group box protein 1; MAC, membrane attack complex; MMP, matrix metalloproteinase; OA, osteoarthritis; PRR, pattern recognition receptor; RAGE, receptor for advanced glycation end products; TGF-β, transforming growth factor β; TLR, toll-like receptor. (Reprinted from Liu-Bryan and Terkeltaub [82], with permission from Springer Nature)

chondrocyte hypertrophy and increased MMP and ADAMTS expression [89, 91–95]. TGF-β has also been implicated in early osteophyte formation and subchondral bone sclerosis [96, 97].

Fibroblast growth factors (FGFs) have also been shown to play an important role in OA. In particular, FGF-2 is released from the pericellular matrix following injury, inhibiting anabolic growth factors BMP-7 and IGF-1 [98] and inducing expression of matrix degrading factors MMP-13 and ADAMTS-5, and reactive oxygen species. This suggests that FGF-2 plays a dual anti-anabolic and pro-catabolic role in OA [99–102]. In contrast, FGF-18 appears to promote cartilage synthesis through inhibition of noggin, a known BMP inhibitor, while FGF-2 increases noggin expression [102]. A phase II clinical trial using intra-articular recombinant FGF-18 for treatment of OA is currently underway, with early results showing that the drug may increase or at least help to maintain cartilage thickness in patients with moderate knee OA [103].

Wnt signaling pathways are important in skeletal development, health, and disease, modulating both chondrogenesis and osteogenesis through both canonical Wnt/β-catenin or non-canonical pathways. As described in a recent review, several studies have shown that Wnt/β-catenin signaling is upregulated in OA, promoting chondrocyte hypertrophy and matrix degradation [104]. The use of a small-molecule inhibitor of the Wnt pathway has shown some promise in phase II clinical trials [105]. Importantly, while some studies have found that inhibition of Wnt signaling can reduce OA progression, others have found that it may lead to increased cell death and cartilage destruction [104, 106]. Taken together, this suggests that Wnt signaling is tightly controlled in cartilage homeostasis, with significant disruption in either direction increasing the risk of OA.

Due to its avascularity, chondrocytes reside in a relatively hypoxic environment. A group of transcription factors known as hypoxia-inducible factors (HIFs) play an important role in cartilage development and homeostasis. HIF-1α is expressed by healthy chondrocytes and promotes expression of several anabolic cartilage genes including Sox9 and type II collagen. However, under abnormal mechanical loading or inflammatory conditions, pro-inflammatory cytokines induce expression of HIF-2α through NF-κB. HIF-2α induces chondrocyte hypertrophic differentiation by increasing Runx2, IHH, and VEGF expression and cartilage degradation by increasing MMP and ADAMTS expression [107]. Suppressing HIF-2α while maintaining HIF-1α expression is another potential target in inhibiting the chondrocyte hypertrophy and cartilage degradation typically seen in OA.

Finally, it is important to discuss the role of aging in OA. The incidence of OA increases dramatically with age; however, recent research has suggested that aging in and of itself does not cause OA. Instead, changes that occur with normal aging increase cartilage susceptibility to damage in the setting of trauma or altered joint kinematics. Changes secondary to aging that increase this risk can be seen at cellular, tissue, and systemic level.

One of the main hallmarks of aging is cell senescence, an irreversible arrest in the cell cycle. Senescence can occur either through replicative senescence via telomere shortening that occurs with each cell division or through stress-induced

senescence triggered by oxidative stress. Since mature chondrocytes rarely divide, stress-induced senescence has been hypothesized to be the main driver of chondrocyte senescence or "chondrosenescence" and likely contributes to development of OA [108]. Oxidative stress can be induced through intracellular processes as described below or by external dysfunction such as abnormal mechanical loading or inflammatory cytokines released from surrounding tissues. Chondrosenescence increases susceptibility to OA due to decreased responsiveness to anabolic growth factors and increased production of catabolic factors [108].

Inflammation has also been implicated in aging. As cells age, their anti-inflammatory responses gradually become less robust and are eventually unable to neutralize the pro-inflammatory processes, resulting in a chronic low-grade inflammation known as "inflammaging" [109, 110]. Increased longevity has been associated with reduced inflammatory and more robust anti-inflammatory responses. The intrinsic effects of aging on cells contributes to the development of inflammaging and OA. Aging cells have been shown to progressively lose their ability to remove dysfunctional proteins and organelles through lysosomal degradation in a process known as autophagy. Loss of autophagic capacity results in protein aggregation, mitochondrial dysfunction, and accumulation of oxygen species (ROS) such as NO. ROS activate the NF-κB signaling pathway, increasing production of pro-inflammatory cytokines including IL-1β and TNF-α. Autophagy may be further suppressed in OA through inhibition of the HIF-2α pathway described previously [109].

These changes in the cellular environment due to aging eventually lead to development of fibrillations on the cartilage surface, decreased size of proteoglycan aggregates due to reduced length of glycosaminoglycan side chains, increased cross-linking of collagen, and decreased total water content, all of which contribute to decreased stiffness and strength that may make the tissue more susceptible to injury [111, 112]. Regenerative therapies that increase cellular resilience to oxidative stress through inhibition of premature chondrosenescence and loss of autophagy could potentially prevent or delay the onset of OA.

