23 Ligament Histology, Composition, Anatomy, Injury, and Healing Mechanisms

John G. Lane and David Amiel

Contents

J.G. Lane, M.D. (✉) • D. Amiel, Ph.D.
Department of Orthopedic Surgery, Connective Tissue Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0630, USA
e-mail: fshepherd@ucsd.edu; jglane@san.rr.com

23.1 Introduction

23.1.1 Ligaments and Collagen

Ligaments are connective tissues with densely packed collagen fiber bundles aligned in parallel along the length of the tissue substance to allow for the most efficient resistance of tensile loads. Ligament insertions to bone are well adapted to their intended function. Force dissipation is achieved through a gradual transition from ligament to fibrocartilage to bone. Disruption is less likely to occur in this transition region than in the bone or peri-insertional tissue substance [1–3]. Classified as dense, regularly arranged connective tissue [4–6], ligaments are composed primarily of fibrillary collagen. These short bands of fibrous tissue bind bone to bone, limiting and guiding motion as well as providing support for internal organs.

Collagen is the single most abundant animal protein in mammals, accounting for up to 30% of all proteins [7]. Collagen molecules assemble into characteristic fibers responsible for the functional integrity of tissues such as bone, cartilage, skin, ligament, and tendon [8]. They contribute a structural framework for most organs. Cross-links between adjacent molecules are a prerequisite for collagen fibers to withstand the physical stresses to which they are exposed. Many disabling conditions result from changes in the nature and organization of collagen [7].

A. Gobbi et al. (eds.), *Bio-orthopaedics*, DOI 10.1007/978-3-662-54181-4_23

23.2 Structure of Ligaments and Tendons

Although many histology texts described both ligament and tendon tissues as "dense, regular connective tissue," [9–12] these two structures have sufficiently unique histologic characteristics to be distinguishable from each other [13]. The variables considered are collagen bundle width, cell morphology, and size as well as "crimp." Crimp is a feature of tendon and ligament and represents a regular sinusoidal pattern in the matrix. The periodicity and amplitude of the crimp appear structure-specific and are best evaluated under polarized light. A simple functional explanation for this accordion-like pattern is that it provides a buffer in which slight longitudinal elongation may occur without fibrous damage. This provides a mechanism for control of tension and acts as a shock absorber along the length of the tissue. However, when the physiologic mechanical limits of the ligament crimp pattern are exceeded, irreversible changes occur in the ligamentous structure [14]. This chapter focuses on knee ligaments and, in particular, the anterior cruciate ligament (ACL) and medial collateral ligament (MCL), as their properties have been well delineated. Crimping patterns differ between tendons and ligaments.

In the canine ACL, the centrally located fascicles in the ACL are either straight or undulate in a planar wave pattern, whereas those located at the periphery are arranged in a helical wave pattern. In the patellar tendon (PT), all the fascicles undulate in the helical wave pattern. Recognizing the structures of the ligaments facilitates understanding the repair of ACL injury and the readaptation of the tissues after surgical implantation. These histologic features are important in h ligament injury.

Histologic assessment of the ACL demonstrates longitudinally oriented bundles of collagen with a width of about 20 μm, as seen in the patellar tendon (PT). The crimp period in the ACL is much shorter (45–60 μm) and the amplitude is less than 5 μm. Fibroblasts are located on either side of the collagenous bundles, but the ligament is considerably more cellular than the tendon. ACL fibroblasts are round to ovoid and substantially different in appearance from the fibroblasts in the patellar tendon. They measure approximately 5–8 μm in diameter and 12–15 μm in length. Cells are arranged longitudinally along the borders of the fascicles (Fig. 23.1).

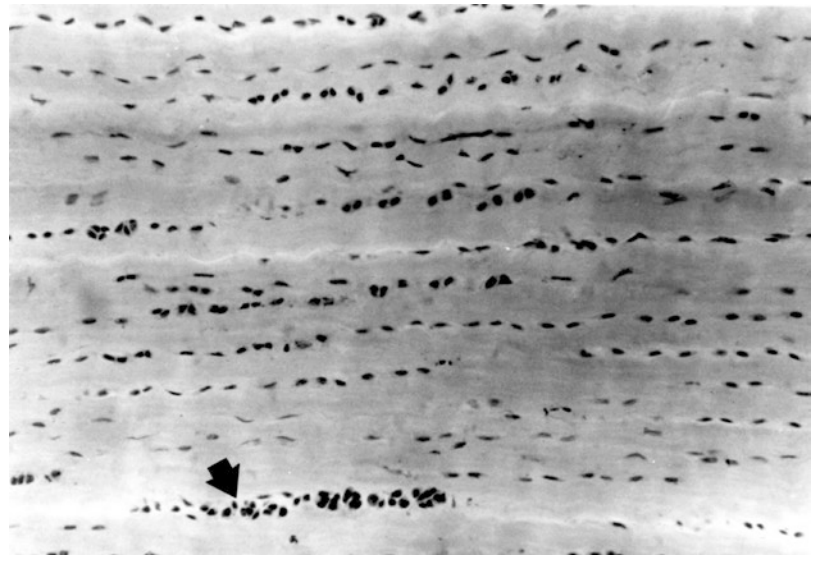

Fig. 23.1 Histology of normal ACL [H&E stain, original magnification ×50]. Note rounded fibroblasts, fine fibrillar crimp, and cluster of potential reserve cells (*arrow*). (From: Khatod M and Amiel D: Chapter 3, Ligament Biochemistry. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz R, Akeson WH and O'Connor eds, LWW, NY, NY, 2003, Pg 34)

In contrast, the MCL of the knee is notable for rod-shaped and spindle-shaped cells that are intermediate in length compared with PT and ACL cells. The MCL has cells that are 15 μm long and 25 μm wide. The crimp pattern measures approximately 45 μm with a 10 μm amplitude, and the collagen bundle width is approximately 20 μm as seen in the other structures discussed. Measurements of cell size, shape, crimp specifics, and bundle width of the tendon and ligaments are provided (Table 23.1).

These substantial differences in morphology and ultrastructure may reflect the functional and environmental differences between the ACL and MCL. The cellular morphological characteristics of the MCL are those of fibroblasts, whereas the ACL cellular characteristics are similar to fibrocartilage cells. These observations lead to a series of profound and important questions concerning the differences in function, homeostasis, and repair between the ACL and MCL.

The biochemical parameters used to assess the constitutional properties of collagenous tissue include collagen structure and type, collagen reducible and non-reducible cross-link analysis, proteoglycan content and glycoprotein. The value of each of these variables is related to its importance in the study of both (a) soft tissue injury and healing, and (b) the response to exercise (and the deleterious effects of immobilization). A more complete understanding of these problems could improve various treatment modalities and place them on firm scientific ground. This is particularly the case because most investigations of tissue injury and healing involve skin, not ligaments [15–17].

23.2.1 Collagen in Ligaments

Collagen is the major protein in ligaments, and it is not a single entity. It is the single most abundant animal protein in mammals, accounting for up to 30% of all proteins. The collagen molecules, after being secreted by the cells, assemble into characteristic fibers responsible for the functional integrity of tissue such as bone, cartilage, skin, ligaments, and tendon [8]. They contribute a structural framework to other tissues, such as blood vessels and most organs. Cross-links between adjacent molecules are a prerequisite for the collagen fibers to withstand the physical stresses to which they are exposed. Significant progress has been made toward understanding the functional groups on the molecules involved in the formation of such cross-links, their nature and location, etc. A variety of human conditions, normal and pathologic, relate to repair and regeneration of the collagenous framework of tissues. Some of these conditions are characterized by excessive deposition of collagen (e.g., cirrhosis, scleroderma, keloid, pulmonary fibrosis, diabetes). After trauma or surgery, abnormal deposition of collagen may impair function (adhesions following repair or scar formation during healing). In addition, many disabling conditions result from changes in the nature of collagen (heart valve lesions, osteoarthritis, rheumatoid arthritis, and congenital collagen diseases such as Marfan's and Ehler–Danlos syndromes and osteogenesis imperfecta.

Table 23.1 Summary of histologic observations of rabbit periarticular connective tissue

Tissue width	Collagen bundle (μm)	Crimp period (μm)	Crimp amplitude (μm)	Cell shape (μm)	Cell size (μm × μm)
Patellar tendon	20	120	15	Spindle	3–5 × 15
Achilles tendon	20	120	40	Spindle	3–5 × 15
Anterior cruciate ligament (ACL)	20	45–60	<5	Round to ovoid	5–8 × 12–15
Medial collateral ligament (MCL)	20	45	10	Rod to spindle	3–5 × 15

From: Amiel D, Kleiner JB: Biochemistry of tendon and ligament. In: Nimni ME, Olsen B, eds. Collagen Biotechnology, Vol III. Cleveland: CRC Press, 1988, with permission. Also from Khatod, M, Amiel D: Ligament Biocemistry and Physiology. In: Pedowitz R, Akeson WH, O'Connor, eds. Daniel's Knee Injuries, 2nd edition, LWW, NY, NY,2003, Pg 34

23.2.2 The Collagen Molecule

The arrangement of amino acids in the collagen molecules is shown schematically in Fig. 23.2. Every third amino acid is glycine. Proline and hydroxyproline follow each other relatively frequently, and the sequence (gly, pro, hyp) makes up about 10% of the molecule. This triple helical structure generates a symmetrical pattern of three left-handed helical chains that are, in turn, slightly displaced to the right, superimposing an additional "supercoil" with a pitch of approximately 8.6 nanometers (nm). These chains, known as alpha chains, have a molecular weight of around 100 kDa and contain approximately 1000 amino acids for the interstitial collagen Types I, II, and III (Fig. 23.3). The amino acids within each chain are displaced by a distance of $h = 0.201$ nm, with a relative twist of 100°, making the number of residues per turn 3.27, and the distance between each third glycine 0.87 nm.

