

Regenerative Medicine for Spine and Joint Pain

Grant Cooper
Joseph Herrera
Jason Kirkbride
Zachary Perlman
Editors



Springer

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Grant Cooper
Princeton Spine and Joint Center
Princeton, NJ
USA

Jason Kirkbride
Princeton Spine and Joint Center
Princeton, NJ
USA

Joseph Herrera
Department of Rehabilitation Medicine
Mount Sinai Hospital
New York, NY
USA

Zachary Perlman
Princeton Spine and Joint Center
Princeton, NJ
USA

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Contributors

Walter Alomar-Jimenez, MD, JD Department of Rehabilitation and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Allison C. Bean, MD, PhD Department of Physical Medicine and Rehabilitation, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Tina Bijlani, DO Rehabilitation Medicine, The Mount Sinai Hospital, Mount Sinai, New York, NY, USA

Eliana Cardozo, DO Sports Medicine and Interventional Spine, Department of Rehabilitation Medicine and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Richard G. Chang, MD, MPH Department of Rehabilitation & Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Grant Cooper, MD Princeton Spine and Joint Center, Princeton, NJ, USA

Andrew Creighton, DO Physiatry, Hospital for Special Surgery, Rehabilitation Medicine, Weill Cornell Medicine, New York, NY, USA

Emily N. Fatakhov, MD Rehabilitation Medicine, The Mount Sinai Hospital, Mount Sinai, New York, NY, USA

Alfred C. Gellhorn, MD Department of Rehabilitation Medicine, Weill Cornell Medicine, New York, NY, USA

Michael Guthrie, MD University of Pittsburgh Medical Center, Department of PM&R, Pittsburgh, PA, USA

Behnum Habibi, MD NewYork-Presbyterian University Hospital, Columbia University Medical Center and Weill Cornell Medical Center, New York, NY, USA

Joseph Herrera, DO Department of Rehabilitation Medicine, Mount Sinai Hospital, New York, NY, USA

Jason Kirkbride, MD Princeton Spine and Joint Center, Princeton, NJ, USA

Jonathan S. Kirschner, MD Physiatry, Hospital for Special Surgery, Rehabilitation Medicine, Weill Cornell Medicine, New York, NY, USA

Anthony J. Mazzola, MD Department of Rehabilitation and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Dayna McCarthy, DO Sports Medicine and Interventional Spine, Department of Rehabilitation and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Anokhi Mehta, MD Department of Rehabilitation and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Gerardo Miranda-Comas, MD Department of Rehabilitation and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Kentaro Onishi, DO University of Pittsburgh School of Medicine, Department of PM&R, Department of Orthopaedic Surgery, Pittsburgh, PA, USA

Zachary Perlman, DO Princeton Spine and Joint Center, Princeton, NJ, USA

Jonathan Ramin, DO Sports Medicine and Interventional Spine, Department of Rehabilitation Medicine and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Krutika Parasar Raulkar, MD Department of Physical Medicine and Rehabilitation, NewYork-Presbyterian Hospital – The University Hospital of Columbia and Cornell, New York, NY, USA

Stephen Schaaf, MD University of Pittsburgh Medical Center, Department of PM&R, Pittsburgh, PA, USA

Caroline Schepker, DO NewYork-Presbyterian University Hospital, Columbia University Medical Center and Weill Cornell Medical Center, New York, NY, USA

Allison N. Schroeder, MD University of Pittsburgh Medical Center, Department of PM&R, Pittsburgh, PA, USA

David A. Spinner, DO, RMSK, CIPS, FAAPMR Department of Rehabilitation and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Katherine V. Yao, MD Weill Cornell Medical College, New York, NY, USA

Xiaoning (Jenny) Yuan, MD, PhD Department of Rehabilitation Medicine, NewYork-Presbyterian Hospital, New York, NY, USA

Mariam Zakhary, DO Department of Rehabilitation and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Nadia N. Zaman, DO Sports Medicine and Interventional Spine, Department of Rehabilitation and Human Performance, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Chapter 1

Introduction to Regenerative Medicine



Grant Cooper, Joseph Herrera, Jason Kirkbride, and Zachary Perlman

Regenerative Medicine Intro Combined

Patients suffering from musculoskeletal ailments frequently seek additional treatment options after more traditional methods have failed. Though eager for alternative methods, they may have reservations over the safety and efficacy of the broad range of regenerative medicine techniques, which can make regenerative medicine a somewhat controversial topic [1, 2]. A large pool of anecdotal evidence exists, but there is no standardization of techniques, and evidence-based research has strained to catch up (Table 1.1). As is the case with cutting-edge treatments, research is continually emerging. By reviewing, evaluating, and exploring the current state of regenerative medicine research, we hope to provide a foundation upon which the practitioner can converse with the patient. Organizing the book based on the anatomic site of injury will allow the medical practitioner to easily reference evidence-based regenerative medicine treatment options and help guide open discussion with their patients about additional treatments that may be appropriate to offer.