Intervertebral Disc Degeneration

Most individuals will experience back pain at some point in their lives. As the leading cause of disability globally, back pain carries a high social and economic burden [113]. In the United States alone, healthcare costs for treatment of low back and neck pain are estimated to exceed $85 billion per year [114]. The most common underlying etiology in the development of low back pain is IVD degeneration [115]. While less is known about the mechanisms underlying IVD degeneration compared to articular cartilage, many similarities are apparent. First, the primary driver of progressive IVD degeneration is biomechanical stress; however, aging, genetics, and other systemic factors also play an important secondary role. Additionally, loss of the delicate balance between anabolic and catabolic processes triggers a

degenerative cascade, leading to upregulation of the same inflammatory mediators and tissue degrading enzymes that are active in articular cartilage degeneration. Lastly, aging and cellular senescence also appear to play an important role in IVD degeneration [116–118]. Despite this overlap, the structure of the IVD is significantly different than that of articular cartilage; thus, regenerative therapies targeting the IVD will likely require a unique approach. Below, we will briefly discuss the pathophysiology of IVD degeneration, focusing on cellular and ECM structure and function in the diseased state.

The first pathologic change noted in IVD degeneration is dysfunction of the NP. Over time, aggrecan content in the NP decreases, leading to a loss of hydrophilicity and compressive resistance. This causes shifting of mechanical loads onto surrounding structures including the AF and EP, increasing mechanical stresses on these tissues. As described above for articular cartilage, persistent mechanical stress disrupts the balance between anabolic and catabolic processes, including upregulation of pro-inflammatory cytokines and matrix degrading enzymes. Under stress, aggrecan and type II collagen within the NP are progressively replaced with fibrous type I collagen leading to worsening dehydration and loss of disc height. The lamellae composed of predominately type I collagen in the outer AF also become increasingly disorganized, increasing susceptibility to disc bulging or AF rupture leading to NP herniation [116, 119].

Nutrients are delivered to the NP and AF primarily by diffusion from the vertebral bodies through the avascular, cartilaginous EP. With degeneration and aging, the EP becomes increasingly thin and calcified. Decreased nutrient perfusion and altered mechanical properties can lead to increased apoptosis and progressive IVD degeneration [118]. NP herniation through the EP into the adjacent vertebral body due to EP mechanical failure results in development of a calcification called a "Schmorl's node" [119].

Development of regenerative therapies for treatment of IVD degeneration require considerations of the different cell types and tissue structure within the IVD. Targeting replacement or preventing loss of aggrecan in the NP may be the most beneficial, as it is the earliest change that is seen with IVD degeneration and is the workhorse in dissipating mechanical forces on the spine. Similar to articular cartilage, regenerative therapies that effectively disrupt the pro-catabolic cycle that occurs in IVD degeneration may also slow progression of the disease and ease symptoms. Finally, restoration of appropriate mechanical loading will need to accompany any regenerative therapy in order to prevent reignition of the pathological processes following treatment.

Tendon and Ligament Degeneration

Tendon and ligament injuries are estimated to account for nearly 50% of musculoskeletal injuries and are common among athletes as well as the general population [120]. While tears or ruptures may occur acutely due to trauma or sudden

mechanical overload, injuries of these tissues occur more frequently as a result of progressive degenerative changes secondary to repetitive mechanical overloading in combination with other factors including aging, genetics, and systemic disorders. In this chapter, we will focus on the pathogenesis of tendinopathy as this is the most frequently encountered and commonly investigated. Similar concepts to those discussed with tendinopathy may also be applied to ligamentous injuries.

Tendinopathy is characterized by several changes including tenocyte proliferation, disruption and disorganization of collagen fibers, increase in the ratio of type III to type I collagen, and increase in non-collagenous matrix proteins including GAGs [121]. The water content of the tissue increases, leading to increased cross-sectional area. Vascular ingrowth, often accompanied by sensory nerves, has also been noted [122].

Much remains to be understood regarding pathophysiology of tendinopathy, and key components continue to be debated. Like other degenerative joint diseases, tendinopathy is considered to be the result of a failed healing response, with a loss of balance between anabolic and catabolic factors. Mechanical loading is a central regulator, with appropriate physiologic loading resulting in an increase in anabolic activity, particularly in the periphery, while underloading or overloading can induce factors that promote tissue degeneration [123]. One of the most popular models proposed to describe the pathophysiology of tendinopathy is the continuum model. Initially published in 2009 [124] and updated in 2016 [125], this model suggests that tendon pathology occurs as a potentially reversible continuum across three stages in the setting of abnormal mechanical loading: reactive tendinopathy, tendon disrepair, and degenerative tendinopathy (Fig. 2.4). As the tendon moves toward the degenerative tendinopathy stage, it becomes more difficult to reverse the pathologic process. A fourth stage, reactive-on-degenerative, was added in the 2016 model to highlight the potential for only a portion of a tendon to have progressed to the degenerative stage, while another area may be in a reactive stage.