The individual residues are nearly fully extended in the collagen structure, as the maximum displacement within a fully stretched chain would be approximately 0.36 nm. This separation will not allow interchain bonds to form, and only interchain hydrogen bonds are possible. In addition to these intramolecular conformational patterns, there appears to be a supermolecular coiling. A process of self-assembly causes the collagen molecules to organize into fibers.

23.2.3 Biosynthesis

Development of an extracellular network of collagen fibers requires cells involved in biosynthesis to synthesize procollagen. This molecule is then enzymatically trimmed of its nonhelical ends, and the resultant collagen molecule assembles into fibers in the extracellular space. Procollagen molecules have been identified as precursors of three interstitial collagens (Types I, II, and III). Several of the N-(amino) and C-(carboxy) terminal peptides (propeptides) have been characterized and the primary sequences determined.

The carboxyterminal propeptides of both α_1 and α_2 chains have molecular weights of 30,000 to 35,000 Da, and globular conformations without any collagen-like domain. These peptides contain asparagine-linked oligosaccharide units composed of *N*-acetylglucosamine and mannose. Once the molecule is completed and translocated to the cell surface, the extensions are enzymatically removed from those collagens, which then form fibrils. Enzymes that selectively remove these extensions can be found in a variety of connective tissues and in the culture media derived from collagen-secreting cells.

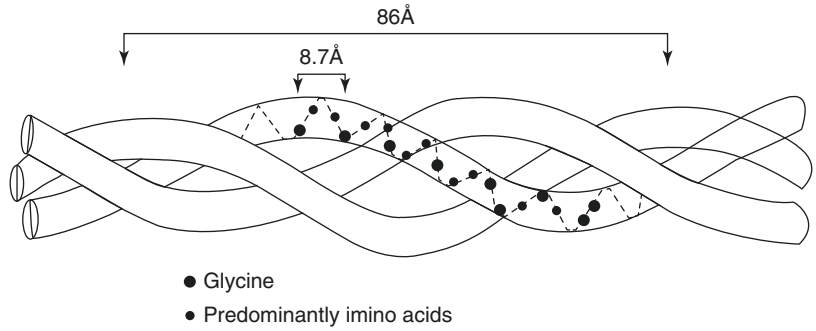

Fig. 23.2 The collagen triple helix. The individual α chains are left-handed helices with approximately three residues per turn. The chains are in turn coiled around each other following a right-handed twist. The hydrogen bonds which stabilize the triple helix (not shown) form between opposing residues in different chains (interpeptide hydrogen bonding) and are therefore quite different from α helices which occur between amino acids located within the same polypeptide. (From: Amiel D, Sano S: Periarticular Ligamentous Tissue. In: Practical Orthopaedic Sports Medicine and Arthroscopy. Eds Johnson D and Pedowitz R. LWW, Philadelphia PA. Chapter 1, pg 6, 2007)

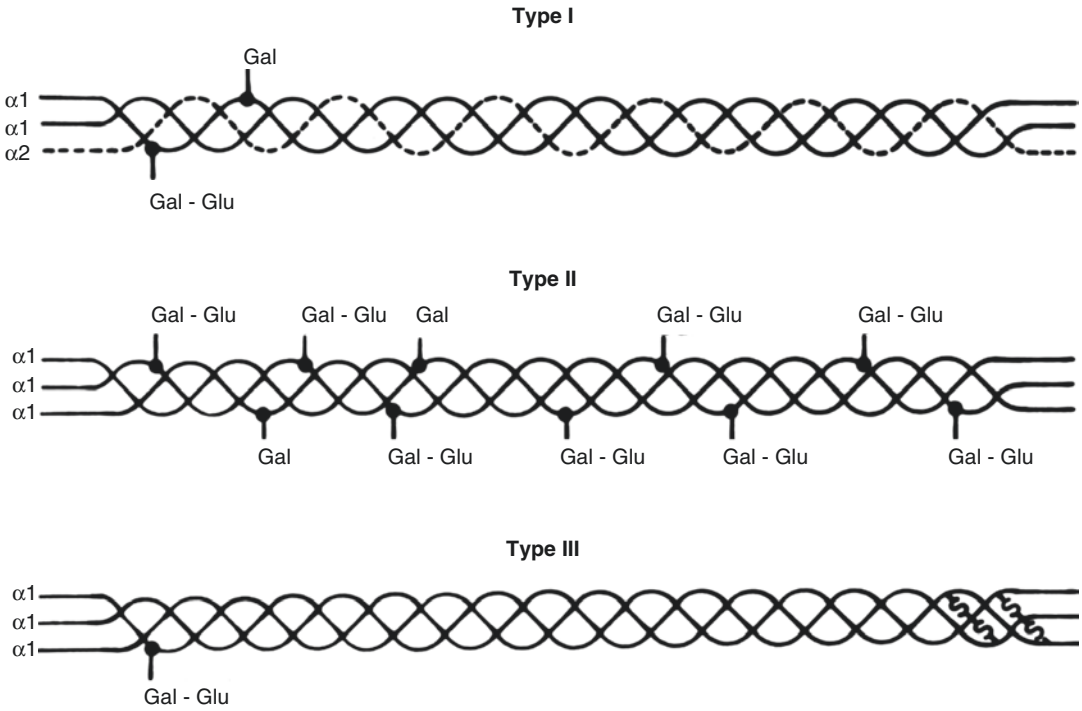

Fig. 23.3 Diagram of three interstitial types of collagen. Type I is present in skin, bone, ligaments and tendon, etc.; type II is present in cartilage; and type III is present in blood vessels and developing tissues and as a minor component in skin and other tissues. There are differences in the chain composition and degrees of glycosylation. Disulfide cross-links are only seen in type III collagen. (From: Amiel D, Sano S: Periarticular Ligamentous Tissue. In: Practical Orthopaedic Sports Medicine and Arthroscopy. Eds Johnson D and Pedowitz R. LWW, Philadelphia PA. Chapter 1, pg 6, 2007)

23.2.4 Gene Expression

Over the past 40 years since the discovery of type II collagen in cartilage, many other species of collagen have been identified. Types I, II, III, V, and XI collagen are categorized as fiber-forming collagens. They all exhibit lengthy, uninterrupted collagenous domains and are first synthesized as biosynthetic precursors (procollagens). Gene cloning experiments have demonstrated that Group I collagen genes are evolutionarily related, for they share a common ancestral gene structure. Human chromosome number 17 contains the coding information for the α_1 chain of Type I collagen, while chromosome 7 codes for its complementary α_2 chain. A comparison of the five fibrillary collagens described shows that, with one exception, (Types III and α_2 (V) are located on chromosome 2), all other genes are located on different chromosomes.

The gene codings for fiber-forming collagens are large, about 10 times the size of the functional mRNA. Many of the exons (coding sequences) are 54 base pairs (bp) in length, and are separated from each other by large intervening sequences (introns) that range in size from ~80 to 2000 bp. The gene itself contains 38,000 bp and is very complex. The finding that most exons of these genes have identical lengths suggests that the ancestral gene for collagen was assembled by multiple duplications of single genetic units containing an exon of 54 bp (Fig. 23.4).

As this chapter focuses on ligaments, the major characteristics of the two collagen types present in the ACL, namely Types I and III, are described. Type V collagen seems to be present at less than 1%.