In the broad sense of the term, “regenerative medicine” is delivering cells or products to diseased tissues or organs in the attempt to restore tissue or organ function. What we are interested in is connective tissue and bone regeneration [3]. The rationale for using these therapies is that the injected product will stimulate repair of these damaged structures as opposed to only treating the patient’s symptoms. To understand these regenerative options, it is important to look back at the history of platelet-rich plasma (PRP) and stem cell therapy.

G. Cooper (✉) · J. Kirkbride · Z. Perlman
Princeton Spine and Joint Center, Princeton, NJ, USA
e-mail: drcooper@princetonajc.com

J. Herrera
Department of Rehabilitation Medicine, Mount Sinai Hospital, New York, NY, USA

Table 1.1 Regenerative Medicine Questions

What is the particular “injectate”?
Are we using leukocyte-reduced or leukocyte-enriched PRP?
How are you harvesting your stem cells?
Bone marrow aspirate, bone marrow concentrate, or mesenchymal from adipose tissue?
What about embryonic products?
Which therapy is the most powerful “regenerator”?
How do we price them?
How much volume is just right for your patient?
Do they need a single or series of multiple injections?
If we are treating an arthritic condition, then what stage of arthritis responds best?
How active is the patient?/What level of competition?
Gender?
What is the post-injection rehabilitation protocol?
As you can see, there are many possible combinations of answers to the above questions, which makes this field very intimidating to patients and practitioners because of the many variables.

Platelet-rich plasma was developed in the field of hematology in the 1970s [4]. PRP releases growth factors, which are also known as bioactive proteins. These proteins aid in stimulating the body’s natural ability to heal. Hematologists were treating thrombocytopenia with a product that was plasma with a platelet count higher than peripheral blood. In the late 1980s, it was used during open heart surgery. Then, in the 1990s, maxillofacial surgeons were using PRP to aid in healing skin flaps. Next, it was used in musculoskeletal medicine. The first documented case in *Sports Medicine* was in 1999, when Dr. Allan Mishra used PRP to treat San Francisco 49ers quarterback Steve Bono’s Achilles tendon injury. In 2006, PRP use for elbow tendonitis was published in the *American Journal of Sports Medicine*. That study showed 60% improvement in pain levels immediately, 81% improvement in 6 months, and 93% improvement at 2 years. This is when PRP gained significant popularity and many well-known professional athletes began using PRP therapy including Kobe Bryant and Tiger Woods [5]. It then gained popularity among orthopedic surgeons for treating fracture nonunion, arthritis, tendonitis, muscle strains, cartilage injuries, and more [6]. Today, PRP is being used in pediatric surgery, gynecology, urology, plastic surgery, dermatology, and ophthalmology.

Stem cells are cells that have the ability to differentiate or change into a particular cell. A specific type of stem cell known as a mesenchymal stem cell can transform into a bone, cartilage, muscle, or fat cell. The first scientists who defined the key properties of stem cells were Ernest McCulloch and James Till in the 1960s. They discovered that the cells can divide and differentiate into mature cell types [7]. Then, in 1996, scientists were crossing ethical boundaries when attempting to clone “Dolly the sheep” by using stem cells. In the early 2000s, Dr. Shinya Yamanaka discovered skin cells can be converted into stem cells by altering gene expression. This was the birth of induced pluripotent stem cells, or iPS. Since then, stem cells have been used in musculoskeletal medicine and many other areas including gene therapy for inheritable disorders.

As you will find, the research is not unambiguous. Patients who have been failed by more traditional treatment options are frequently desperate for additional potential treatments. Demand for regenerative medicine is growing as the amount of evidence increases. Oftentimes, patients are initiating the conversation about regenerative medicine and it is important for the physician to be well prepared for such a discussion. Practitioners must be ready to acknowledge the lack of clear-cut evidence at times and be open to frank discussions regarding the risks of treatment and potential benefits [8]. Informed consent is paramount and cannot be stressed enough. The ability to counsel on the risks and benefits of different regenerative medicine techniques based on the current literature is the first step to offering regenerative medicine treatment options. Though it is important to remain hopeful that regenerative treatment will allow for improvements when more conservative measures have failed, it is essential to develop realistic goals with the patient.