The role of inflammation in tendinopathy has remained controversial. In the 1970s, histological studies on degenerated tendons demonstrated an absence of acute inflammatory cells, leading to a shift away from an inflammatory etiology for chronic tendon pain and toward a degenerative model [127, 128]. However, recent studies using more advanced techniques have confirmed the presence of macrophages, lymphocytes, and mast cells in acutely injured and chronically degenerated tendons [129–132]. Interestingly, macrophages found in chronic tendinopathy typically express the M2 phenotype, which produces immunosuppressive cytokines to reduce inflammatory responses, unlike the M1 phenotype, which is pro-inflammatory. Similar to OA pathogenesis, inflammatory mediators appear to play an important role in modulating matrix composition and tenocyte phenotype. By binding to cell surface receptors and inducing downstream pathways, pro-inflammatory cytokines including interleukins and TNF-α, as well as PGE2 and NO among others, can enhance inflammation, induce collagen remodeling, increase tenocyte proliferation, and promote angiogenesis [133–135].

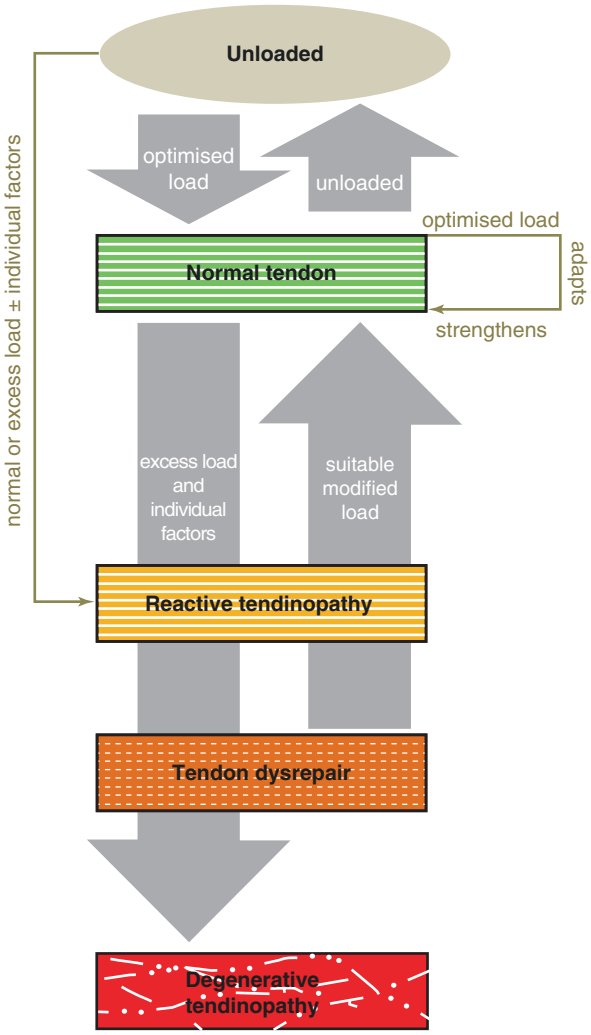

Fig. 2.4 Continuum model of tendinopathy. (Reprinted from Rudavsky and Cook [126] with permission from Elsevier)

Studies investigating the changes that occur in otherwise healthy tendons with aging, particularly in humans, are somewhat limited. Results from in vitro studies suggest that aging may cause a decline in tenocyte migration and proliferation capacity. However, there is no clear evidence that aging leads to impairment in the ability of tenocytes to synthesize collagen, consistent with findings that aging does not independently lead to reduced cross-sectional area. Whether aging affects mechanical properties of the tendon remains uncertain, though physical activity appears to increase tissue stiffness independent of aging [123].

Conclusion

Unlike many current therapies that focus primarily on reducing physical symptoms, regenerative medicine has the potential to either halt or even reverse tissue disease and degeneration. The high prevalence of pathology and disability related to degeneration of skeletal tissues including articular cartilage, IVD, and ligaments/tendons makes them an important focus for regenerative therapies. As the field advances, understanding the biology of tissue development and disease can provide invaluable insight into potential therapeutic targets. Potential targets for regenerative therapies include inhibition of catabolic pathways including the inflammatory cascade, matrix degrading enzymes and their upstream effectors, vascular and neural ingrowth, and cellular senescence, as well as enhancing anabolic pathways by increasing cell proliferation and the availability of anabolic growth factors and antioxidants. Finally, as altered mechanical loading is often the sentinel change that leads to progressive degeneration, it is imperative that normal kinematics and mechanical stress on the tissue are restored and that appropriate cyclic loading through exercise is continued in conjunction with the use of regenerative therapies.