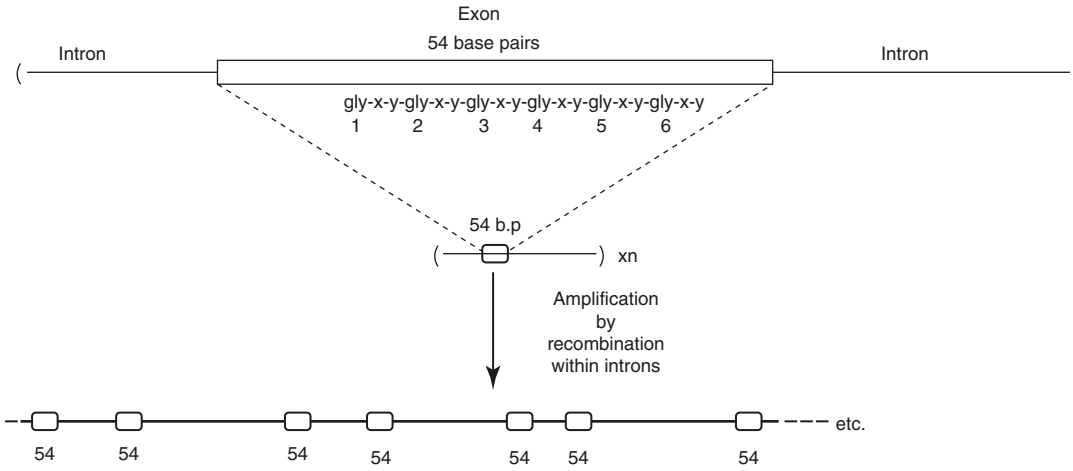

Fig. 23.4 The collagen gene is made up of multiple units containing 54 base pairs, each of which corresponds to sequences of 18 amino acids. *The conservation of this minimum sequence and the fact that it is repeated in such an exacting fashion provide valuable information to investigators interested in the process of evolution of proteins.* (From: Amiel D, Nimni ME: The collagen in normal ligaments. Iowa Orthop J 1993;13:49–55, with permission. Also In: Khatod, M, Amiel D: Ligament Biochemistry and Physiology. In: Pedowitz R, Akeson WH, O'Connor, eds. Daniel's Knee Injuries, 2nd edition, Lippincott, William and Wilkins, NY, NY, Pg 38)

23.3 Proteoglycans

Proteoglycans consist of small amounts of protein bound to negatively charged polysaccharide chains referred to as GAGs. In articular cartilage, proteoglycans form a large portion of the macromolecular framework (commonly about 30–35% of the tissue dry weight), but in ligaments they form only a small portion of this framework, usually less than 1% of the dry weight [13, 18, 19]. Nonetheless, proteoglycans may have an important role in organizing the extracellular matrix and interacting with the tissue fluid [20–27].

Like tendon, meniscus, and articular cartilage, ligaments contain two known classes of proteoglycans: large articular-type proteoglycans containing long, negatively charged chains of chondroitin and keratin sulfate (syndecan) and smaller proteoglycans that contain dermatan sulfate [22–24]. Because of their long chains of negative charges, the articular cartilage-type proteoglycans tend to expand to their maximum domain until restrained by the collagen fibril network. As a result, they maintain water within the tissue, alter fluid flow within the tissue during loading, and exert a swelling pressure, thereby contributing to the mechanical properties of the tissue and filling the region between collagen fibrils.

23.4 Noncollagenous Proteins (Glycoproteins)

These molecules are composed primarily of protein, but many of them also contain a few monosaccharides and oligosaccharides [19, 22, 24]. Although noncollagenous proteins such as fibronectin contribute only a few percentage points to the dry weight of ligaments, they have an important role in the complex interaction of ligament cells and their environment during growth, healing, and remodeling. This role, however, is poorly understood.

Fibronectins are important in an array of cellular functions, particularly those involving a cell's interaction with its surrounding extracellular matrix. They are high-molecular-weight extracellular glycoproteins whose functions include modulating intra- and extracellular matrix morphology, cellular adhesion (both cell-to-cell and cell-to-substratum), and cell migration. Examined by electron microscopy, fibronectins appear as fine filaments or granules coating the surface of fibrillary collagens or associated with cell membranes. Fibronectins have an adhesive domain specific to fibrin, actin, hyaluronic acid, cell surface factors, and collagen. They function to attract and couple key elements in normal healing and in growing tissue.

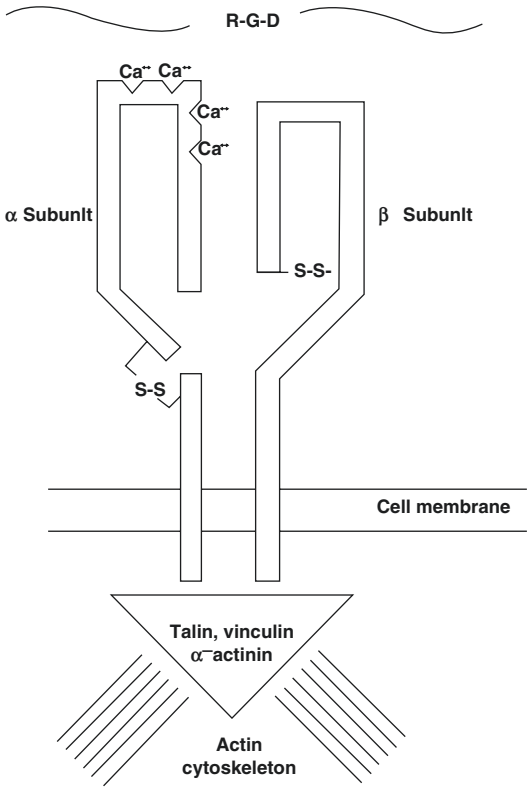

Fig. 23.5 Integrin structure. This schematic representation of a typical integrin demonstrates the large globular extracellular region, the single short membrane-spanning domain, and the carboxyterminal cytoplasmic domain of each subunit. The extracellular ligand-binding domain of a particular integrin is created by an association of the amino-terminal domains of both α and β chains (Hynes RO: Cell 1987; 48:549–554). Ligand recognition by these binding pockets uses specific amino acid sequences within the ligand peptide; the best described is the tripeptide sequence arginine-glycine-aspartic acid (R-G-D). This sequence is involved in binding a variety of ligands, including fibronectin, fibrinogen, thrombospondin, vitronectin, laminin, and type I collagen (Ruoslahti E: Annu Rev Biochem 1988; 57:375–413). The cytoplasmic domains of the integrin are physically linked to the actin-containing cytoskeleton, probably through intermediary cytoplasmic proteins, including talin, vinculin, and α-actinin (Burridge et al.: Annu Rev Cell Biol 1988; 4:487–525; Horwitz et al.: Nature 1986; 320:531–533; Otey CA et al.: J Cell Biol 1990; 111:721–729). (From Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 196)

Quantitative studies of fibronectin concentrations in rabbit ligaments demonstrate significantly (two to three times) higher amounts of fibronectin in the cruciate ligaments as compared to the collateral ligaments [13]. This difference may reflect the fact that the cruciate ligaments are surrounded by a synovial sheath, and therefore have a higher degree of cellularity relative to the extraarticular ligaments.

The maintenance of ligament tissue and its ability to respond to load changes depend on interactions between the cells and matrix. Normally, the matrix macromolecules are slowly but continually degraded and replaced. The cells must synthesize new macromolecules to balance the losses due to normal degradation or microtrauma. The matrix provides to the cells protection from mechanical injury during normal loading and transmits signals generated by loading to the cells.

Cells bind to the matrix primarily through a family of cell surface proteins called integrins. These molecules mechanically link the matrix macromolecules, including fibronectin, to the internal cell cytoskeleton. They participate in cell adhesion, migration, and proliferation, and in regulation of cell synthesis of new matrix macromolecules (Fig. 23.5).

23.5 Growth Factors

A vast and rapidly growing amount of literature abounds on a class of peptides commonly called growth factors. Accelerated healing of skin wounds has been reported after local application of several growth factors [28–30].

After injury the platelets travel to the wound site, form a clot, and hemostasis is obtained. Platelets secrete peptides such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β). Both PDGF and TGF-β play an important role in the initiation of repair processes after injury. These factors are chemotoxic for inflammatory cells, and appear to regulate proliferation and differentiation of fibroblasts [31–36]. Inflammatory cells at the wound site then release other peptides such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). bFGF has demonstrated stimulatory effects on

angiogenesis, urokinase-type plasminogen activator (implicated in the neovascular response), and wound healing [37]. PDGF has demonstrated beneficial effects upon ligament healing upon testing in a medial collateral ligament injury model in rats [38]. The administration of platelet-rich plasma has been shown to enhance collagen remodelingand hypercellularity with increased metabolic activity during the healing process [39]. Collagen PRP hydrogel has been shown to improve healing of the ACL with improved wound site healing in a canine injury model [40]. VEGF has been shown to increase healing via neogenesis [41, 42].

Because synovial fluid washes clots away from the ligament injury site, it is hypothesized that a deficiency of growth factors exists at the wound site. Without the necessary stimulus from growth factors and other clot-derived substances, the response to injury is poor.