As the number of degenerative and chronic conditions continues to climb among the population, demand for regenerative medicine is increasing. Regenerative medicine and tissue engineering have been identified as top research priorities by the Medical Research Council in the United Kingdom and the National Research Council of the United States [9]. With increased interest and research into regenerative medicine, the ambition to transition healthcare from a focus on symptomatic treatment to a more curative treatment approach grows [10]. Because of this growing expectation, significant controversies exist. Concerns include research misconduct and tumor development [11, 12], while unproven therapies are creating an entire stem cell tourism industry with little safety oversight for patients desperate for therapeutic treatment [13]. In addition, there are multiple manufacturers of the systems that isolate the injectate, so not every physician offering regenerative medicine is using the same concentration of growth factors. Another major barrier to administering regenerative medicine to patients is that insurance companies generally do not cover these injections. It is difficult to say if the Food and Drug Administration (FDA) could administer an approval since “the injectate” is not a product of a pharmaceutical laboratory, but it stems from the patient themselves. Despite these obstacles, regenerative medicine continues to make progress with regard to safety and its use of evidence-based treatment options. As the number of clinical trials continue to increase, regenerative medicine is at the cutting edge of translational research and will require a collaborative effort among a vast array of interdisciplinary researchers and clinicians [14]. This further cements the need for an evidence-based, practitioner-friendly guide to regenerative treatments.

References

1. Matthews KR, Iltis AS. Unproven stem cell-based interventions and achieving a compromise policy among the multiple stakeholders. *BMC Med Ethics*. 2015;16:75. <https://doi.org/10.1186/s12910-015-0069-x>.
2. Alta CR. On the road (to a cure?) — stem-cell tourism and lessons for gene editing. *N Engl J Med*. 2016;374:901–3. <https://doi.org/10.1056/NEJMp1600891>.

3. <https://www.mayo.edu/research/centers-programs/center-regenerative-medicine/patient-care/about-regenerative-medicine>.
4. Andia I, Abate M. Platelet rich plasma: underlying biology and clinical correlates. *Regen Med*. 2013;8:645–58.
5. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sports Med*. 2006;34(11):1774–8.
6. <https://www.movementortho.com/2017/11/03/the-history-of-prp-therapy/>.
7. <http://sitn.hms.harvard.edu/flash/2014/stem-cells-a-brief-history-and-outlook-2/>.
8. Bubela T, Li MD, Hafez M, Bieber M, Atkins H. Is belief larger than fact: expectations, optimism and reality for translational stem cell research. *BMC Med*. 2012;10:133. <https://doi.org/10.1186/1741-7015-10-133>.
9. O'Dowd A. Peers call for UK to harness “enormous” potential of regenerative medicine. *BMJ*. 2013;347:f4248. <https://doi.org/10.1136/bmj.f4248>.
10. Nelson TJ, Behfar A, Terzic A. Strategies for therapeutic repair: the “R3” regenerative medicine paradigm. *Clin Transl Sci*. 2008;1:168–71.
11. Berkowitz AL, Miller MB, et al. Glioproliferative lesion of the spinal cord as a complication of “stem-cell tourism”. *N Engl J Med*. 2016;375:196–8. <https://doi.org/10.1056/NEJMc1600188>.
12. Wang Y, Han Z-B, Song Y-P, Han ZC. Safety of mesenchymal stem cells for clinical application. *Stem Cells Int*. 2012;2012:4. <https://doi.org/10.1155/2012/652034.652034>.
13. Brown C. Stem cell tourism poses risks. *CMAJ*. 2012;184(2):E121–2. <https://doi.org/10.1503/cmaj.109-4073>.
14. Jessop ZM, et al. Transforming healthcare through regenerative medicine. *BMC Med*. 2016;14(1):115. <https://doi.org/10.1186/s12916-016-0669-4>.