References

1. Yalcinkaya TM, Sittadjody S, Opara EC. Scientific principles of regenerative medicine and their application in the female reproductive system. Maturitas. 2014;77:12–9.
2. Ono N, Kronenberg HM. Developmental biology of musculoskeletal tissues for tissue engineers. In: Developmental biology and musculoskeletal tissue engineering. Elsevier Academic Press; 2018. p. 1–24.
3. Wu M, Chen G, Li Y-P. TGF-β and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. Bone Res. 2016;4:16009.
4. Berendsen AD, Olsen BR. Bone development. Bone. 2015;80:14–8.
5. Teti A. Bone development: overview of bone cells and signaling. Curr Osteoporos Rep. 2011;9:264–73.
6. Kronenberg HM. Developmental regulation of the growth plate. Nature. 2003;423:332–6.
7. Colnot C, Lu C, Hu D, Helms JA. Distinguishing the contributions of the perichondrium, cartilage, and vascular endothelium to skeletal development. Dev Biol. 2004;269:55–69.
8. Bi W, Deng JM, Zhang Z, Behringer RR, de Crombrugghe B. Sox9 is required for cartilage formation. Nat Genet. 1999;22:85–9.
9. Long F, Chung U, Ohba S, McMahon J, Kronenberg HM, McMahon AP. Ihh signaling is directly required for the osteoblast lineage in the endochondral skeleton. Development. 2004;131:1309–18.
10. Hu H, Hilton MJ, Tu X, Yu K, Ornitz DM, Long F. Sequential roles of Hedgehog and Wnt signaling in osteoblast development. Development. 2005;132:49–60.
11. Chung UI, Lanske B, Lee K, Li E, Kronenberg H. The parathyroid hormone/parathyroid hormone-related peptide receptor coordinates endochondral bone development by directly controlling chondrocyte differentiation. Proc Natl Acad Sci U S A. 1998;95:13030–5.

12. Duan X, Murata Y, Liu Y, Nicolae C, Olsen BR, Berendsen AD. Vegfa regulates perichondrial vascularity and osteoblast differentiation in bone development. Development. 2015;142:1984–91.
13. Coskun S, Hirschi KK. Establishment and regulation of the HSC niche: roles of osteoblastic and vascular compartments. Birth Defects Res C Embryo Today. 2010;90:229–42.
14. Shen G. The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage. Orthod Craniofac Res. 2005;8:11–7.
15. Kobayashi T, Kronenberg HM. Overview of skeletal development. Methods Mol Biol. 2014;1130:3–12.
16. Decker RS. Articular cartilage and joint development from embryogenesis to adulthood. Semin Cell Dev Biol. 2017;62:50–6.
17. Shwartz Y, Viukov S, Krief S, Zelzer E. Joint development involves a continuous influx of Gdf5-positive cells. Cell Rep. 2016;15:2577–87.
18. Pacifici M, Koyama E, Shibukawa Y, Wu C, Tamamura Y, Enomoto-Iwamoto M, Iwamoto M. Cellular and molecular mechanisms of synovial joint and articular cartilage formation. Ann N Y Acad Sci. 2006;1068:74–86.
19. Greene GW, Banquy X, Lee DW, Lowrey DD, Yu J, Israelachvili JN. Adaptive mechanically controlled lubrication mechanism found in articular joints. Proc Natl Acad Sci U S A. 2011;108:5255–9.
20. Poole AR, Kojima T, Yasuda T, Mwale F, Kobayashi M, Laverty S. Composition and structure of articular cartilage: a template for tissue repair. Clin Orthop Relat Res. 2001;391:S26–33.
21. Bowles RD, Setton LA. Biomaterials for intervertebral disc regeneration and repair. Biomaterials. 2017;129:54–67.
22. Whatley BR, Wen X. Intervertebral disc (IVD): structure, degeneration, repair and regeneration. Mater Sci Eng C. 2012;32:61–77.
23. Sivakamasundari V, Lufkin T. Bridging the gap: understanding embryonic intervertebral disc development. Cell Dev Biol. 2012;1(2):103.
24. Raj PP. Intervertebral disc: anatomy-physiology-pathophysiology-treatment. Pain Pract. 2008;8:18–44.
25. McCann MR, Tamplin OJ, Rossant J, Séguin CA. Tracing notochord-derived cells using a Noto-cre mouse: implications for intervertebral disc development. Dis Model Mech. 2012;5:73–82.
26. Choi K-S, Cohn MJ, Harfe BD. Identification of nucleus pulposus precursor cells and notochordal remnants in the mouse: implications for disk degeneration and chordoma formation. Dev Dyn. 2008;237:3953–8.
27. Cox MK, Serra R. Development of the intervertebral disc. In: Shapiro IM, Risbud MV, editors. The intervertebral disc. Vienna: Springer Vienna; 2014. p. 33–51.
28. Chu G, Shi C, Wang H, Zhang W, Yang H, Li B. Strategies for annulus fibrosus regeneration: from biological therapies to tissue engineering. Front Bioeng Biotechnol. 2018;6:90.
29. Tozer S, Duprez D. Tendon and ligament: development, repair and disease. Birth Defects Res C Embryo Today. 2005;75:226–36.
30. Schweitzer R, Zelzer E, Volk T. Connecting muscles to tendons: tendons and musculoskeletal development in flies and vertebrates. Development. 2010;137:2807–17.
31. Rothrauff BB, Yang G, Tuan RS. Tendon resident cells—functions and features in section I—developmental biology and physiology of tendons. In: Tendon regeneration. Elsevier Academic Press; 2015. p. 41–76.
32. Blitz E, Viukov S, Sharir A, Shwartz Y, Galloway JL, Pryce BA, Johnson RL, Tabin CJ, Schweitzer R, Zelzer E. Bone ridge patterning during musculoskeletal assembly is mediated through SCX regulation of Bmp4 at the tendon-skeleton junction. Dev Cell. 2009;17:861–73.
33. Benjamin M, Kaiser E, Milz S. Structure-function relationships in tendons: a review. J Anat. 2008;212:211–28.
34. Chal J, Pourquié O. Making muscle: skeletal myogenesis in vivo and in vitro. Development. 2017;144:2104–22.