Additional detail regarding effects of growth factors will be discussed in the following chapters on *Orthobiologic Approaches to Ligament Injuries and Biologic Augmentation in ACL injuries.*

23.6 Cellular-Biologic ACL–MCL Differences

Lyon et al. [43] further revealed cellular alignment differences. The deep portion of the central one-third of the MCL had compact collagen fiber bundles oriented along the longitudinal axis of the ligament. Spindle-shaped MCL fibroblasts were interspersed throughout the collagen fiber bundles. Transillumination of the MCL using polarized light demonstrated a high-amplitude, low frequency crimp pattern of the collagen fibers, which parallels that of adjacent fiber bundles. MCL fibroblasts were oriented at angles corresponding to the angle of collagen fiber crimp (Fig. 23.6a). The deep portion of the central third of the ACL also had compact, parallel collagen fiber bundles oriented along the long axis of the ligament. However, the fiber bundles were separated by narrow spaces containing ovoid cells arranged in columns like pearls on a string. The crimp pattern for the ACL collagen fiber bundles demonstrated a low amplitude, high frequency pattern (Fig. 23.6b). Furthermore, the cells within the narrow spaces did not conform to the crimp pattern of the adjacent fibers. The anteromedial bundle of human ACLs distinguished three

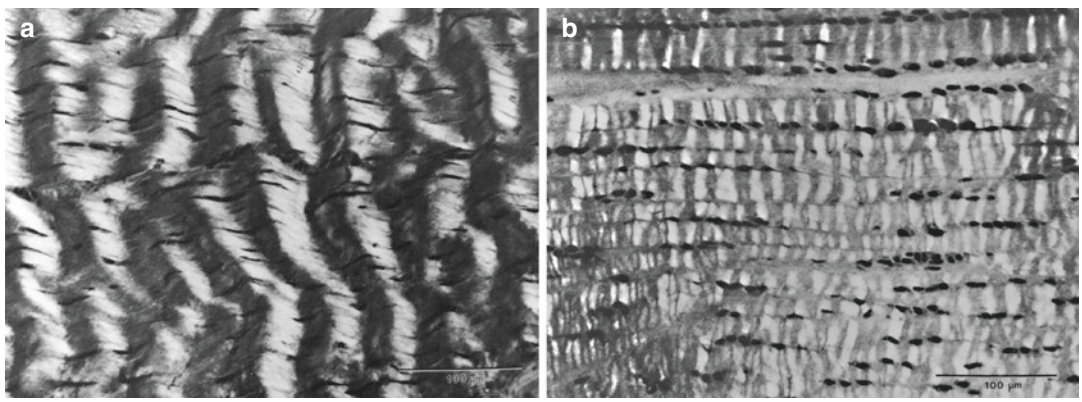

Fig. 23.6 (**a**) Polarized light photomicrograph of the midportion of the medial collateral ligament showing the sharp waveforms roughly parallel to each other across the section. The cell bodies and processes closely follow the waveform configurations (hematoxylin and eosin [H&E], ×50). (From Sherman MF, Bonamo JR. Primary repair of the anterior cruciate ligament. Clin Sports Med 1988;7:739–750, with permission.) (**b**) Polarized light appearance of the rabbit anterior cruciate ligament shows lack of register of waveforms of adjacent bundles. Cells are not tightly adherent to matrix. They do not deform in register with the waveforms of the matrix (H&E, ×50). (From Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 191)

different zones along the length of the ligament. These zones were characterized by fusiform, ovoid, and spheroid cell shapes. Fusiform and ovoid cells occupied the proximal quarter of the anteromedial bundle of the ACL and were found to express the alpha smooth muscle actin isoform. In the spheroid zone, which constitutes the distal three fourths of the ACL, only a portion of cells expressed the alpha smooth muscle actin isoform [44]. These cellular alignment differences may represent a spectrum among ligamentous cells with the classic fibroblast-fibrocyte at one end and the chondroblast-chondrocyte at the other end [44]. But in general, the MCL histologically appears to favor the fibroblast-fibrocyte phenotype, whereas the ACL cell tends to favor a fibrocartilage phenotype [43].

Cellular and biologic processes need to be discussed to understand the responses to ligament injury. The cellular difference in the ACL versus the MCL includes differences in phenotype, cellular alignment, proliferation, migration, adhesion to substrates, responses to mechanical forces, and signaling. Nagineni et al. [45] studied knee ligament histology and found that MCL cells exhibited a typical fibroblastic morphology. The cells were elongated and spindle shaped. ACL cells, however, were slightly larger and more ovoid in shape. Lyon et al. [43] in our laboratory reported that ACL cells had a more fibrocartilage characteristic.

Cellular cytoplasmic processes were found to be different. The MCL fibroblast has long cytoplasmic processes extending outward into the surrounding matrix. In striking contrast, the ACL cells are devoid of any long cytoplasmic processes, and the cell membrane and the adjacent collagen fibrils are separated by an amorphous matrix [43]. When these cells were stained for fibronectin, both cell types showed intense staining in the area of the cell membrane. The major difference was that the fibronectin stain followed the MCL processes far out into the matrix. Because the ACL cells lack the long processes, they did not have the same staining pattern [43] (Fig. 23.7). Burridge and Chrzanowska-Wodnicka [46] found more bundles of microfilaments (representing stress fibers) in the ACL than in the MCL. The result supported the conclusion that the ACL cells are able to form more stable adhesion plaques than the MCL cells.

Proliferative differences between ACL and MCL cells have been well demonstrated. The outgrowth of cells from ACL explants was slower than that from MCL explants and slower in closing in vitro confluent culture streak wounds [45]. Growth curves of ACL and MCL cultures at both passage numbers two and six showed a significantly slower rate of proliferation of ACL cells than MCL cells (Fig. 23.8) [47–49]. DNA synthesis measured in terms of tritiated thymidine incorporation of both log phase and confluent cultures supports the conclusion that differential proliferation rates of these cells exist in culture [45]. Furthermore, an in vitro wound created in a confluent layer of ACL and MCL cells revealed that 48 h after injury, the cell-free zones created in ACL cultures were occupied partially by single cells in a nonconfluent fashion. In contrast, the wounded zone in the MCL cultures was almost completely covered by cells [45] (Fig. 23.9). These results demonstrate a lower proliferation and migration potential of ACL cells in comparison with MCL cells in response to injury.

Another factor important in the migration of ligament fibroblasts is the expression of fibronectin [50]. The ACL and PCL each contain twice the amount of fibronectin found in either MCL or patellar tendon. The cellular-biologic characteristics of phenotype, cellular alignment and crimp pattern, proliferation, and migration reveal differences in the intrinsic properties of the normal ACL versus MCL cells. These different intrinsic properties between the cells of these ligaments have been proposed as important factors in their differential repair mechanisms.

23.7 Ligament Injury

Ligamentous injuries of the knee joint are among the most common ligament injuries encountered by orthopedists [51]. The anterior cruciate ligament and medial collateral ligament are major ligaments contributing to the stability and normal functioning of the knee

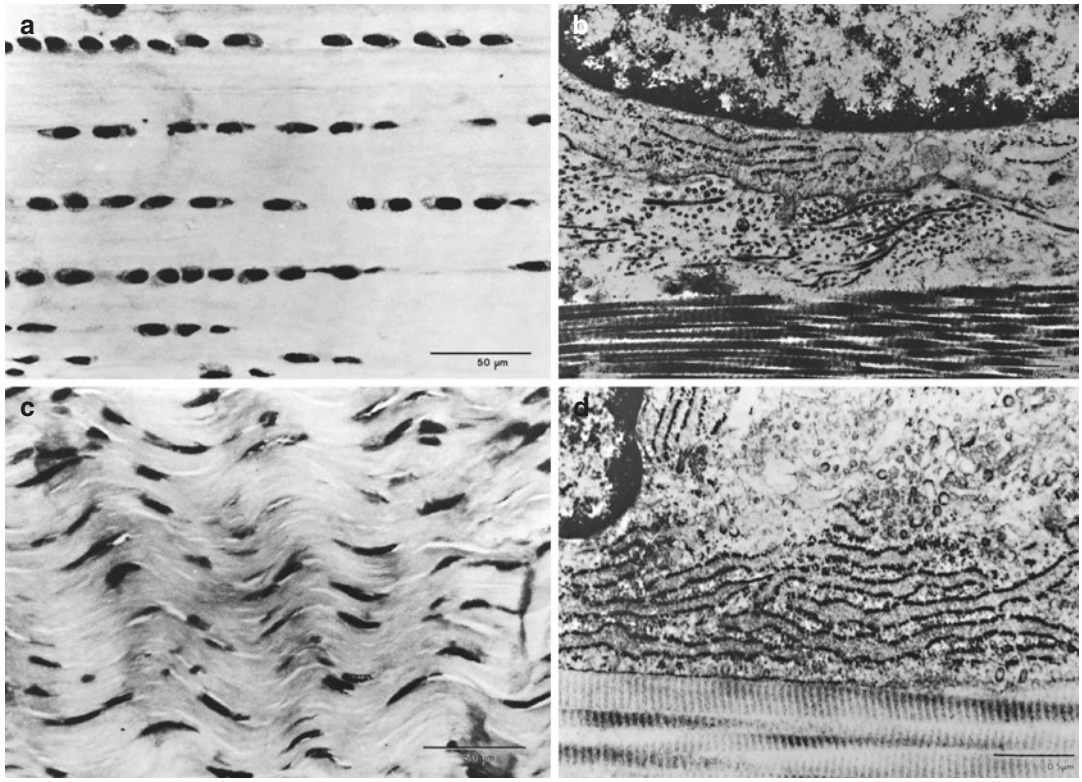

Fig. 23.7 (**a**) Longitudinal section from the deep midportion of the rabbit anterior cruciate ligament (ACL). The cells are strung out like pearls on a string and lack long cellular processes [hematoxylin and eosin (H&E), ×100]. (**b**) Photomicrograph of longitudinal section from the deep midportion of a rabbit medial collateral ligament (MCL). The cells are spindle-shaped with long cytoplasmic processes extending distances many times the length of the cell body (H&E ×100). (**c**) High-power transmission electron microscopy (TEM) of rabbit ACL reveals an amorphous matrix separating the cell membrane and the adjacent collagen fibrils. The mature collagen fibrils are not closely approximated by the cell membrane. (**d**) High-power TEM of rabbit MCL shows a fibroblast whose cell membrane is in close proximity to the mature collagen fibrils adjacent to it (From O'Donoghue DH, Frank GR, Jeter GL, et al. Repair and reconstruction of the anterior cruciate ligament in dogs: factors influencing long-term results. J Bone Joint Surg Am 1971;53:710–718, with permission.) (Also from Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 190)

