TENDON AND LIGAMENT

Structure

Tendons and ligaments are both dense, regularly arranged connective tissues. The surface of the tendon is enveloped in a white, glistening, synovial-like membrane, called the epitenon, which is continuous on its inner surface with the endotenon, a thin layer of connective tissue that binds collagen fibers and contains lymphatics, blood vessels, and nerves. In some tendons, the epitenon is surrounded by a loose areolar tissue called the paratenon, which functions as an elastic sheath through which the tendon can slide. In some tendons, the paratenon is replaced by a true synovial sheath or bursa consisting of two layers lined by synovial cells, called the tenosynovium, within which the mesotendon carries important blood vessels to the tendon. In the absence of a synovial lining, the paratenon often is called a tenovagina. Together the epitenon and the paratenon compose the peritenon (Fig. 1.1). The blood supply to tendons has several sources, including the perimysium, periosteal attachments, and surrounding tissues. Blood supplied through the surrounding tissues reaches the tendon through the paratenon, mesotenon, or vincula. Vascular tendons are surrounded by a paratenon and receive vessels along their borders; these vessels then coalesce within the tendon. The relatively avascular tendons are contained within tendinous sheaths, and the mesotenons within these sheaths function as vascularized conduits called vincula. The muscle-tendon and tendon-bone junctions, along with the mesotenon, are the three types of vascular supply to the tendon inside the sheath. Other sources of nutrition include diffusional pathways from the synovial fluid, which provide an important supply of nutrients for the flexor tendons of the hand, for example. The nervous supply to a tendon involves mechanoreceptors located near the musculotendinous junction, which provide proprioceptive feedback to the central nervous system.

Ligaments grossly appear as firm, white fibrous bands, sheets, or thickened strips of joint capsule securely anchored to bone. They consist of a proximal bone insertion, the substance of the ligament or the capsule, and a distal bone insertion. Because most insertions are no more than 1 mm thick, they contribute only a small amount to the volume and the length of the ligament. Bundles of collagen fibrils form the bulk of the ligament substance. Some ligaments consist of more than one band of collagen fibril bundles. For example, the anterior cruciate ligament (ACL) has a continuum of fiber lengths; different fibers become taut throughout the range of motion. The alignment of collagen fiber bundles within the ligament substance generally follows the lines of tension applied to the ligament. This is in contrast to the alignment of collagen fiber bundles within the tendon, which is generally parallel to its longitudinal axis. In addition, thinner collagen fibrils extend the entire length of the tendon. Light microscopic examination has shown that the collagen bundles have a wave or crimp pattern. The crimp pattern of matrix organization may allow slight elongation of the ligament without incurring damage to the tissue. In some regions, the ligament cells align themselves in rows between collagen fiber bundles, but in other regions, the cells lack apparent orientation relative to the alignment of the matrix collagen fibers. Scattered blood vessels penetrate the ligament substance, forming small-diameter, longitudinal vascular channels that lie parallel to the collagen bundles. Nerve fibers lie next to some vessels, and, like tendon, nerve endings with the structure of mechanoreceptors have been found in some ligaments.

Tendon and ligament insertions vary in size, strength, angle of the ligament collagen fiber bundles relative to the bone, and proportion of ligament collagen fibers that penetrate directly into bone. Based on the angle between the collagen fibrils and the bone and the proportion of the collagen fibers that penetrate directly into bone, investigators group tendon and ligament insertions into two types: direct and indirect. Direction insertions typically occur at the apophysis or epiphysis of bone, often within or around a synovial joint, and consist of sharply defined regions where the collagen fibers appear to pass directly into the cortex of the bone. Although the thin layer of superficial collagen fibers of direct insertions joins the fibrous layer of the periosteum, most of the tendon or ligament insertions consist of deeper fibers that directly penetrate the cortex, often at a right angle to the bone surface. The deeper collagen fibers pass through four zones with increasing stiffness: ligament substance, fibrocartilage, mineralized fibrocartilage, and bone. This four-zone interface is known as the fibrocartilaginous enthesis. Dissipation of force is achieved effectively through this gradual transition from tendon to fibrocartilage to bone. A larger area of fibrocartilage can be found on one side of the insertion, which is thought to be an adaptation to the compressive forces experienced by the tendon or ligament on that side. Conversely, indirect or oblique insertions, such as the tibial insertion of the medial collateral ligament of the knee or the femoral insertion of the lateral collateral ligament, typically occur at the metaphysis.
Abstract
Musculoskeletal structures contain tissue-specific cells, extracellular matrix, and fiber arrangements which impart their unique biologic and mechanical properties. Tendon, ligament, meniscus, articular cartilage, and bone all have different structures that determine their specific function. Furthermore, the healing potential and response to injury of these tissues is highly variable and dependent on a number of factors, including the presence of a surrounding vasculature and the ability of intrinsic cells to replicate and remodel the injured matrix. In this chapter, we review the structure, biology, healing response to injury, and potential augmentative therapies of tendon, ligament, meniscus, articular cartilage, and bone.

Keywords
- tendon
- ligament
- meniscus
- cartilage
- articular
- bone
- physiology
or diaphysis of bone without an intervening fibrocartilaginous zone. They usually cover more bone surface area than do direct insertions, and their boundaries cannot be easily defined because the collagen fibers pass obliquely along the bone surface rather than directly into the cortex.

### Extracellular Matrix

Tendons and ligaments consist of relatively few cells and an abundant extracellular matrix primarily containing collagen, proteoglycans, and water. Tenocytes (tendon-specialized fibroblasts) are the dominant cell of tendons, whereas fibroblasts are the dominant cells of ligaments. Tenocytes and fibroblasts form and maintain the extracellular matrix. Within ligaments, fibroblasts vary in shape, activity, and density among regions of the same tissue and with the age of the tissue. Both tenocytes and fibroblasts are spindle shaped, with fibroblasts being rounder, and extend between the collagen fibrils. Endothelial cells of small vessels and nerve cell processes are also present. Studies have shown that tendon and ligament contain a small population of resident stem cells which function to maintain tissue homeostasis during growth and repair.

Type I collagen, which is the major component of the molecular framework, composes more than 90% of the collagen content of ligaments. Type III collagen constitutes approximately 10% of the collagen, and small amounts of other collagen types also may be present. Ligaments have a higher content of type III collagen than do tendons. All types of collagen have in common a triple helical domain, which is combined differently with globular and nonhelical structural elements. The triple helix conformation of collagen is stabilized mainly by hydrogen bonds between glycine residues and between hydroxyl groups of hydroxyproline. This helical conformation is reinforced by hydroxyproline-forming and proline-forming hydrogen bonds to the other two chains. The physical properties of collagen and its resistance to enzymatic and chemical breakdown rely on covalent cross-links within and between the molecules.

Elastin is a protein that allows connective tissues to undergo large changes in geometry while expending little energy in the process. Tendons of the extremities possess small amounts of this structural protein, whereas most ligaments have little elastin (usually less than 5%), although a few, such as the nuchal ligament and the ligamentum flavum, have high concentrations (up to 75%). In most tendons, elastin is found primarily at the fascicle surface, comprising less than 1% of the tendon by dry weight, and it is responsible for the crimp pattern of the tendon when viewed by a light microscope. Elastin forms protein fibrils or sheets, but elastin fibrils lack the cross-banding pattern of fibrillar collagen and differ in amino acid composition, including two amino acids not found in collagen (desmosine and isodesmosine). In addition, unlike collagen, elastin amino acid chains form random coils when the molecules are unloaded. This conformation of the amino acid chains makes it possible for elastin to undergo some deformation without rupturing or tearing and then, when the load is removed, to return to its original size and shape.