35. Valdivia M, Vega-Macaya F, Olguín P. Mechanical control of myotendinous junction formation and tendon differentiation during development. Front Cell Dev Biol. 2017;5:26.
36. Charvet B, Ruggiero F, Le Guellec D. The development of the myotendinous junction. A review. Muscles Ligaments Tendons J. 2012;2:53–63.
37. Asahara H, Inui M, Lotz MK. Tendons and ligaments: connecting developmental biology to musculoskeletal disease pathogenesis. J Bone Miner Res. 2017;32:1773–82.
38. Jensen PT, Lambertsen KL, Frich LH. Assembly, maturation, and degradation of the supraspinatus enthesis. J Shoulder Elb Surg. 2018;27:739–50.
39. Apostolakos J, Durant TJ, Dwyer CR, Russell RP, Weinreb JH, Alaee F, Beitzel K, McCarthy MB, Cote MP, Mazzocca AD. The enthesis: a review of the tendon-to-bone insertion. Muscles Ligaments Tendons J. 2014;4:333–42.
40. Sugimoto Y, Takimoto A, Akiyama H, Kist R, Scherer G, Nakamura T, Hiraki Y, Shukunami C. Scx+/Sox9+ progenitors contribute to the establishment of the junction between cartilage and tendon/ligament. Development. 2013;140:2280–8.
41. Blitz E, Sharir A, Akiyama H, Zelzer E. Tendon-bone attachment unit is formed modularly by a distinct pool of Scx- and Sox9-positive progenitors. Development. 2013;140:2680–90.
42. Schwartz AG, Long F, Thomopoulos S. Enthesis fibrocartilage cells originate from a population of Hedgehog-responsive cells modulated by the loading environment. Development. 2015;142:196–206.
43. Dyment NA, Breidenbach AP, Schwartz AG, et al. Gdf5 progenitors give rise to fibrocartilage cells that mineralize via hedgehog signaling to form the zonal enthesis. Dev Biol. 2015;405:96–107.
44. Thomopoulos S, Kim H-M, Rothermich SY, Biederstadt C, Das R, Galatz LM. Decreased muscle loading delays maturation of the tendon enthesis during postnatal development. J Orthop Res. 2007;25:1154–63.
45. WHO|Chronic rheumatic conditions 2019. https://www.who.int/chp/topics/rheumatic/en/. Accessed 14 June 2019.
46. Barbour KE, Helmick CG, Boring M, Brady TJ. Vital signs: prevalence of doctor-diagnosed arthritis and arthritis-attributable activity limitation – United States, 2013–2015. MMWR Morb Mortal Wkly Rep. 2017;66:246–53.
47. Mobasheri A, Batt M. An update on the pathophysiology of osteoarthritis. Ann Phys Rehabil Med. 2016;59:333–9.
48. Houard X, Goldring MB, Berenbaum F. Homeostatic mechanisms in articular cartilage and role of inflammation in osteoarthritis. Curr Rheumatol Rep. 2013;15:375.
49. Ripmeester EGJ, Timur UT, Caron MMJ, Welting TJM. Recent insights into the contribution of the changing hypertrophic chondrocyte phenotype in the development and progression of osteoarthritis. Front Bioeng Biotechnol. 2018;6:18.
50. Rigoglou S, Papavassiliou AG. The NF-κB signalling pathway in osteoarthritis. Int J Biochem Cell Biol. 2013;45:2580–4.
51. Xia B, Chen D, Zhang J, Hu S, Jin H, Tong P. Osteoarthritis pathogenesis: a review of molecular mechanisms. Calcif Tissue Int. 2014;95:495–505.
52. Marcu KB, Otero M, Olivotto E, Borzi RM, Goldring MB. NF-kappaB signaling: multiple angles to target OA. Curr Drug Targets. 2010;11:599–613.
53. Haseeb A, Haqqi TM. Immunopathogenesis of osteoarthritis. Clin Immunol. 2013;146:185–96.
54. Mariani E, Pulsatelli L, Facchini A. Signaling pathways in cartilage repair. Int J Mol Sci. 2014;15:8667–98.
55. Andriacchi TP, Koo S, Scanlan SF. Gait mechanics influence healthy cartilage morphology and osteoarthritis of the knee. J Bone Joint Surg Am. 2009;91(Suppl 1):95–101.
56. Hinterwimmer S, Krammer M, Krötz M, Glaser C, Baumgart R, Reiser M, Eckstein F. Cartilage atrophy in the knees of patients after seven weeks of partial load bearing. Arthritis Rheum. 2004;50:2516–20.
57. Vanwanseele B, Eckstein F, Knecht H, Spaepen A, Stüssi E. Longitudinal analysis of cartilage atrophy in the knees of patients with spinal cord injury. Arthritis Rheum. 2003;48:3377–81.