joint [52–55]. Injuries to these ligaments can be clinically classified as described by Rockwood et al. [56] into three degrees. A first degree sprain involves a tear of a minimum number of fibers (microtears) or less than one-third of the ligament. There is minimal hemorrhage and swelling, localized tenderness, and no clinical instability or laxity. Second degree sprains involve a tear of more ligamentous fibers (one-third to two-thirds of the ligament) with a greater loss of function, localized tenderness, and an effusion, but there is no laxity or noticeable instability. Third degree injuries have greater disruption (greater than two-thirds of the ligament), more tenderness, and demonstrable laxity of the knee joint. Laxity of the knee can be evaluated clinically on a grade 1–3 scale (grade 1 = 0–5 mm, grade 2 = 5–10 mm, grade 3 = >10 mm). Gradation is dependent on the distance of translation of the joint on physical exam, reflecting injury severity in the ligament.

It is well known that the ACL mounts a poor to negligible repair response to injury [57–61], whereas the MCL heals readily without even the need for surgical repair in most cases [59–62].

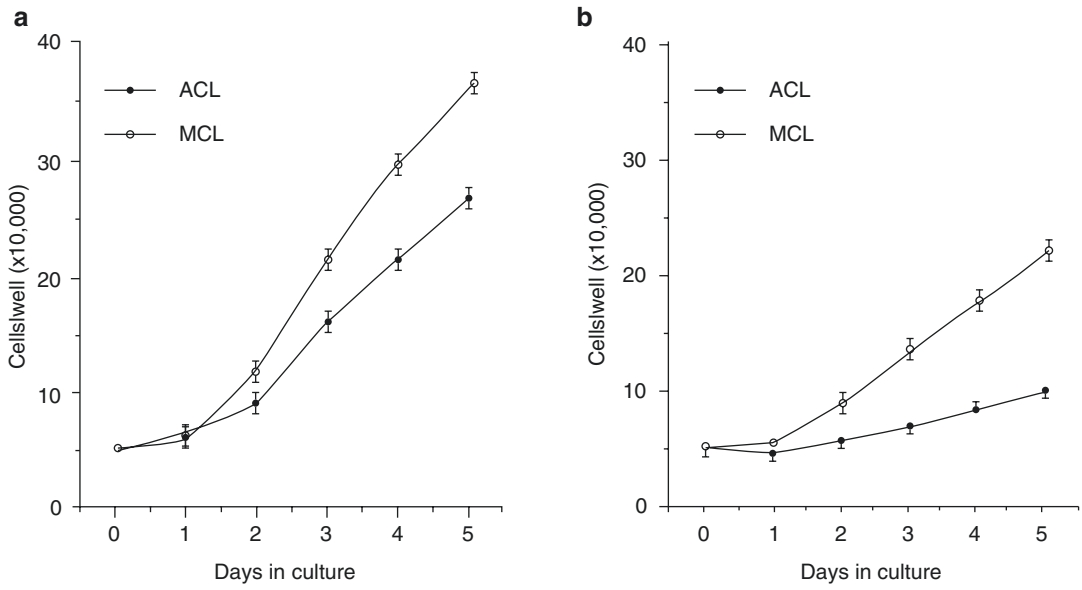

Fig. 23.8 Growth curves of anterior cruciate ligament (ACL) and medial collateral ligament (MCL) at passages two (**a**) and six (**b**). Results are means ± SEM of duplicate samples of six batches of cultures derived from ACL and MCL tissues of six rabbits. (From Nagineni CN, AMiel D, Green MH, et al. Characterization of the intrinsic properties of the anterior cruciate and medial collateral ligament cells: and in vitro cell culture study. J Orthop Res 1992;10(4)::470, with permission)

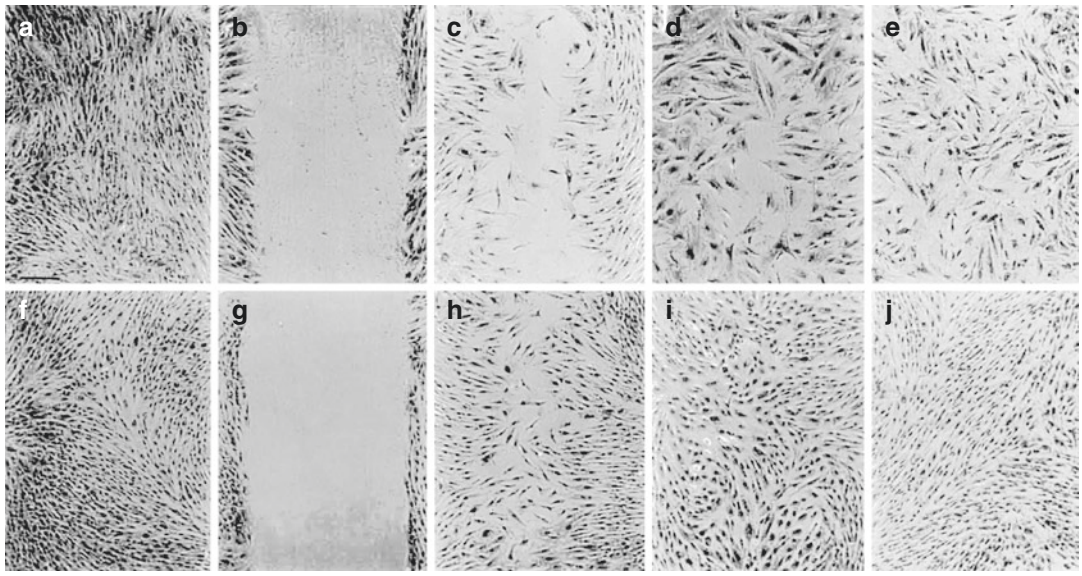

Fig. 23.9 (**a**–**j**) Representative pictures of anterior cruciate ligament (ACL) and medial collateral ligament (MCL) cultures subjected to in vitro wounding and allowed subsequent healing. (**a**–**e**) ACL cultures. (**f**–**j**) MCL cultures. (**a**, **f**) Control cultures. (**b**, **g**) Immediately after wounding. (**c**, **h**) Twenty-four hours after wounding. (**d**, **i**) Forty-eight hours after wounding. (**e**, **j**) Seventy-two hours after wounding. Bar in (**a**) represents 200 μm. All pictures are at the same magnification. (From Nagineni CN, Amiel D, Green MH, et al. Characterization of the intrinsic properties of the anterior cruciate and medial collateral ligament cells: and in vitro cell culture study. J Orthop Res 1992;10:473, with permission)

Multiple structural factors influence knee ligament injury and repair: (a) location of the injury within the ligament determines the type of reparative cell, osteoblast versus fibroblast; (b) the size of the injury in the collateral ligament potentially affects the mechanical strength of the repair scar; (c) the blood supply to each ligament affects its nutrition [63] has also postulated that variation of oxygen tension in ligaments during healing may be an important modulator of successful repair; and (d) the location of the ligament, intrasynovial versus extrasynovial, may play a role in its ability to mount a repair process.

23.7.1 Morphology Issues in Knee Ligament Repair

To understand the healing response of the ligament to injury, one must examine the structural factors involved in the injured ligament. Location of the injury within the ligament is one such factor. Sherman and Bonamo [64] found that proximal stump tears (those injuries involving the proximal 20% of the ligament) accounted for more than 80% of ACL tears. Midsubstance or "mop end" tears accounted for another 10% of all tears, and avulsions accounted for less than 5% of ACL injuries. Lyon et al. [43] found that the only exception to poor ACL healing is seen in avulsion injuries of the ligament from its attachment to bone. The repair response in the avulsion injuries is mounted by the bone cells, not by the cells of the ACL. The cells of the ACL do not mount an effective repair response. The ACL will occasionally drop down after injury and become affixed to the PCL [65], but in this altered location it is not functionally adequate in the athletically inclined patient. The location of injury within the ligament substance of the MCL does not seem to alter its ability to undergo functional repair but, as mentioned earlier, if the injury occurs in combination with ACL tears, the MCL will tend to heal with laxity unless the ACL is first reconstructed [66].