Approximately 1% of the total dry weight of tendon and ligament is composed of ground substance, which consists of proteoglycans, glycosaminoglycans, structural glycoproteins, plasma proteins, and a variety of small molecules. Most ligaments have a higher concentration of glycosaminoglycans than do tendons, due to the functional need for more rapid adaptation. Proteoglycans and glycosaminoglycans both have important roles in organizing the extracellular matrix and control the water content of the tissue. Tendon and ligaments contain two known classes of proteoglycans. Larger proteoglycans contain long negatively charged chains of chondroitin and keratan sulfate. Smaller proteoglycans contain dermatan sulfate. Because of their long chains of negative charges, the large articular cartilage-type proteoglycans tend to expand to their maximal domain in solution until restrained by the collagen fibril network. As a result, they maintain water within the tissue and exert a swelling pressure, thereby contributing to the mechanical properties of the tissue and filling the regions between the collagen fibrils. The small leucine-rich proteoglycans usually lie directly on the surface of collagen fibrils and appear to affect formation, organization, and stability of the extracellular matrix, including collagen fibril formation and diameter. They may also control the activity of growth factors by direct association.

Although noncollagenous proteins contribute only a small percentage of the dry weight of dense fibrous tissues, they appear to help organize and maintain the macromolecular framework of the collagen matrix, aid in the adherence of cells to the framework, and possibly influence cell function. One noncollagenous protein, fibronectin, has been identified in the extracellular matrix of ligaments and may be associated with several matrix component molecules and with blood vessels. Other noncollagenous proteins undoubtedly exist within the matrix, but their identity and their functions have not yet been defined. Many of the noncollagenous proteins also contain a few monosaccharides and oligosaccharides.

### Injury

Acute strains and tears to tendons and ligaments disrupt the matrix, damage blood vessels, and injure or kill cells. Damage to cells, matrices, and blood vessels and the resulting hemorrhage...
start a response that leads to a sequential process of inflammation, repair, and remodeling. These events form a continuous sequence of cell, matrix, and vascular changes that begins with the release of inflammatory mediators and ends when remodeling ceases. As with any injury to biologic tissue, acute inflammation lasts 48 to 72 hours after the injury and then gradually resolves as repair progresses. Some of the events that occur during inflammation, including the release of cytokines or growth factors, may help to stimulate tissue repair. These mediators promote vascular dilation and increase vascular permeability, leading to exudation of fluid from vessels in the injured region, which causes tissue edema. Blood escaping from the damaged vessels forms a hematoma that temporarily fills the injured site. Fibrin accumulates within the hematoma, and platelets bind to fibrillar collagen, thereby achieving hemostasis and forming a clot consisting of fibrin, platelets, red cells, and cell and matrix debris. The clot provides a framework for vascular and fibroblast cell invasion. As they participate in clot formation, platelets release vasoactive mediators and various cytokines or growth factors (e.g., transforming growth factor-β [TGF-β] and platelet-derived growth factor). Polymorphonuclear leukocytes appear in the damaged tissue and the clot. Shortly thereafter, monocytes arrive and increase in number until they become the predominant cell type. Enzymes released from the inflammatory cells help to digest necrotic tissue, and monocytes phagocytose small particles of necrotic tissue and cell debris. Endothelial cells near the injury site begin to proliferate, creating new capillaries that grow toward the region of tissue damage. Release of chemotactic factors and cytokines from endothelial cells, monocytes, and other inflammatory cells helps to stimulate migration and proliferation of the fibroblasts that begin the repair process.

Overuse tendon injury is one of the more common forms of musculoskeletal injury and clinical causes of pain, although controversy exists in the literature about a universal classification and the responsible pathologic entities. A classification of Achilles tendon disorders provides a guide to the structural manifestations of overuse injury as follows: (1) peritendinitis, or inflammation of the peritenon; (2) tendinosis with peritendinitis; (3) tendinosis without peritendinitis; (4) partial rupture; and (5) total rupture. Other classifiers have added a sixth category, tendinitis, in which the primary site of injury is the tendon, with an associated reactive peritendinitis. The classification is not universal because some tendons lack a paratenon and instead have synovial sheaths; furthermore, it is unclear if certain histopathologic conditions are actually separate entities. For instance, human biopsy studies have been unable to show histologic evidence of acute inflammation within the tendon substance. Because of uncertainty regarding the histologic features of these conditions, several authors have suggested use of the term tendinopathy rather than tendinitis.

Studies have shown that in cases of chronic tendinosis, the pathologic lesion is typical of a degenerative process rather than an inflammatory one and that this degeneration occurs in areas of diminished blood flow. Several authors have documented the existence of areas of marked degeneration without acute or chronic inflammatory cell accumulation in most of these cases. These changes are separate and distinct from the site of rupture. A review of patients with chronic tendinitis syndrome revealed similar findings of tendon degeneration. Nirschl described the pathology of chronic tendinitis as “angiofibroblastic hyperplasia.” A characteristic pattern of fibroblasts and vascular, atypical, granulation-like tissue can be seen microscopically. Cells characteristic of acute inflammation are virtually absent. These observations suggest that factors other than mechanical overuse play an important role in the pathogenesis of these tendon lesions.

In several studies, a correlation between age and the incidence of chronic tendinopathy has been identified. In vitro studies have shown decreased proliferative and metabolic responses of aging tendon tissue. Other causative factors include the lack of blood flow in certain areas (e.g., supraspinatus and Achilles tendon) that may predisperse a tendon to rupture or may result in chronic tendinopathy. Biopsy specimens of young patients with symptoms of chronic tendinopathy have revealed a change in the morphology of tenocytes adjacent to areas of collagen degeneration.

**Repair**

Tendons and ligaments may possess both intrinsic and extrinsic capabilities for healing, and the contribution of each of these two mechanisms probably depends on the location, extent, and mechanism of injury and the rehabilitation program used after the injury. Several studies have suggested that the inflammatory response is not essential to the healing process and that these tissues possess an intrinsic capacity for repair. Recent research has isolated intrinsic stem cells within tendon and ligament, although their in vivo identities, niche, and role in healing remain controversial. Lindsay and Thomson were the first to show that an experimental tendon suture zone can be isolated from the perisheath tissues and that healing progressed at the same rate as when the perisheath tissues were intact. Later, in isolated segments of profundus tendon in rabbits, these researchers found anabolic and catabolic enzymes, which showed that an active metabolic process existed in the isolated tendon segments.

As in other areas in the body, tendon healing proceeds in three phases: (1) an inflammatory stage, (2) a reparative or collagen-producing stage, and (3) a remodeling phase.