58. Haapala J, Arokoski J, Pirttimäki J, Lyyra T, Jurvelin J, Tammi M, Helminen HJ, Kiviranta I. Incomplete restoration of immobilization induced softening of young beagle knee articular cartilage after 50-week remobilization. Int J Sports Med. 2000;21:76–81.
59. Palmoski M, Perricone E, Brandt KD. Development and reversal of a proteoglycan aggregation defect in normal canine knee cartilage after immobilization. Arthritis Rheum. 1979;22:508–17.
60. Jurvelin J, Kiviranta I, Tammi M, Helminen JH. Softening of canine articular cartilage after immobilization of the knee joint. Clin Orthop Relat Res. 1986;207:246–52.
61. Alexander PG, Song Y, Taboas JM, Chen FH, Melvin GM, Manner PA, Tuan RS. Development of a spring-loaded impact device to deliver injurious mechanical impacts to the articular cartilage surface. Cartilage. 2013;4:52–62.
62. Bonnevie ED, Delco ML, Fortier LA, Alexander PG, Tuan RS, Bonassar LJ. Characterization of tissue response to impact loads delivered using a hand-held instrument for studying articular cartilage injury. Cartilage. 2015;6:226–32.
63. Jeffrey JE, Gregory DW, Aspden RM. Matrix damage and chondrocyte viability following a single impact load on articular cartilage. Arch Biochem Biophys. 1995;322:87–96.
64. Kurz B, Jin M, Patwari P, Cheng DM, Lark MW, Grodzinsky AJ. Biosynthetic response and mechanical properties of articular cartilage after injurious compression. J Orthop Res. 2001;19:1140–6.
65. Loening AM, James IE, Levenston ME, et al. Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. Arch Biochem Biophys. 2000;381:205–12.
66. Torzilli PA, Grigiene R, Borrelli J, Helfet DL. Effect of impact load on articular cartilage: cell metabolism and viability, and matrix water content. J Biomech Eng. 1999;121:433–41.
67. Kiviranta I, Tammi M, Jurvelin J, Arokoski J, Säämänen AM, Helminen HJ. Articular cartilage thickness and glycosaminoglycan distribution in the canine knee joint after strenuous running exercise. Clin Orthop Relat Res. 1992;283:302–8.
68. Arokoski J, Kiviranta I, Jurvelin J, Tammi M, Helminen HJ. Long-distance running causes site-dependent decrease of cartilage glycosaminoglycan content in the knee joints of beagle dogs. Arthritis Rheum. 1993;36:1451–9.
69. Carbone A, Rodeo S. Review of current understanding of post-traumatic osteoarthritis resulting from sports injuries. J Orthop Res. 2017;35:397–405.
70. Andriacchi TP, Mündermann A, Smith RL, Alexander EJ, Dyrby CO, Koo S. A framework for the in vivo pathomechanics of osteoarthritis at the knee. Ann Biomed Eng. 2004;32:447–57.
71. Brandt KD, Dieppe P, Radin E. Etiopathogenesis of osteoarthritis. Med Clin North Am. 2009;93(1–24):xv.
72. Lotz MK, Otsuki S, Grogan SP, Sah R, Terkeltaub R, D'Lima D. Cartilage cell clusters. Arthritis Rheum. 2010;62:2206–18.
73. Temenoff JS, Mikos AG. Review: tissue engineering for regeneration of articular cartilage. Biomaterials. 2000;21:431–40.
74. Buckwalter JA. Articular cartilage: injuries and potential for healing. J Orthop Sports Phys Ther. 1998;28:192–202.
75. van der Kraan PM. The changing role of TGFβ in healthy, ageing and osteoarthritic joints. Nat Rev Rheumatol. 2017;13:155–63.
76. Lories RJ, Luyten FP. The bone-cartilage unit in osteoarthritis. Nat Rev Rheumatol. 2011;7:43–9.
77. Ashraf S, Wibberley H, Mapp PI, Hill R, Wilson D, Walsh DA. Increased vascular penetration and nerve growth in the meniscus: a potential source of pain in osteoarthritis. Ann Rheum Dis. 2011;70:523–9.
78. Mapp PI, Walsh DA. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. Nat Rev Rheumatol. 2012;8:390–8.
79. Malemud CJ. Inhibition of mmps and ADAM/ADAMTS. Biochem Pharmacol. 2019;165:33–40.