The size of the injury was also found to be of importance in the eventual mechanical strength of healing in the collateral ligament. Loitz-Ramage et al. [67] created an 8mm gap injury compared with a 4mm Z-plasty injury in the MCL of a rabbit knee. Mechanical testing at 40, 78, and 104 weeks showed the scar material properties in both injury models remained markedly inferior to normal, and gap injuries showed significantly inferior structural properties at all intervals. These results suggest that a large initial gap between ligament ends in the extraarticular space predisposes scars to long-term structural weakness. Still, clinical studies have generally found no benefit of suture versus closed management of the MCL.

A surgical model was developed to explore the role of surgical repair methods in repair of the ACL [68] which allows assessing the structural issues of intrinsic healing mechanisms in the knee ligaments. Initial attempts to repair a complete laceration of the ACL were carried out on rabbits with complete midsubstsance lacerations, using a technique described by Marshall et al. [69] (Fig. 23.10). Six weeks after surgery, none of the repaired ligaments showed evidence of healing. All specimens were in the process of resorption, and a large gap spanned by suture was uniformly evident. A second group was operated on in a similar manner, except the sutures were placed before lacerating the ACL in an attempt to decrease the interstump gap. The same resorptive process and lack of healing response were observed in all animals 6 weeks after surgery.

To limit stump retraction and ensure accurate approximation of the lacerated portions of the ACL, a Z-plasty repair was attempted (Fig. 23.10b). Six weeks later, no evidence of healing was observed. Failure of this technique was again related to the retraction of lacerated ACL stumps, followed by resorption of the exposed ligament ends.

A model was developed where only the midportion of the ligament was transected (Fig. 23.10e). This model minimally disturbs the biomechanical stability of the ligament by retaining lateral and medial ligament continuity. Thus, the lacerated ends stay in close proximity to each other during the post-laceration recovery period (Fig. 23.11).

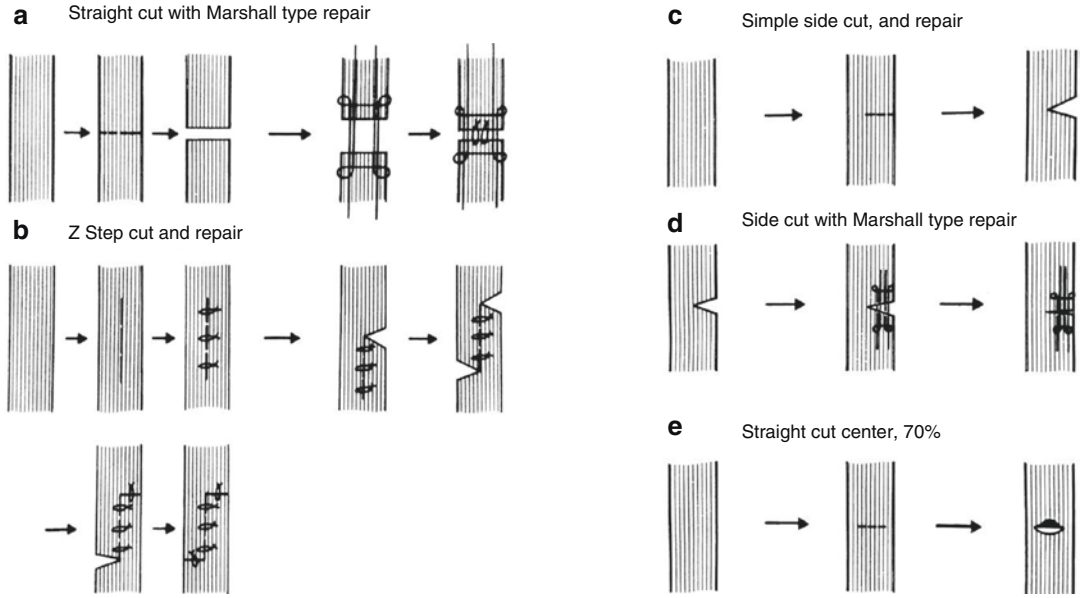

Fig. 23.10 Surgical models for evaluation of anterior cruciate ligament healing. (**a**, **b**) Complete laceration models. (**c**–**e**) Partial laceration models. (From Amiel D, Kleiner JB. Biochemistry of tendon and ligament. In: Nimni M. Olsen B, eds. Collagen: biotechnology. Vol. 3. Cleveland, OH: CRC Press. 1988, with permission.) (Also from Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 186)

The surface area of the ligament exposed to joint fluid is also limited in this model to the site of the perforation into the ligament by a 2 mm wide, razor thin, square edged Beaver blade. Access to the area of injury by potentially harmful enzymes contained in the synovial fluid is thereby restricted. The model largely, although not totally, eliminates two mechanisms proposed to be responsible for failure of ACL healing: (a) destabilizing biomechanical forces at the injury site and (b) enzymatic degradation of ligament substance along with inhibition of fibroblast activity by synovial fluid. Using the surgical ACL laceration model described herein, a partial healing response was observed in a small percentage (5%) of ACLs tested in a reproducible fashion (Fig. 23.12).

Another morphological reason postulated for the difference in healing responses between the ACL and the MCL is their differences in blood supply. The MCL has a rich blood supply which it derives from the inferior medial geniculate artery and from its osseous attachments [70]. The blood supply to the ACL is described as poor [70–72]. A paraligamentous network of vessels courses through the synovial membrane. These vessels enter the ligament transversely and anastamose freely with endoligamentous vessels. The core of the midportion of the cruciate ligament is less well vascularized than the proximal and distal cores.

Of the many morphologic factors important in ligament healing—mechanical forces, blood supply, and local environment—it is the local environment that has often been used as an explanation for the poor healing capacity of the cruciate ligaments [73]. The cruciate ligaments reside in a unique environment. Both ligaments are intracapsular, and both are enveloped by a synovial membrane, effectively making them extrasynovial. The synovial membrane is only a few cells thick and separates the cruciates from the synovial fluid that bathes the other intracapsular knee joint structures. During ACL injury the synovial membrane is usually torn, exposing the frayed ligament ends to the synovial fluid and a host of potentially destructive enzymes released by the breakdown of hemarthrosis fluid in the injured joint. This local environment has been referred to as the "hostile" environment of the synovial joint space.

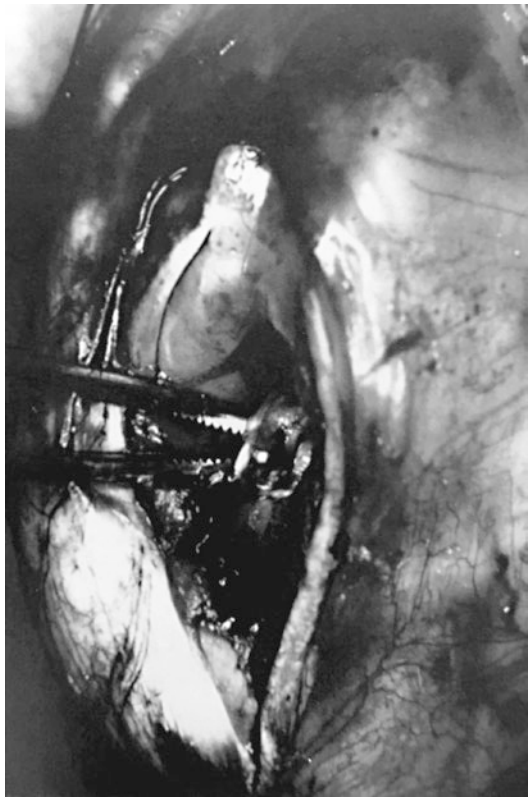

Fig. 23.11 Midsubstance partial laceration in rabbit anterior cruciate ligament. (From Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 187)

Synovial fluid, formed from an ultrafiltrate of blood [74], has been shown to adversely affect ligament fibroblasts and to stimulate them [75, 76]. Andrish and Holmes [75] demonstrated that ACL fibroblast proliferation was diminished in vitro when exposed to synovial fluid. Synovial fluid has also been shown to be a physiologically important nutrient delivery pathway for the ACL [77]. Nickerson et al. [76] found that bovine synovial fluid stimulates proliferation of rabbit ACL and MCL cells. Maximum stimulation occurred at 20% concentration with diminishing stimulation at higher concentrations; however, even high concentrations were not inhibitory.

Our laboratory studied the role of synovial fluid in providing nutrition to rabbit knee ligaments and menisci, in vivo, by intra-articular injection of titrated proline (a collagen precursor) [77]. Measurement of 3H hyp incorporation showed that all knee structures tested utilized synovial fluid-derived proline. The cruciate ligaments demonstrated the highest uptake of 3H hyp (Fig. 23.13). Control ligaments and menisci showed no detectable isotopes. These findings indicate that intra-articular structures can derive nutrition from a synovial source.