**Inflammatory Phase**

Tendon and ligament healing begins with hematoma formation and an inflammatory reaction that includes an accumulation of fibrin and inflammatory cells. A clot forms between the two ends and is invaded by cells resembling fibroblasts and migratory capillary buds. Within 2 to 3 days of the injury, fibroblasts within the wound begin to proliferate rapidly and synthesize new matrix. They replace the clot and the necrotic tissue with a soft, loose fibrous matrix containing high concentrations of water, glycosaminoglycans, and type III collagen. Inflammatory cells and fibroblasts fill this initial repair tissue. Within 3 to 4 days, vascular buds from the surrounding tissue grow into the repair tissue and then canalize to allow blood flow to the injured tissue and across small tissue defects. This vascular granulation tissue fills the tissue defect and extends for a short distance into the surrounding tissue but has little tensile strength. The inflammatory phase is evident until the 8th to 10th day after injury.
Reparative Phase
As the repair progresses during the next several weeks, proliferating fibroblasts continue to produce fibrous tissue containing a high proportion of type III collagen. Collagen synthesis reaches its maximal level after approximately 4 weeks, and at 3 months, collagen synthesis continues at a rate 3 to 4 times that of normal tissue. Over time, water, glycosaminoglycan, and type III collagen concentrations decline, the inflammatory cells disappear, and the concentration of type I collagen increases. Newly synthesized collagen fibrils increase in size and begin to form tightly packed bundles, and the density of fibroblasts decreases. Matrix organization increases, as the fibrils begin to align along the lines of stress, the number of blood vessels decreases, and small amounts of elastin may appear within the site of injury. The tensile strength of the repair tissue increases as the collagen concentration increases.

Remodeling Phase
Repair of many tendon and ligament injuries results in an excessive volume of highly cellular tissue with limited mechanical properties and a poorly organized matrix. Remodeling reshapes and strengthens this tissue by removing, reorganizing, and replacing cells and matrix. In most tendon and ligament injuries, evidence of remodeling appears within several weeks of injury as fibroblasts and macrophages decrease, fibroblast synthetic activity decreases, and fibroblasts and collagen fibrils assume a more organized appearance. As these changes occur in the repair tissue, collagen fibrils grow in diameter, the concentration of collagen and the ratio of type I to type III collagen increase, and the water and proteoglycan concentrations decline. During the months after the injury occurs, the matrix continues to align, presumably in response to loads applied to the repair tissue. The most apparent signs of remodeling disappear within 4 to 6 months of injury. However, removal, replacement, and reorganization of repair tissue continue to some extent for years. The mechanical strength of the healing tendon and ligament increases as the collagen becomes stabilized by cross-links and the fibrils assemble into fibers.

Factors Affecting Healing
Among the most important variables that affect healing of tendon and ligament are the type of tendon or ligament, the size of the tissue defect, and the amount of load applied to the repair tissue. For example, injuries to capsular and extra-articular ligaments stimulate production of repair tissue that will fill most defects, but injuries to intra-articular ligaments, such as the ACL, often fail to produce a successful repair response. Treatments that achieve or maintain apposition of torn tissue and that stabilize the injured site decrease the volume of repair tissue necessary to heal the injury, which can benefit the healing process. Such treatments may also minimize scarring and help to provide near-normal tissue length. For these reasons, avoidance of wide separation of ruptured tendon or ligament ends and selection of treatments that maintain some stability at the injured site during the initial stages of repair are generally desirable.

Early excessive loading in the immediate postoperative period may have a deleterious effect on tendon and ligament healing by disrupting the repair tissue, leading to gap formation and ischemia, adverse changes in tendon matrix, and possible rupture. However, controlled loading of tendon and ligament repair tissue can promote healing and enhance the mechanical and biologic characteristics of tendon-to-bone healing. The optimal amount of tension necessary to promote an acceptable clinical response is currently not well understood and depends on the type of tissue and healing environment, but it is clear that remodeling of collagen scar tissue into mature tendon tissue depends on the presence of tensile forces. The concept of immediate passive mobilization after flexor tendon repair in the hand was introduced by Kleinert and coworkers, who showed that, during limited active extension, reciprocal relaxation of the flexor tendons occurs, allowing passive extension of the repaired tendon. This controlled passive motion was found to be effective experimentally and clinically in decreasing the tethering effect of adhesions and in improving the rates of tendon repair, gliding function, and strength of the tendon.

Methods for Augmentation of Tendon and Ligament Healing
A large body of research has demonstrated the potential for growth factors to improve tendon and ligament tissue healing by stimulation of cell proliferation, chemotaxis, matrix synthesis, and cell differentiation (summarized in Table 1.1). In addition to multifunctional cytokines such as TGF-β and platelet-derived growth factor, work has focused on recapitulating the cellular and molecular signals that are expressed during embryonic tendon development, such as scleraxis and TGF-β3. However, challenges in the delivery of these growth factors, specifically regarding the optimal carrier vehicles and proper dosing regimen, to the desired site still remain.

Platelet-rich plasma (PRP), an autologous blood concentrate, can be used to locally deliver a high concentration “cocktail” of cytokines and has gained popularity as a treatment modality for tendon and ligament injuries. Recent studies have reported potentially promising results with the use of PRP to augment healing of rotator cuff repair and patellar tendinopathy. However, the results of PRP for augmentation of tendon and ligament healing have been variable, which can partially be attributed to the lack of understanding of the optimal PRP formulation for different tissues and pathologies, as well as the tremendous variability in the methods of PRP production among commercial systems. To complicate matters further, within a given separation technique, there is a high degree of intersubject and intrasubject variability in the composition of PRP produced.

Cell-based approaches appear promising for tendon and ligament tissue engineering and improvement of healing. Therapies using mesenchymal stem cells (MSCs) derived from adipose and bone marrow to augment tendon and ligament healing have garnered the most attention due to their multipotent potential and ability to exert a paracrine effect to modulate and control inflammation, stimulate endogenous cell repair and proliferation, inhibit apoptosis, and improve blood flow. However, like PRP augmentation therapy, continued research is needed to identify the optimal cell source and the ideal treatment protocol needed to drive differentiation of these or neighboring
IGF-1, insulin-like growth factor-1; small intestine submucosa has been used as a collagen scaffold for tendon repair and ligament reconstruction. Porcine-derived cells into mature tenocytes and fibroblasts. Recent studies have identified resident tissue-specific stem cells in the perivascular regions of native tendon and ligament that detach from vessels in response to injury, migrate into the interstitial space, and deposit extracellular matrix, although their precise potential for use in augmenting tendon and ligament healing remains to be elucidated.

Research has also investigated scaffold materials to augment tendon repair and ligament reconstruction. Porcine-derived small intestine submucosa has been used as a collagen scaffold to augment Achilles tendon and rotator cuff tendon repair. However, negative clinical results have been reported, including inflammatory/immunologic response to the small intestine submucosa material believed to be due to residual porcine DNA in the implant. Various other allografts and xenografts, such as collagen allograft matrices and porcine dermal xenografts, are commercially available and differ from porcine small intestine submucosa in both biologic and mechanical composition.

TABLE 1.1 Growth Factors in Soft Tissue Repair

<table>
<thead>
<tr>
<th>Biologic Factor</th>
<th>Functions</th>
<th>Reference</th>
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<tr>
<td>HGF</td>
<td>Enhanced angiogenesis Enhanced collagen deposition</td>
<td>Ueshima K et al: J Orthop Sci 2011</td>
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bFGF, Basic fibroblast growth factor; BMP-12, bone morphogenetic protein-12; GDF, growth/differentiation factor; HGF, human growth factor; IGF-1, insulin-like growth factor-1; PDGF-β, platelet-derived growth factor-β; PRP, plasma-rich protein; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.
nimate extracellular matrix. Multiphasic scaffolds are being used to create bone-ligament composites. In addition to various scaffold materials and cell types, it has become clear that mechanical stimulation of the neotissue is also critical to optimize the structure and composition of the tissue. The specific scaffold can be modified in vitro by seeding marrow stromal cells on the scaffold and applying cyclic stretching to increase the alignment of cells, as well as to improve the production and orientation of collagen. When applied in vivo, such a tissue-engineered scaffold could serve to accelerate the healing process, ultimately helping to make a better neoligament or tendon.