80. Clouet J, Vinatier C, Merceron C, Pot-vaucel M, Maugars Y, Weiss P, Grimandi G, Guicheux J. From osteoarthritis treatments to future regenerative therapies for cartilage. Drug Discov Today. 2009;14:913–25.
81. Identifier NCT03595618, a study to assess efficacy and safety of GLPG1972/S201086 in patients with knee osteoarthritis (Roccella). In: ClinicalTrials.gov 2018. https://clinicaltrials.gov/ct2/show/NCT03595618?term=GLPG1972&recrs=d&rank=1. Accessed 30 Apr 2019.
82. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. Nat Rev Rheumatol. 2015;11:35–44.
83. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum. 2012;64:1697–707.
84. Kandahari AM, Yang X, Dighe AS, Pan D, Cui Q. Recognition of immune response for the early diagnosis and treatment of osteoarthritis. J Immunol Res. 2015;2015:192415.
85. Zeng C, Lane NE, Hunter DJ, Wei J, Choi HK, McAlindon TE, Li H, Lu N, Lei G, Zhang Y. Intra-articular corticosteroids and the risk of knee osteoarthritis progression: results from the osteoarthritis initiative. Osteoarthr Cartil. 2019;27:855–62.
86. McAlindon TE, LaValley MP, Harvey WF, Price LL, Driban JB, Zhang M, Ward RJ. Effect of intra-articular triamcinolone vs saline on knee cartilage volume and pain in patients with knee osteoarthritis: a randomized clinical trial. JAMA. 2017;317:1967–75.
87. Malemud CJ. Anticytokine therapy for osteoarthritis: evidence to date. Drugs Aging. 2010;27:95–115.
88. Kim J-R, Yoo JJ, Kim HA. Therapeutics in osteoarthritis based on an understanding of its molecular pathogenesis. Int J Mol Sci. 2018;19(3):674. https://doi.org/10.3390/ijms19030674.
89. Furumatsu T, Matsumoto E, Kanazawa T, Fujii M, Lu Z, Kajiki R, Ozaki T. Tensile strain increases expression of CCN2 and COL2A1 by activating TGF-β-Smad2/3 pathway in chondrocytic cells. J Biomech. 2013;46:1508–15.
90. Bougault C, Aubert-Foucher E, Paumier A, Perrier-Groult E, Huot L, Hot D, Duterque-Coquillaud M, Mallein-Gerin F. Dynamic compression of chondrocyte-agarose constructs reveals new candidate mechanosensitive genes. PLoS One. 2012;7:e36964.
91. Yang X, Chen L, Xu X, Li C, Huang C, Deng CX. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. J Cell Biol. 2001;153:35–46.
92. Ballock RT, Heydemann A, Wakefield LM, Flanders KC, Roberts AB, Sporn MB. TGF-beta 1 prevents hypertrophy of epiphyseal chondrocytes: regulation of gene expression for cartilage matrix proteins and metalloproteases. Dev Biol. 1993;158:414–29.
93. van de Laar IMBH, Oldenburg RA, Pals G, et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet. 2011;43:121–6.
94. Yao J-Y, Wang Y, An J, Mao C-M, Hou N, Lv Y-X, Wang Y-L, Cui F, Huang M, Yang X. Mutation analysis of the Smad3 gene in human osteoarthritis. Eur J Hum Genet. 2003;11:714–7.
95. Patwari P, Cook MN, DiMicco MA, Blake SM, James IE, Kumar S, Cole AA, Lark MW, Grodzinsky AJ. Proteoglycan degradation after injurious compression of bovine and human articular cartilage in vitro: interaction with exogenous cytokines. Arthritis Rheum. 2003;48:1292–301.
96. Zhen G, Wen C, Jia X, et al. Inhibition of TGF-β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. Nat Med. 2013;19:704–12.
97. Blaney Davidson EN, Vitters EL, van der Kraan PM, van den Berg WB. Expression of transforming growth factor-beta (TGFbeta) and the TGFbeta 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.
98. Loeser RF, Chubinskaya S, Pacione C, Im H-J. Basic fibroblast growth factor inhibits the anabolic activity of insulin-like growth factor 1 and osteogenic protein 1 in adult human articular chondrocytes. Arthritis Rheum. 2005;52:3910–7.