Fig. 23.12 Laceration site, 12 weeks after surgery (hematoxylin and eosin, ×50). (From Amiel D, Kleiner JB. Biochemistry of tendon and ligament. In: Nimni M, Olsen B, eds. Collagen: biotechnology. Vol. 3. Cleveland, OH: CRC Press. 1988, with permission.) (Also from Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 187)

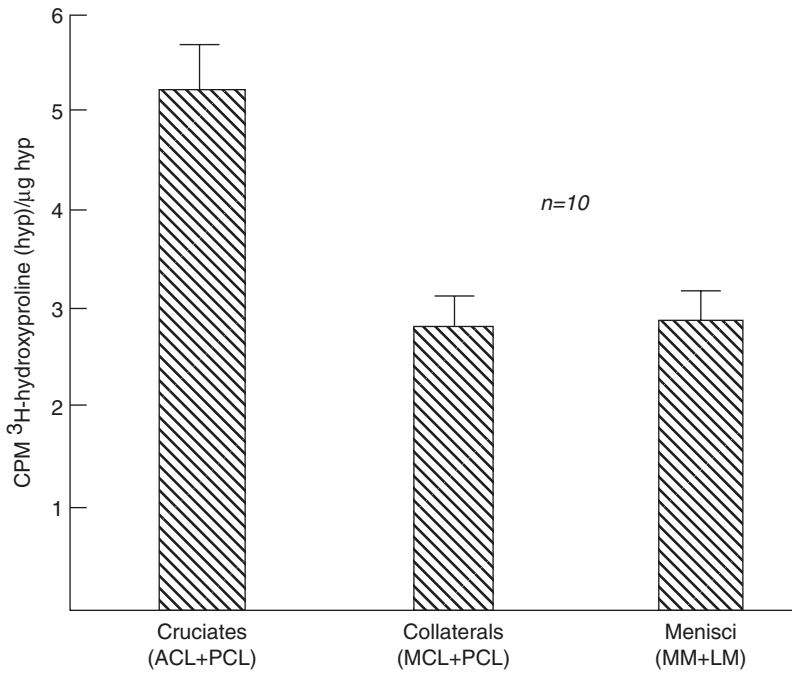

Fig. 23.13 Nutrient uptake of the various periarticular connective tissue structures. (From Amiel D, Abel MF, Kleiner JB, et al. Synovial fluid nutrient delivery in the diarthrial joint: an analysis of rabbit knee ligaments. J Orthop Res 1986;4:90–95, with permission.) (Also from Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 188)

Rapid degeneration of the ACL occurs after acute rupture. Warren [78] described this phenomenon clinically wherein ruptured ACL ligament substance could completely disappear 6 weeks after injury. These findings were confirmed by Kohn [79], who noted either complete disappearance or only remnant of the ACL in patients who underwent arthroscopy.

To test the hypothesis that ligament resorption after ACL injury represents a cellular response of intrinsic ligamentous cells to degrade their extracellular matrix, a rabbit model of ACL injury was created by our laboratory with the development of an in vitro assay for collagenase activity [80]. A collagenase assay was used because collagen represents the major structural protein of the cruciate ligament. The left ACL was transected off its tibial insertion, whereas the right knee served as a sham-operated control. The ACL and menisci were harvested 10 days after surgery, placed in tissue culture, and assayed 3 days later. Results demonstrated a relatively large increase (82%) in injured ACL collagenase content compared with control ACLs (Fig. 23.14). This was consistent with the average net loss of 34% in total collagen mass from the injured ACLs.

In addition, the transected ACLs were swollen and retracted (Fig. 23.15). The free transected ligament ends displayed a relative hypocellularity and loss of collagen matrix organization, histologically confirming the observation that, once ruptured, the free ends of the ACL undergo rapid degeneration [71, 78, 79]. The transected ACL tissue itself may be responsible for this degenerative process. Cells within the ACL may respond to injury by degrading their collagenous matrix.

Collagenase release has been documented from other articular structures such as synovium [81, 82] and articular cartilage [83, 84]. These structures synthesize and release a latent form of collagenase. The data from our experiment indicate that the ACL and menisci secrete only active enzymes, which may be detrimental to intra-articular structures [85]. The ACL may be a privileged intra-articular structure, because it possesses a synovial covering that allows it to be protected from the intra-articular environment. With acute rupture of the ACL and resultant synovial injury, this protective barrier may be lost. Subsequent exposure of ligament substance to the intra-articular environment may produce changes in the ligament and

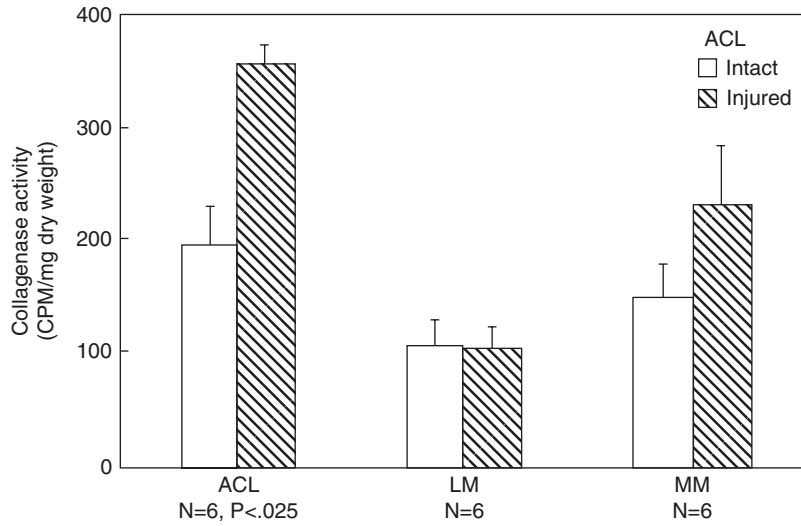

Fig. 23.14 Collagenase activity. Note significant increase in collagenase activity in injured anterior cruciate ligaments. No differences were noted in menisci. (From Amiel D, Ishizue KK, Harwood FL, et al. Injury of the ACL: the role of collagenase in ligament degeneration. J Orthop Res 1989;7:486–493, with permission.) (Also from Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 189)

may explain, in part, the poor results reported with attempts to primarily repair the ACL.

23.8 Ligament Repair

Andriacchi et al. [86] and Arnoczsky [87] described four phases of healing in the injured ligament. According to their description, phase I occurs within the first 72 h after injury, and encompasses the acute inflammatory response. This phase has two distinct components important for understanding potential therapeutic control approaches to stimulate or to reduce scar proliferation. Hematoma formation is the first element with its associated platelet aggregation and degranulation. The platelet release of growth factors stimulates the second element, the trafficking of white cells into the area of injury. Phase II lasts over the next 6 weeks and is associated with a marked proliferation of both cellular and extracellular components. This phase involves the inward migrating monocyte transformation into macrophages, which in turn stimulate fibroplasia. Remodeling in the Andriacchi–Arnoczsky conceptual framework involves two phases. Phase III occurs from 6 weeks to several months and involves the initial remodeling of the early scar. Finally, in phase IV, the final remodeling of the area of injury occurs with maturation of the repair tissue, which can continue for years. Neurath et al. [88] reviewed these phases in human ACL injuries and found that in phase I, erythrocytes, lymphocytes, and mononuclear macrophages were common. In the extracellular matrix, fibrinous exudate and cell debris was observed predominately near the ruptured area. At the end of this phase, Day 3, a marked proliferation of fibroblasts occurred with a markedly enhanced proliferation of fibroblasts occurred with a markedly enhanced expression of type III procollagen in the pericellular area. Phase II revealed proliferation of fibroblasts in the stumps of the ruptured ACL. This proliferation was even more pronounced than in phase I. The cellular ultrastructure of these cells was increasingly distorted. There were only a few myofibroblasts in the ligament stumps. Phase III demonstrated increased variance from patient to patient. The repair tissue in the ACL stump however never approached normal ligament characteristics.