**MENISCUS**

**Structure**

Human menisci are semilunar in shape and consist of a sparse distribution of cells surrounded by an abundant extracellular matrix. The meniscus functions to optimize force transmission and provide stability to the knee. The medial meniscus is the dominant secondary stabilizer in an ACL-deficient knee during the Lachman maneuver, whereas the lateral meniscus is the dominant secondary stabilizer in an ACL-deficient knee during the pivot shift maneuver. Within the meniscus lies an anisotropic, inhomogeneous, and highly ordered arrangement of collagen fibrils. The meniscal surface is composed of a randomly woven mesh of fine collagen type II fibrils that lie parallel to the surface. Below this surface layer, large, circumferentially arranged collagen fiber bundles (mostly type I) spread through the body of the tissue (Fig. 1.2). These circumferential collagen bundles give menisci great tensile stiffness and strength parallel to their orientation. The collagen bundles insert into the anterior and the posterior meniscal attachment sites on the tibial plateau, providing for rigid and strong attachment sites. Fig. 1.2A illustrates these large fiber bundles and the thin superficial surface layer. Fig. 1.2B is a photograph of a bovine medial meniscus with the surface layer removed, showing the large collagen bundles of the deep zone.

Radial sections of meniscus (Fig. 1.3) show radially oriented bundles of collagen fibrils, or “radial tie fibers,” among the circumferential collagen fibril bundles, weaving from the periphery of the meniscus to the inner rim in every radial section throughout the meniscus. They are more abundant in the posterior sections and gradually diminish in number as the sections progress toward the anterior region of the meniscus. (Modified from Kelly MA, Fithian DC, Chern KY, et al. Structure and function of meniscus: basic and clinical implications. In: Mow VC, Ratcliffe A, Woo SL, eds. Biomechanics of Diarthrodial Joints. Vol 1. New York: Springer-Verlag; 1990.)

Unlike articular cartilage, the peripheral 25% to 30% of the lateral meniscus and the peripheral 30% of the medial meniscus have a blood supply, and the peripheral regions of the meniscus, especially the meniscal horns, have a nerve supply as well. Branches from the geniculate arteries form a capillary
plexus along the peripheral borders of the menisci, with the medial inferior geniculate artery supplying the peripheral medial meniscus and the lateral inferior geniculate artery supplying the peripheral lateral meniscus. Small radial branches project from these circumferential parameniscal vessels into the meniscal substance. The central aspects of the meniscus do not have a direct arterial supply and instead receive nutrients primarily through synovial fluid diffusion.

**Extracellular Matrix**

The mechanical functions of the menisci depend on a highly organized extracellular matrix consisting of fluid and a macromolecular framework formed of collagen (types I, II, III, V, and VI), proteoglycans, elastin, and noncollagenous proteins, along with the cells that maintain this matrix.

Based on morphologic characteristics, two major types of meniscal cells exist. Near the surface, the cells have flattened ellipsoid or fusiform shapes and are considered more fibroblastic; in the deep zone, the cells are spherical or polygonal and considered more chondrocytic. The superficial and the deep meniscal cells appear to have different metabolic functions and perhaps different responses to loading. Like most other mesenchymal cells, these cells lack cell-to-cell contacts. Because most of the cells lie at a distance from blood vessels, they rely on diffusion through the matrix for transport of nutrients and metabolites. The membranes of meniscal cells attach to matrix macromolecules through adhesion proteins (e.g., fibronectin, thrombospondin, and type VI collagen). The matrix, particularly the pericellular region, protects the cells from damage due to physiologic loading of the tissue. Deformation of the macromolecular framework causes fluid flow through the matrix and influences meniscal cell function. Because meniscal tissue is more fibrous than is hyaline cartilage, some authors have proposed that meniscal cells be called fibrochondrocytes.

Water comprises 65% to 75% of the total weight of the meniscus. Some portion of this water may reside within the intrafibrillar space of the collagen fibers. Most of the water is retained within the tissue in the solvent domains of the proteoglycans due to both their strong hydrophilic tendencies and the Donnan osmotic pressure exerted by the counter ions associated with the negative charge groups on the proteoglycans. Because the pore size of the tissue is extremely small (<60 nm), very large hydraulic pressures are required to overcome the impact of frictional resistance when forcing fluid flow through the tissue. These interactions between water and the macromolecular framework of the matrix significantly influence the viscoelastic properties of the tissue.

Some meniscal regions have a proteoglycan concentration of up to 3% of their dry weight. Like proteoglycans from other dense fibrous tissues, meniscal proteoglycans can be divided into two general types. The large, aggregating proteoglycans expand to fill large volumes of matrix and contribute to tissue hydration and the mechanical properties of the tissue. The smaller, nonaggregating proteoglycans usually have a close relationship with fibrillar collagen. The large aggregating meniscal proteoglycans have the same structure as the large aggregating proteoglycans from articular cartilage. The concentration of large aggregating proteoglycans suggests that they probably contribute less to the properties of meniscus than to the properties of articular cartilage. As with the quantitatively minor collagens, the smaller nonaggregating meniscal proteoglycans may help to organize and stabilize the matrix, but currently their exact function remains unknown.

Noncollagenous proteins also form part of the macromolecular framework of the meniscus and may contribute as much as 10% of the dry weight of the tissue in some regions. Two specific noncollagenous proteins, link protein and fibronectin, have been identified in the meniscus. Link protein is required for the formation of the stable proteoglycan aggregates that are capable of forming strong networks. Fibronectin serves as an attachment protein for cells in the extracellular matrix. Other noncollagenous proteins such as thrombospondin may serve as adhesive proteins in the tissue, thus contributing to the structure and the mechanical strength of the matrix; however, the exact details of their composition and function in the meniscus remain largely unknown.

Finally, elastin contributes less than 1% of the dry weight of the meniscus. The contribution of elastin to the mechanical properties of meniscal tissue is not well understood because the sparsely distributed elastic fibers are unlikely to play a significant role in the organization of the matrix or in determining the mechanical properties of the tissue.

**Injury**

Traumatic meniscal tears occur most frequently in young, active people. Tension, compression, or shear forces that exceed the strength of the meniscal matrix in any direction can lead to tissue failure. Acute traumatic injuries of normal meniscal substance usually produce longitudinal or transverse tears, although the morphology of these tears can be highly variable, including oblique, radial horizontal, bucket-handle, and complex tears. The configuration of tears due to overloading of normal meniscal tissue depends strongly on the direction of the load and the rate of stretch. Unlike acute traumatic tears through apparently normal meniscal tissue, degenerative meniscal tears occur as a result of age-related changes in the tissue. These degenerative tears are most common in persons older than 40 years. Often, these persons do not recall a specific injury, or they recall only a minor load applied to the knee. Degenerative tears often have complex shapes or may appear as horizontal clefts or flaps, as though they were produced by shear failure. Multiple degenerative tears often occur within the same meniscus. These features of degenerative meniscal tears suggest that they result more from age-related changes in the collagen-proteoglycan solid matrix than from specific acute trauma.

The response of meniscal tissue to tears depends on whether the tear occurs through a vascular or an avascular portion of the meniscus. The peripheral, vascularized, dense fibrous tissues do. The tissue damage initiates a sequence of cellular and vascular events including inflammation, repair, and remodeling that can result in healing and restoration of tissue structure and function. Although tears through the vascular regions of the meniscus can typically heal, tears through the avascular regions do not typically heal.
spontaneously, resulting in tissue deficiency. Therefore strategies for meniscal repair in the avascular zone are continuously being explored.