99. Im H-J, Li X, Muddasani P, Kim G-H, Davis F, Rangan J, Forsyth CB, Ellman M, Thonar EJ. Basic fibroblast growth factor accelerates matrix degradation via a neuro-endocrine pathway in human adult articular chondrocytes. J Cell Physiol. 2008;215:452–63.
100. Im H-J, Muddasani P, Natarajan V, Schmid TM, Block JA, Davis F, van Wijnen AJ, Loeser RF. Basic fibroblast growth factor stimulates matrix metalloproteinase-13 via the molecular cross-talk between the mitogen-activated protein kinases and protein kinase Cdelta pathways in human adult articular chondrocytes. J Biol Chem. 2007;282:11110–21.
101. Wang X, Manner PA, Horner A, Shum L, Tuan RS, Nuckolls GH. Regulation of MMP-13 expression by RUNX2 and FGF2 in osteoarthritic cartilage. Osteoarthr Cartil. 2004;12:963–73.
102. Ellman MB, Yan D, Ahmadinia K, Chen D, An HS, Im HJ. Fibroblast growth factor control of cartilage homeostasis. J Cell Biochem. 2013;114:735–42.
103. Hochberg M, Guermazi A, Guehring H, Aydemir A, Wax S, Fleuranceau-Morel P, Reinstrup Bihlet A, Byrjalsen I, Ragnar Andersen J, Eckstein F. OP0059 efficacy and safety of intra-articular sprifermin in symptomatic radiographic knee osteoarthritis: pre-specified analysis of 3-year data from a 5-year randomised, placebo-controlled, phase II study. Wednesday 13 June 2018. BMJ Publishing Group Ltd and European League Against Rheumatism. 2018:80–1.
104. Teufel S, Hartmann C. Wnt-signaling in skeletal development. Curr Top Dev Biol. 2018;133:235–79. https://doi.org/10.1016/bs.ctdb.2018.11.010.
105. Yazici Y, McAlindon TE, Gibofsky A, et al. Results from a 52-week randomized, double-blind, placebo-controlled, phase 2 study of a novel, intra-articular wnt pathway inhibitor (SM04690) for the treatment of knee osteoarthritis. Osteoarthr Cartil. 2018;26:S293–4.
106. Zhu M, Chen M, Zuscik M, Wu Q, Wang Y-J, Rosier RN, O'Keefe RJ, Chen D. Inhibition of beta-catenin signaling in articular chondrocytes results in articular cartilage destruction. Arthritis Rheum. 2008;58:2053–64.
107. Saito T, Kawaguchi H. HIF-2α as a possible therapeutic target of osteoarthritis. Osteoarthr Cartil. 2010;18:1552–6.
108. Loeser RF. Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. Osteoarthr Cartil. 2009;17:971–9.
109. Salminen A, Kaarniranta K, Kauppinen A. Inflammaging: disturbed interplay between autophagy and inflammasomes. Aging (Albany, NY). 2012;4:166–75.
110. Franceschi C, Capri M, Monti D, et al. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev. 2007;128:92–105.
111. Martin JA, Brown TD, Heiner AD, Buckwalter JA. Chondrocyte senescence, joint loading and osteoarthritis. Clin Orthop Relat Res. 2004;427:S96–103.
112. Martin JA, Buckwalter JA. Aging, articular cartilage chondrocyte senescence and osteoarthritis. Biogerontology. 2002;3(5):257–64.
113. GBD 2015 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388:1603–58.
114. Dieleman JL, Baral R, Birger M, et al. US spending on personal health care and public health, 1996–2013. JAMA. 2016;316:2627–46.
115. Kennon JC, Awad ME, Chutkan N, DeVine J, Fulzele S. Current insights on use of growth factors as therapy for Intervertebral Disc Degeneration. Biomol Concepts. 2018;9:43–52.
116. Sakai D, Grad S. Advancing the cellular and molecular therapy for intervertebral disc disease. Adv Drug Deliv Rev. 2015;84:159–71.
117. Dowdell J, Erwin M, Choma T, Vaccaro A, Iatridis J, Cho SK. Intervertebral disk degeneration and repair. Neurosurgery. 2017;80:S46–54.
118. Zhao C-Q, Wang L-M, Jiang L-S, Dai L-Y. The cell biology of intervertebral disc aging and degeneration. Ageing Res Rev. 2007;6:247–61.

119. Adams MA, Roughley PJ. What is intervertebral disc degeneration, and what causes it? Spine. 2006;31:2151–61.
120. James R, Kesturu G, Balian G, Chhabra AB. Tendon: biology, biomechanics, repair, growth factors, and evolving treatment options. J Hand Surg Am. 2008;33:102–12.
121. Longo UG, Ronga M, Maffulli N. Achilles tendinopathy. Sports Med Arthrosc. 2009;17:112–26.
122. Xu Y, Murrell GAC. The basic science of tendinopathy. Clin Orthop Relat Res. 2008;466:1528–38.
123. Magnusson SP, Kjaer M. The impact of loading, unloading, ageing and injury on the human tendon. J Physiol Lond. 2019;597:1283–98.
124. Cook JL, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. Br J Sports Med. 2009;43:409–16.
125. Cook JL, Rio E, Purdam CR, Docking SI. Revisiting the continuum model of tendon pathology: what is its merit in clinical practice and research? Br J Sports Med. 2016;50:1187–91.
126. Rudavsky A, Cook J. Physiotherapy management of patellar tendinopathy (jumper's knee). J Physiother. 2014;60:122–9.
127. Puddu G, Ippolito E, Postacchini F. A classification of Achilles tendon disease. Am J Sports Med. 1976;4:145–50.
128. Rees JD, Stride M, Scott A. Tendons–time to revisit inflammation. Br J Sports Med. 2014;48:1553–7.
129. Schubert TEO, Weidler C, Lerch K, Hofstädter F, Straub RH. Achilles tendinosis is associated with sprouting of substance P positive nerve fibres. Ann Rheum Dis. 2005;64:1083–6.
130. Millar NL, Hueber AJ, Reilly JH, Xu Y, Fazzi UG, Murrell GAC, McInnes IB. Inflammation is present in early human tendinopathy. Am J Sports Med. 2010;38:2085–91.
131. Matthews TJW, Hand GC, Rees JL, Athanasou NA, Carr AJ. Pathology of the torn rotator cuff tendon. Reduction in potential for repair as tear size increases. J Bone Joint Surg Br. 2006;88:489–95.
132. Barbe MF, Barr AE, Gorzelany I, Amin M, Gaughan JP, Safadi FF. Chronic repetitive reaching and grasping results in decreased motor performance and widespread tissue responses in a rat model of MSD. J Orthop Res. 2003;21:167–76.
133. Dakin SG, Martinez FO, Yapp C, et al. Inflammation activation and resolution in human tendon disease. Sci Transl Med. 2015;7:311ra173.
134. Millar NL, Murrell GAC, McInnes IB. Inflammatory mechanisms in tendinopathy – towards translation. Nat Rev Rheumatol. 2017;13:110–22.
135. Tang C, Chen Y, Huang J, Zhao K, Chen X, Yin Z, Heng BC, Chen W, Shen W. The roles of inflammatory mediators and immunocytes in tendinopathy. J Orthop Translat. 2018;14:23–33.