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

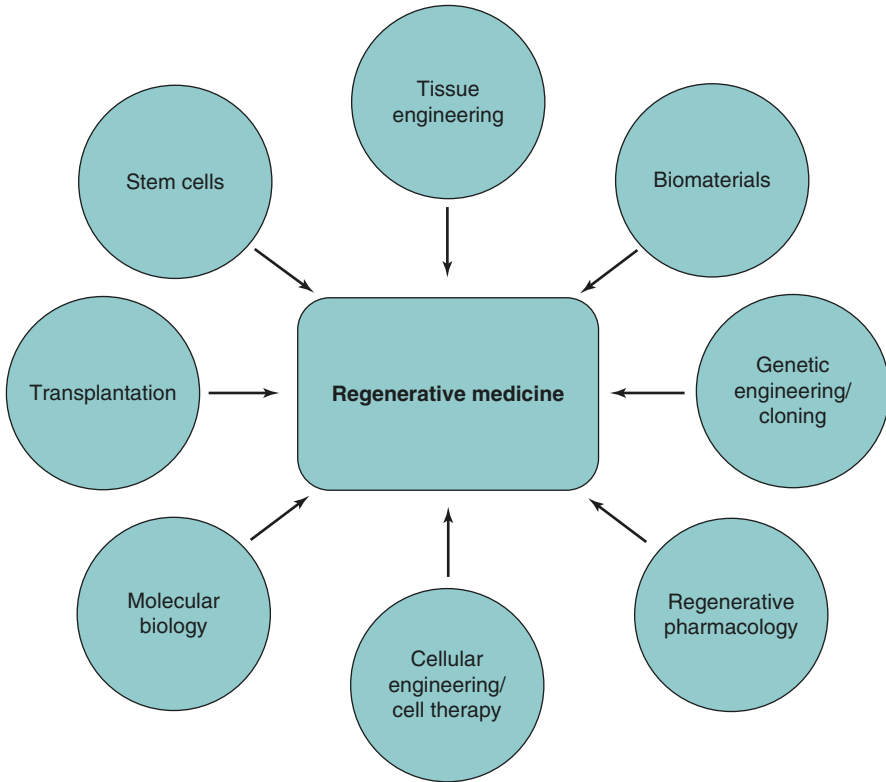


Fig. 2.1 Schematic representation of the various components of regenerative medicine. (Reprinted from Yalcinkaya et al. [1], with permission from Elsevier)

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

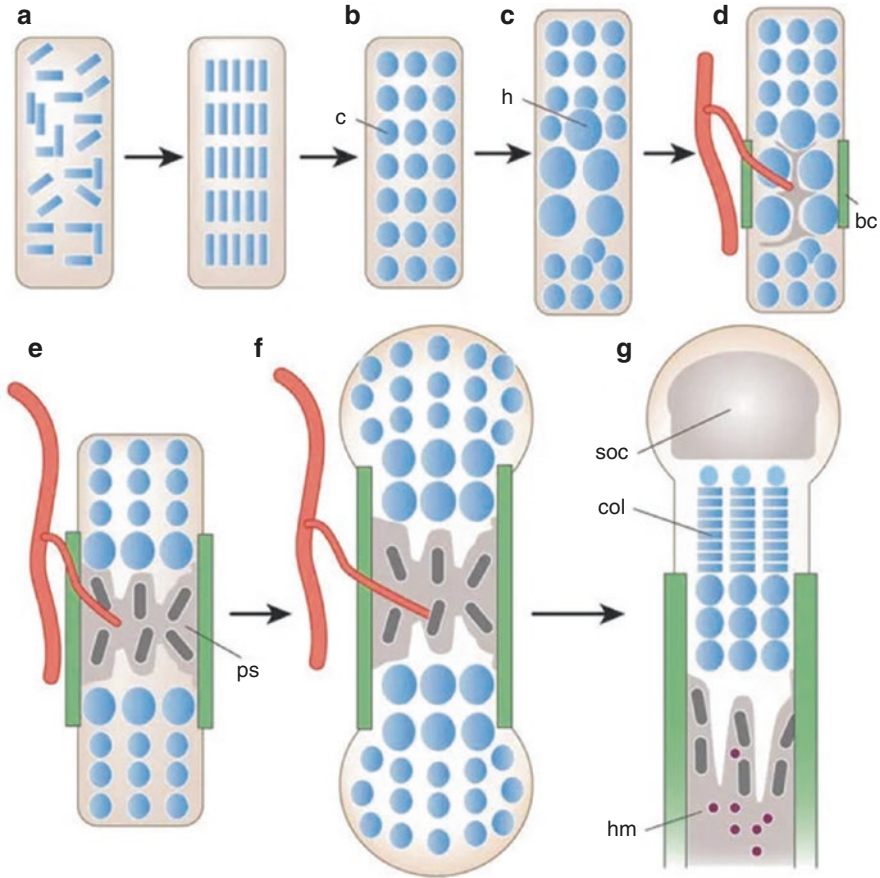


Fig. 2.2 Endochondral bone formation. (a) Mesenchymal cells condense. (b) Cells of condensations become chondrocytes (c). (c) Chondrocytes at the center of condensation stop proliferating and become hypertrophic (h). (d) Perichondrial cells adjacent to hypertrophic chondrocytes become osteoblasts, forming 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 rostro-caudal axis 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-1 β 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- κ B) 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

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-1 α 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-2 α through NF- κ B. HIF-2 α induces chondrocyte hypertrophic differentiation by increasing Runx2, IHH, and VEGF expression and cartilage degradation by increasing MMP and ADAMTS expression [107]. Suppressing HIF-2 α while maintaining HIF-1 α 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 “chondroscenescence” 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. Chondroscenescence 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- κ B signaling pathway, increasing production of pro-inflammatory cytokines including IL-1 β and TNF- α . Autophagy may be further suppressed in OA through inhibition of the HIF-2 α 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 chondroscenescence 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].