Meniscal Repair
Factors Affecting Healing

Repair in vascular regions of the meniscus. Partial meniscal resection through the peripheral vascularized region or complete meniscal resection initiates production of repair tissue that can extend from the remaining peripheral tissue into the joint. Although the repair cells usually fail to replicate normal meniscal tissue, many authors have referred to this phenomenon as meniscal regeneration. Some repaired menisci grossly resemble normal menisci, but the functional capabilities and mechanical properties of this “regenerated” meniscal tissue have not been comprehensively studied. Surgeons have reported meniscal regeneration in many clinical situations. Investigators have also examined the tissue produced by meniscal regeneration in animals. Meniscal regeneration can occur repeatedly in the same knee and occasionally occurs after total knee replacement. In rabbits, meniscal regeneration occurs more frequently on the medial side of the knee than on the lateral side, and development of degenerative changes in articular cartilage after a meniscectomy is inversely correlated with the extent of meniscal regeneration.

Synovectomy appears to prevent meniscal regeneration, which suggests that synovial cells contribute to the formation of meniscal repair tissue. The mechanisms and conditions that promote this type of repair, its functional importance, and the factors related to the predictability and frequency of meniscal regeneration remain unknown.

Repair in avascular portions of the meniscus. The response of meniscal tissue to tears in the avascular portion resembles the response of articular cartilage to lacerations in many respects. Experimental studies show that a penetrating injury to the avascular region of the meniscus causes no apparent repair or inflammatory reaction. Meniscal cells in the injured region, like chondrocytes in the region of an injury limited to the articular cartilage, may proliferate and synthesize new matrix, but they appear to be incapable of migrating to the site of the defect or producing enough new matrix to fill it. The ineffective response of meniscal cells in the avascular region of the meniscus has led investigators to develop novel methods to stimulate repair. Some promising approaches include creation of a vascular access channel to the injury site and stimulation of cell migration to the avascular region using implantation of a fibrin clot, an artificial matrix, or growth factors.

Synovial abrasion has also been shown to stimulate proliferation of the synovial fringe into the meniscus and allows blood vessels to enter the avascular regions. Although early results appear promising, the quality of the repair tissue, its biomechanical properties, and the long-term results of these methods have not been evaluated.

Augmentation of Meniscus Healing

Given the well-established poor intrinsic healing potential of the meniscus, particularly in avascular regions, intense interest exists regarding methods to augment healing using cytokines, exogenous cells, and scaffolds. Fibroblast growth factor-2 and connective tissue growth factor have been evaluated in rabbit models, and vascular endothelial growth factor has been tested in a sheep meniscus tear model. Although these cytokines appear to have a positive effect on basic meniscal fibrochondrocyte biology, the challenge at this time is to identify the optimal carrier vehicles and dosage to translate these preclinical data to clinical trials. Although some studies have suggested that PRP, as a source of cytokines, may confer some benefit in meniscus healing, other studies have demonstrated no differences in outcomes or reoperation rates. In addition, in an animal model of meniscus injury, PRP treatment increased hypertrophic fibrous tissue rather than meniscal cartilage. Thus further investigation is necessary to better elucidate the role of growth factors and PRP in meniscal healing.

Cell-based approaches have also been evaluated for augmentation of biologic healing mechanisms. Various sources of both autogenous and allogeneic cells have been evaluated using different carrier materials. Both differentiated cells, such as chondrocytes, and undifferentiated cells, such as MSCs, have been tested in animal models. Few human studies investigating the role of MSCs for meniscal repair have been performed. Although the authors suggest that MSCs may be effective in repairing meniscal tears, these studies are limited, and more rigorous, placebo-controlled trials are necessary.

Scaffolds. The use of scaffold materials to replace a portion of the damaged meniscus or to replace the entire structure is an appealing option and has the theoretical benefit of providing mechanical stability to the injured site while allowing for cell attachment and proliferation. A collagen-based scaffold (Collagen Meniscus Implant, Menaflex, ReGen Biologics, Glen Rock, NJ) and a resorbable porous polyurethane-based scaffold (Actifit, Orteq Sport Medicine, London, United Kingdom) have demonstrated satisfactory clinical outcome in up to 80% of cases at up to 10 years and 2 years of follow-up, respectively. Both of these devices are designed for partial meniscus replacement. Although it remains unclear whether the use of such scaffolds can affect the long-term sequelae of meniscectomy, early results are promising and may represent a new horizon in the treatment of these complex injuries. Further optimization of these materials may occur by incorporating undifferentiated cells into the scaffold.

ARTICULAR CARTILAGE

Synovial joints allow the rapid controlled movements necessary to support joint motion and to participate in sports. Normal function of these complex diarthrodial structures depends on the structural integrity and macromolecular composition of articular cartilage. Sports-related traumatic disruptions of cartilage structure and alterations in the macromolecular composition or organization change the biomechanical properties of the tissue, compromise joint function, and can lead to progressive pain and disability.

The specialized composition and organization of hyaline articular cartilage impart its unique biomechanical properties that permit normal synovial joint function. In the joint, cartilage distributes the loads of articulation, thereby minimizing peak
stresses acting on the subchondral bone. The tensile strength of the tissue provides its structural integrity under such loads. Alterations in the mechanical properties of cartilage due to injury, disease, or increasing age have not been well defined, but the available information shows that these properties change with age and loss of structural integrity. Cartilage from skeletally immature joints (open growth plates) is much stiffer than cartilage from skeletally mature joints (closed growth plates). Participation in sports often subjects the articular cartilage to intense repetitive, compressive high-energy impact forces that can cause tissue injury. These abnormally large forces generate high shear stresses at the cartilage-subchondral bone junction, causing matrix disruption and death of the articular chondrocytes that may lead to early osteoarthritis. Because cartilage is aneural, patients with pure chondral injuries can remain asymptomatic.

Structure and Composition of Articular Cartilage

Like the dense fibrous tissues and meniscus, articular cartilage consists of cells, matrix water, and a matrix macromolecular framework. Unlike the most dense fibrous tissues, cartilage lacks nerves, blood vessels, and a lymphatic system. The composition, organization, and mechanical properties of the matrix of articular cartilage and the cell morphology and function vary according to the depth from the articular surface (Fig. 1.4). Matrix composition, organization, and function also vary with distance from the cell. Morphologic changes in articular cartilage cells and matrix from the articular surface to the subchondral bone make it possible to identify four zones or layers of articular cartilage.

Zones of Articular Cartilage

Superficial zone. The thinnest zone, the superficial tangential zone, has two layers. A sheet of fine fibrils without cells covers the joint surface (see Fig. 1.4). On phase-contrast microscopy, it appears as a narrow bright line, the “lamina splendens.” In the next layer of the superficial zone, flattened ellipsoid chondrocytes are arranged so that their major axes are parallel to the articular surface (see Fig. 1.4). They synthesize a matrix that has a high collagen concentration and a low proteoglycan concentration relative to the other cartilage zones. Water content is the highest in this zone, averaging 80%. In addition, a specific protein, called lubricin (or PRG4) is also only produced in this zone. Lubricin plays an important role in joint lubrication and in allowing frictionless articulation.

Transitional zone. The transitional (middle) zone has several times the volume of the superficial zone (see Fig. 1.4). The cells of this zone assume a spheroidal shape and synthesize a matrix with collagen fibrils of a larger diameter and a higher concentration of proteoglycans than is found in the superficial zone. In this zone, the proteoglycan concentration is higher than in the superficial zone, but the water and the collagen concentrations are lower.

Fig. 1.4 Normal articular cartilage structure. Histologic (A) and schematic (B) views of a section of normal articular cartilage. The tissue consists of four zones: the superficial tangential zone (STZ), the middle zone, the deep zone, and the calcified zone. Notice the differences in cell alignment among zones. The cells of the superficial zone have an ellipsoidal shape and lie with their long axes parallel to the articular surface. The cells of the other zones have a more spheroidal shape. In the deep zone, they tend to align themselves in columns perpendicular to the joint surface. (Schematic from Nordin M, Frankel VH. Basic Biomechanics of the Musculoskeletal System. 2nd ed. Philadelphia: Lea & Febiger; 1989.)
Deep zone. The chondrocytes in the deep zone resemble those of the middle zone, but they tend to align in columns perpendicular to the joint surface (see Fig. 1.4). This zone contains the collagen fibrils with the largest diameter, the highest concentration of proteoglycans, and the lowest concentration of water. The collagen fibers of this zone pass through the tidemark (a thin basophilic line seen on light microscopic sections that marks the boundary between calcified and uncalcified cartilage) into the calcified zone.146

Calcified cartilage zone. A zone of calcified cartilage lies between the deep zone of uncalcified cartilage and the subchondral bone. The calcified layer plays an integral role in securing the cartilage to bone by anchoring the collagen fibrils of the deep zone to the subchondral bone. In this zone, the cell population is scarce and chondrocytes are hypertrophic. Type X collagen is present in the calcified cartilage.

Chondrocytes

The chondrocyte is the predominant cell in cartilage. Chondrocytes contribute only 5% or less to the total volume of cartilage. Like other mesenchymal cells, chondrocytes surround themselves with their extracellular matrix and rarely form cell-to-cell contacts. In normal cartilage, they are isolated. Because the tissue lacks blood vessels, the cells depend on diffusion through the matrix for their nutrition and rely primarily on anaerobic metabolism.

After completion of skeletal growth, chondrocytes rarely divide, but throughout life they synthesize and maintain the extracellular matrix that gives cartilage its essential material properties. Synthesis and turnover of proteoglycans are relatively fast, whereas collagen synthesis and turnover are very slow.150,151 The limited potential for chondrocyte replication contributes to the limited inherent capacity of cartilage to regenerate or heal after injury.

Extracellular Matrix

Water contributes up to 80% of the wet weight of articular cartilage. The interaction of water with the matrix macromolecules, particularly the large aggregating proteoglycans, significantly influences the material properties of the articular cartilage.109,143,152,153 This tissue fluid contains gases, small proteins, metabolites, and a high concentration of cations that balance the negatively charged proteoglycans.152,154 The interaction between proteoglycans and tissue fluid significantly influences the compressive stiffness and resilience of articular cartilage.139,152

Collagens contribute approximately 60% of the dry weight of cartilage, proteoglycans contribute 25% to 35%, and the non-collagenous proteins and glycoproteins contribute 15% to 20%. Collagens are distributed relatively uniformly throughout the depth of the cartilage except in the collagen-rich region near the surface. The collagen fibrillar meshwork and cross-linking give cartilage its form and tensile strength.139,155 Proteoglycans and noncollagenous proteins bind to the collagenous meshwork or become mechanically entrapped within it, and water fills this molecular framework. Proteoglycans give cartilage its stiffness in compression and its resilience. Some noncollagenous proteins organize and stabilize the matrix macromolecular framework, whereas others bind chondrocytes to the macromolecules of the matrix.

Cell-Matrix Interactions

Maintenance of cartilage depends on continual complex interactions between chondrocytes and the matrix they synthesize. Normal degradation of matrix macromolecules, especially proteoglycans, requires that the chondrocytes continually synthesize new molecules.143,144 If the cells did not replace the lost proteoglycans, the tissue would deteriorate. Mechanical loading also affects cartilage homeostasis.156,157

Chondrocytes respond to changes in patterns of matrix deformation due to persistent changes in joint use. Both mechanical and physicochemical events during matrix deformation likely play significant roles. A chondrocyte embedded in the charged extracellular matrix may exist in either an undeformed state or a deformed state. Deformation during compression alters the charge density around the cells and induces a streaming potential throughout the tissue. These physicochemical effects vary according to proteoglycan concentration relative to depth from the surface in the different zones of the charged collagen-proteoglycan extracellular matrix.143,144 and are important in modulating chondrocyte proteoglycan biosynthesis.152,158-161 In addition to these events, biochemical agents such as growth factors, cytokines, and enzymes are also potent stimulators of chondrocytes. Altogether, studies addressing these important questions offer great challenges for the future.

Relevance for Articular Cartilage Repair

The typical tissue response of vascularized connective tissues to injury follows a cascade of inflammation, repair, and scar remodeling, which is facilitated by cells and other mediators brought in from the surrounding vasculature. However, because hyaline cartilage is avascular, this vital response cannot be generated, and the intrinsic reparative ability of cartilage is very low.163-165

In healthy cartilage, a homeostasis of extracellular matrix metabolism balances the degradation of macromolecules with their replacement through newly synthesized products. Insult to the cartilage can lead to imbalance of this equilibrium and a shift toward degradation, leading to progression of the chondral defect and potentially, osteoarthritis. Factors associated with this physiologic imbalance and repair response include joint loading, depth of the defect, size of the defect, and patient age.

Joint Loading

Acute or repetitive direct blunt trauma or abnormal loading can cause a spectrum of cartilage injuries ranging from those isolated to microscopic matrix damage to those that lead to visible fissures causing matrix disruption and chondrocyte death. Abnormal loading can be caused by mechanical malalignment or concomitant ligament injury that leads to excessive focal stresses on the cartilage. Loss of proteoglycans typically occurs before other signs of tissue injury.166,167 and may be due to either increased degradation or altered synthesis of the molecules, including the collagen fibrils, thus increasing the vulnerability of the tissue to damage from further impact loading.166-168 Disruption of the surface collagen matrix leads to increased hydration, fissuring in the cartilage, and thickening of subchondral bone. A study of the response of human articular cartilage to blunt trauma
showed that impact loads exceeding 25 N/m² (25 megapascal [MPa]) caused chondrocyte death and cartilage fissures.149

**Depth of the Defect**

Articular cartilage defects are generally classified as chondral or osteochondral, depending on the depth. Chondral defects can be further classified into partial thickness or full thickness (i.e., down to subchondral bone). The repair response depends on whether the injury extends down to the subchondral vascular bone marrow. For partial-thickness chondral injuries, the local response depends entirely on chondrocytes near the injury site, which proliferate and increase the synthesis of matrix molecules.109,169 However, the newly synthesized matrix and the proliferating chondrocytes are unable to fill the tissue defect, and soon after injury, the increased proliferative and synthetic activity ceases. Because chondrocytes cannot repair these matrix injuries, the fissures either remain unchanged or progress. Injuries that extend down into the subchondral bone marrow lead to migration of osteoprogenitor cells into the defect region and synthesis of a new fibrocartilaginous tissue. However, this repair tissue is biomechanically and structurally inferior to hyaline cartilage and thus prone to breakdown with time and loading.170,171

**Size of the Defect**

Smaller defects are less likely to affect the stress distribution on the subchondral bone and progress in size, whereas larger defects are more likely to progress due to increased rim stresses and an inadequate repair response. A study in horses revealed that defects less than 3 mm in diameter may lead to complete repair after 9 months, whereas those larger in size (up to 21 mm in diameter) failed to heal.172

**Age**

Articular cartilage undergoes significant structural, matrix composition, and mechanical changes with age.104,119,173,174 As with most type of cells in the body, mitotic and synthetic activities of chondrocytes decline with age.175 These changes are responsible for higher incidence of chondral lesions and development of osteoarthritis in older patients. As a result, any reparative response or ability to maintain extracellular matrix homeostasis decreases with older age. Animal studies in rabbits have demonstrated a better reparative response for chondral defects in younger animals compared with older ones.176,177

**Clinical Relevance and Further Developments**

Small, symptomatic chondral defects may be treated by marrow-stimulating techniques such as microfracture. However, the resultant fibrocartilage repair tissue after microfracture is histologically different and biomechanically inferior to native hyaline cartilage.177 For larger lesions, a myriad of options is available, including osteochondral autografts or allografts, autologous chondrocyte implantation (ACI), or matrix-induced autologous chondrocyte implantation (MACI). Osteochondral grafts are able to treat defects that extend into the subchondral bone, whereas ACI and MACI require well-preserved bone stock at the base of the chondral defect. The use of particulated juvenile cartilage allograft, which may have increased proliferative and restorative potential, has demonstrated promising early results for treatment of cartilage defects of the knee.178,180 Future developments include improved scaffolds,178,181 augmentation with therapeutic factors such as proteins or genes,182 and the use of MSCs.182,183 Furthermore, small molecules and activation of endogenous repair (homing of intrinsic progenitors/MSCs) are potential forthcoming therapeutic avenues.184,185

**BONE**

**Types of Bone**

Normal bone is lamellar and can be classified as cortical or cancellous. Immature bone and pathologic bone are woven and, in contrast to lamellar bone, have more random orientation with more osteocytes, increased turnover, and inferior integrity. Lamellar bone is stress oriented, whereas woven bone is not stress oriented.

Cortical bone (compact bone) (Fig. 1.5) makes up 80% of the skeleton and is composed of tightly packed osteons or haversian systems which are connected by haversian (or Volkmann) canals. These canals contain arterioles, venules, capillaries, nerves, and possibly lymphatic channels. Interstitial lamellae lie between the osteons. Fibrils frequently connect lamellae but do not cross cement lines. Cement lines define the outer border of an osteon and represent the area where bone resorption has stopped and new bone formation has begun. Nutrition occurs through the intraosseous circulation, which involves networks of canals and
canaliculi. Radiating processes of bone osteocytes, also known as filopodia, project into the canaliculi and allow for osteocyte interaction.

Cortical bone is characterized by a slow turnover rate, a relatively high Young's modulus (E), and a high resistance to torsion and bending. Cancellous bone (spongy or trabecular bone) (see Fig. 1.5) is less dense than cortical bone and undergoes more remodeling according to lines of stress (Wolff’s law). Cancellous bone has a higher turnover rate and a smaller Young's modulus and is more elastic than cortical bone.

**Cellular Biology**

Osteoblasts are responsible for bone formation and are derived from undifferentiated mesenchymal cells. More differentiated, metabolically active cells line bone surfaces, and less active cells in “resting regions” or entrapped cells maintain the ionic milieu of bone. Disruption of the lining cell layer activates these cells.

Osteocytes (see Fig. 1.5) make up 90% of the cells in the mature skeleton and serve to maintain bone. These cells consist of former osteoblasts that have been trapped within newly formed matrix, which they help to preserve. Osteocytes are not as active in matrix production as are osteoblasts.

Osteoclasts are responsible for bone resorption. These multinucleated, irregularly shaped giant cells originate from hematopoietic tissues. Bone resorption occurs in depressions known as Howship lacunae and is more rapid than bone formation; however, bone formation and resorption are linked (“coupled”).

Osteoclasts have specific receptors for calcitonin, osteoprotegerin, and other molecules which allow them to directly regulate bone resorption. Interleukin-1 (IL-1) is a potent stimulator of osteoclastic bone resorption in nonphysiologic situations and has been found in the membranes surrounding loose total joint implants. IL-10 suppresses osteoclast formation.

**Bone Matrix**

Bone matrix is composed of both organic and inorganic components. The organic components make up 40% of the dry weight of bone. Organic components include collagen (mainly type I) proteoglycans, noncollagenous matrix proteins (glycoproteins, phospholipids, and phosphoproteins), growth factors, and cytokines. Collagen is responsible for the tensile strength of bone.

Proteoglycans are partially responsible for the compressive strength of bone. Matrix proteins include osteocalcin, osteonectin, osteopontin, and others. Growth factors and cytokines, which are present in small amounts in bone matrix, include TGF-β; insulin-like growth factor; interleukins (e.g., IL-1 and IL-6); and bone morphogenetic proteins. These proteins aid in bone cell differentiation, activation, growth, and turnover. The inorganic or mineral component of bone matrix makes up 60% of the dry weight of bone. Calcium hydroxyapatite \[Ca_{10}(PO_4)_6(OH)_2\] is responsible for the compressive strength of bone. Calcium hydroxyapatite makes up most of the inorganic matrix and is responsible for matrix mineralization.

**Bone Remodeling**

Bone remodeling is affected by mechanical stress according to Wolff’s law. Removal of external stresses can lead to significant bone loss, but this situation can be reversed to varying degrees on remodeling. In addition to remodeling in response to stress, bone remodels in response to piezoelectric charges. The compression side is electronegative, stimulating osteoblasts and bone formation; the tension side is electropositive, stimulating osteoclasts and bone resorption.

Both cortical bone and cancellous bone are continuously remodeled by osteoclastic and osteoblastic activity. Bone remodeling occurs in small packets of cells known as basic multicellular units. This bone remodeling is modulated by systemic hormones and local cytokines. Bone remodeling occurs throughout life. The Huer-Volkmann law (i.e., compressive forces inhibit growth and tensile forces stimulate growth) suggests that mechanical factors can influence longitudinal growth, bone remodeling, and fracture repair. Cancellous bone remodels by osteoclastic resorption followed by osteoblastic bone formation.

**Bone Circulation**

As an organ system, bones receive 5% to 10% of the cardiac output. The long bones receive blood from three sources: the nutrient artery system, the metaphyseal-epiphyseal system, and the periosteal system. Bones with a tenuous blood supply include the scaphoid, the talus, the femoral head, and the odontoid.

The nutrient artery enters the diaphyseal cortex through the nutrient foramen and then enters the medullary canal. In the medullary canal, the nutrient artery branches into ascending and descending small arteries, which, in turn, branch into arterioles which penetrate the endosteal cortex to supply the inner two-thirds of mature diaphyseal cortex through vessels that traverse the haversian system. The metaphyseal-epiphyseal system arises from the periarticular vascular plexus. The periosteal system is composed primarily of capillaries that supply the outer one-third of the mature diaphyseal cortex. Although the nutrient artery system is a high-pressure system, the periosteal system is a low-pressure system.

At the site of bony injury, the initial response is decreased flow to a fracture as a result of disruption of the nutrient artery system at the fracture site. However, within hours to days, bone blood flow increases (as part of the regional acceleratory phenomenon) and peaks at approximately 2 weeks. Blood flow returns to baseline between 3 and 5 months. The arterial system of bone has great potential for vasoconstriction (from the resting state) and much less potential for vasodilation. The vessels within bone possess a variety of vasoactive receptors, which may be useful in the future for pharmacologic treatment of bone diseases related to aberrant circulation (e.g., osteonecrosis and fracture nonunion).

**Tissue Surrounding Bone**

The periosteum is the connective tissue membrane that covers bone. It is more highly developed in children because of its role in the deposition of cortical bone, which is responsible for growth in bone diameter. The inner, or cambium, layer of periosteum is loose and more vascular and contains cells that are capable of becoming osteoblasts. These cells are responsible for enlarging the diameter of bone during growth and forming periosteal...
callus during fracture healing; the outer, fibrous layer is less cellular and is contiguous with joint capsules.

Bone marrow is the soft, gelatinous tissue within the interior of bones, which consists of both stromal and progenitor cells. Red marrow is more commonly found in flat bones, contains hematopoietic stem cells and MSCs, and slowly changes to yellow marrow with age. Yellow marrow is most commonly found in the long bones, contains primarily fat cells, and has a lower water content than red marrow.

**Types of Bone Formation**

**Endochondral Bone Formation and Mineralization**

In the process of endochondral bone formation, undifferentiated cells secrete cartilaginous matrix and differentiate into chondrocytes. This matrix mineralizes and is invaded by vascular buds that bring in osteoprogenitor cells. Osteoclasts then resorb calcified cartilage, and osteoblasts form bone. Bone replaces the cartilage model. Examples of endochondral bone formation include embryonic long-bone formation, longitudinal growth (physis), development of fracture callus, and the formation of bone via the use of demineralized bone matrix.

**Intramembranous Bone Formation**

Intramembranous bone formation occurs without a cartilage model. Undifferentiated mesenchymal cells aggregate into layers (or membranes). These cells differentiate into osteoblasts and deposit organic matrix that mineralizes to form bone. Examples of intramembranous bone formation include embryonic flat-bone formation (e.g., the pelvis, clavicle, and vault of the skull), bone formation during distraction osteogenesis, and blastema bone formation (which occurs in young children with amputations).

**Appositional Ossification**

In the process of appositional ossification, osteoblasts align themselves on an existing bone surface and lay down new bone. Examples of appositional ossification include periosteal bone enlargement (width) and the bone formation phase of bone remodeling.

**Biology of Fracture Healing**

**Overview**

Fracture healing involves a series of cellular events: inflammation, fibrous tissue formation, cartilage formation, and endochondral bone formation. The cellular events of fracture healing are influenced by undifferentiated cells in the vicinity of the fracture, osteoinductive growth factors released into the fracture environment, and the mechanical loading environment.

**Fracture Repair**

The response of bone to injury can be thought of as a continuum of histologic processes, beginning with inflammation, proceeding through repair (soft callus followed by hard callus), and finally ending in remodeling. Fracture repair is unique in that healing is completed without the formation of a scar. Fracture healing may be influenced by a variety of biologic and mechanical factors.

In the inflammation phase, bleeding from the fracture site and surrounding soft tissues creates a hematoma, which provides a source of hematopoietic cells capable of secreting growth factors. Subsequently, fibroblasts, mesenchymal cells, and osteoprogenitor cells accumulate at the fracture site, and fibrovascular tissue forms around the fracture ends. Osteoblasts from surrounding osteogenic precursor cells, fibroblasts, or both proliferate.

In the repair phase, primary callus response occurs within 2 weeks. If the bone ends are not in continuity, a bridging (soft) callus occurs. Fibrocartilage develops and stabilizes the bone ends. The soft callus (fibrocartilage) later is replaced by woven bone (hard callus) through the process of endochondral ossification. Another type of callus—medullary callus—supplements the bridging callus, although it forms more slowly and occurs later in the repair process. The amount and type of callus formation are dependent upon the method of treatment (Table 1.2).

Primary cortical healing, which resembles normal remodeling, occurs with rigid immobilization and anatomic (or near-anatomic) reduction with the bone ends in continuity. With rigidly fixed fractures (such as with a compression plate), direct osteonal or primary bone healing occurs without visible callus formation. In contrast, “endochondral healing,” with periosteal bridging callus formation, occurs in the setting of nonrigid fixation.

The remodeling phase begins during the middle of the repair phase and continues long after the fracture has clinically healed (can be for several years). Remodeling allows the bone to assume its normal configuration and shape based on the stresses to which it is exposed (Wolff’s law). Throughout the process, woven bone formed during the repair phase is replaced with lamellar bone. Fracture healing is complete when repopulation of the marrow space occurs.

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**TABLE 1.2** Type of Fracture Healing Based on Type of Stabilization

<table>
<thead>
<tr>
<th>Type of Immobilization</th>
<th>Predominant Type of Healing</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cast (closed treatment)</td>
<td>Periosteal bridging callus</td>
<td>Enchondral ossification</td>
</tr>
<tr>
<td>Compression plate</td>
<td>Primary cortical healing (remodeling)</td>
<td>Cutting cone-type remodeling</td>
</tr>
<tr>
<td>Intramedullary nail</td>
<td>Early: periosteal bridging callus</td>
<td>Enchondral ossification</td>
</tr>
<tr>
<td></td>
<td>Late: medullary callus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dependent on extent of rigidity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Less rigid: periosteal bridging callus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>More rigid: primary cortical healing</td>
<td></td>
</tr>
<tr>
<td>Inadequate</td>
<td>Hypertrophic nonunion</td>
<td>Failed endochondral ossification; type II collagen predominates</td>
</tr>
</tbody>
</table>

The different factors affecting bone are summarized in Table 1.3.

For a complete list of references, go to ExpertConsult.com.

### SELECTED READINGS

**Citation:**

**Level of Evidence:**
V

**Summary:**
An excellent textbook covering all aspects of the basic science of musculoskeletal tissues.

**Citation:**

**Level of Evidence:**
V

**Summary:**
A review focusing on the challenges and important questions regarding tendinopathies and tendon healing.

**Citation:**

**Level of Evidence:**
V

**Summary:**
A review of the approaches for bioenhanced ACL repair and the group’s journey to carry their novel technology from bench to bedside.

**Citation:**

**Level of Evidence:**
V

**Summary:**
A comprehensive review of the biology of cartilage repair and the science supporting current reconstructive surgical techniques and future tissue engineering endeavors.

**Citation:**

**Level of Evidence:**
V

**Summary:**
A review focusing on the challenges and important questions regarding tendinopathies and tendon healing.

**Citation:**

**Level of Evidence:**
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**Summary:**
A comprehensive review of the biology of fracture healing at the tissue, cellular, and molecular levels.

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<table>
<thead>
<tr>
<th>TABLE 1.3</th>
<th>Factors Affecting Bone and Bone Healing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor</strong></td>
<td><strong>Example</strong></td>
</tr>
<tr>
<td>Growth factors</td>
<td>BMPs</td>
</tr>
<tr>
<td></td>
<td>TGF-β</td>
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<tr>
<td></td>
<td>IGF-I and IGF-II and IGFBPs 1–6</td>
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<tr>
<td></td>
<td>Platelet-derived growth factor</td>
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<tr>
<td>Ultrasound</td>
<td>Low-intensity pulsed ultrasound</td>
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<tr>
<td>Electricity</td>
<td>Direct current</td>
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<tr>
<td></td>
<td>Alternating current</td>
</tr>
<tr>
<td></td>
<td>Pulsed electromagnetic fields</td>
</tr>
</tbody>
</table>

*BMP,* Bone morphogenetic protein; *IGF,* insulin-like growth factor; *IGFBP,* insulin-like growth factor–binding protein; *TGF-β,* transforming growth factor-β.
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