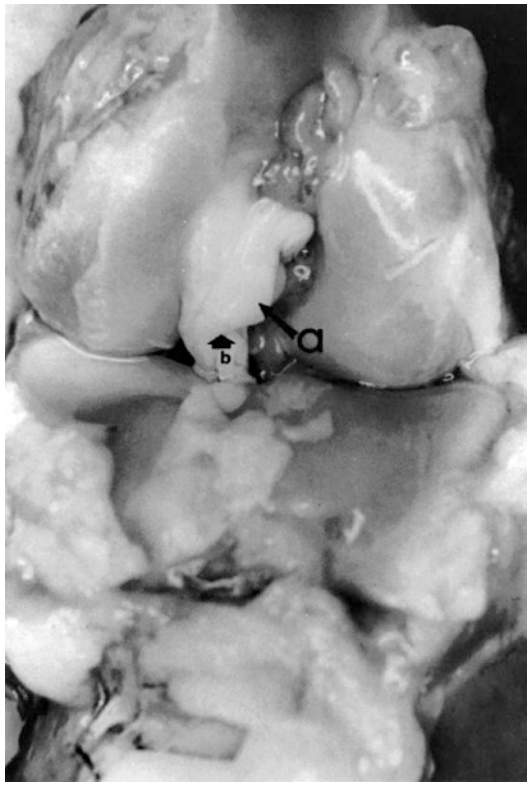

Fig. 23.15 Gross morphology showing transected anterior cruciate ligament. Note swollen (*a*) and retracted (*b*) appearances. (From Amiel D, Ishizue KK, Harwoo FL, et al. Injury of the ACL: the role of collagenase in ligament degeneration. J Orthop Res 1989;7:486–493, with permission.) (Also from Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 189)

The MCL is found to have a distinctly different repair process. Frank et al. [62] reviewed the healing of the MCL in rabbits. Grossly, all ligaments in this experiment "healed" by bridging their gap injury with "scar tissue." The MCL defect was filled with vascular inflammatory tissue by 10 days as seen by histology. In the "scar zone" itself, the preponderance of inflammatory tissue then subsided and active fibroblasts then subsided and active fibroblasts then dominated most fields by 3 weeks. Between 3 and 6 weeks, there was a decrease in fibroblast numbers and size, and some evidence of longitudinal (along the long axis of the ligament) alignment of their nuclei. At 14 weeks, continued remodeling had occurred, with improving realignment and a further decrease in cell numbers. Between 14 and 40 weeks, few changes were noted. Cells remained larger and more numerous than those seen in normal MCLs (Fig. 23.16).

23.8.1 Maturation of the Extracellular Matrix During the Healing Process

As the MCL heals, the biochemical and biomechanical properties of the healing tissue are altered from normal values [64]. Biochemically, the water content of the injury site is significantly elevated at 10 days and at 3 weeks. By 6 weeks, however, it returns to normal values. The glycosaminoglycan (GAG) content, a measurement of the proteoglycan content in the matrix, also increases significantly. This increase occurs early and remains elevated at all postinjury intervals. There was a trend toward normal, but the GAG content remained elevated at 40 weeks. The collagen content is found to be normal at 10 days but drops significantly by 3 weeks. A subsequent return toward normal values is seen, but never completely attained. Collagen typing also shows an altered ratio of type III to type I collagen. Type III collagen, an immature form of collagen, is found to be significantly increased in the ligament scar at all time intervals. DNA content, as well, is significantly elevated at all intervals except 40 weeks where the content is slightly, but not significantly, elevated. Type III collagen is thought to be of particular use in the early repair process because of its ability to form rapid cross-links that stabilize the repair site [89]. In the Sherman study [64], the authors concluded that despite the apparent healing of the MCL, a continuous remodeling of the ligament primarily at the site of injury was still occurring even at 1 year after injury.

Structural alterations of the healing ligament include changes in the cross-sectional area of the midsubstance of the ligament scar tissue. This is significantly increased over normal at all time intervals. The increased diameter is greatest by 3 weeks, corresponding to the increased water content, and then undergoes a gradual decrease from 3

to 14 weeks. The diameter remains stable through 40 weeks. Ligament scar laxity was significantly increased at 3 weeks. The laxity decreased slightly by 6 weeks and was not significantly different from normal at 14 weeks. This decrease in laxity is presumably caused by contraction of the scar by myofibrils. At 40 weeks, however, a significant amount of laxity is once again detected in the healed MCL. Another mechanical property, load at ligament failure, was significantly lower for all experimental ligaments compared with their contralateral shams. These experiments reveal that the injured MCL will heal without primary repair, but that the resultant ligament scar will have altered biochemical and biomechanical properties. The remodeling that occurs appears to favor a return of

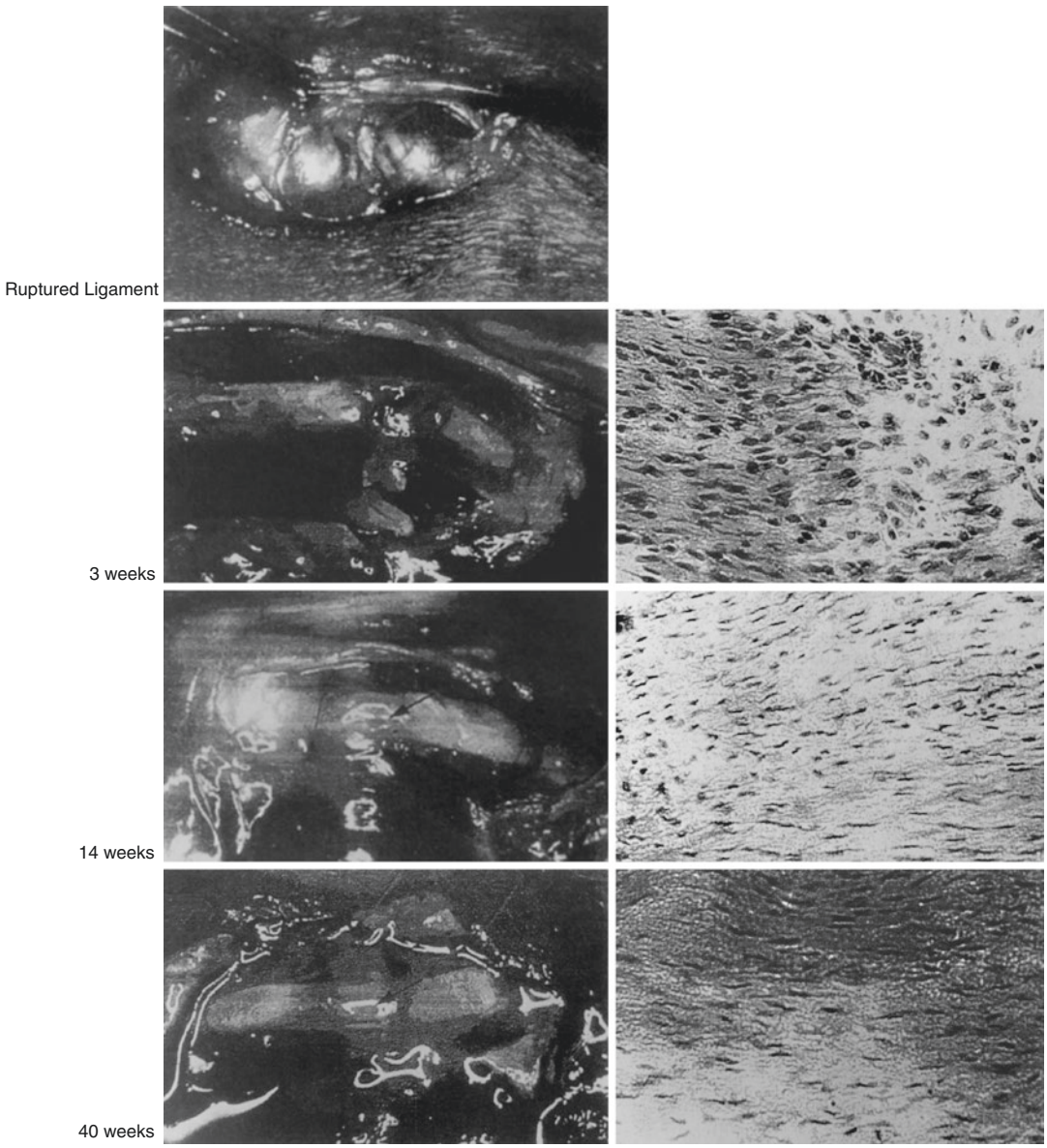

Fig. 23.16 Representative sections of scar tissue taken from midsubstance areas of healing medial collateral ligaments at different intervals. Progressive changes in cell numbers, size (metabolic activity), distribution, and orientation are noted as stages of inflammation, proliferation, and remodeling take place (Hematoxylin and eosin, ×30). (Khatod M, Akeson WH and Amiel D: Ligament Injury and Repair, Chapter 11. In: Daniel's Knee Injuries, 2nd Edition/Pedowitz, Akeson and O'Connor, LWW, NY, NY, 2003, Pg 194)

the biochemical properties such as the water content, GAG content, and collagen content at the expense of biomechanical measurements such as ligament laxity and load at failure. Although further remodeling may occur to create functional recovery of the MCL, studies have not shown a similar biologic recovery of the ligament scar. The ACL mounts neither a functional nor a biologic recovery.

Characteristics of ligaments can be acquired when structures are transferred to a new environment. We have evaluated the process of ligamentization of a patellar tendon graft which was used for ACL reconstruction in a patient. At the time of harvest, histologic findings demonstrated remodeled to a ligament structure including a change in crimp pattern (Figs. 23.17, 23.18, and 23.19) (Lane et al. [90]).

In conclusion, ligament healing is dependent upon many factors. Structural factors such as laxity, location of tear, and size gap affect healing. Growth factors, integrins, and other molecular factors also play a crucial role in ligament healing. The explosion of studies evaluating ligament healing and the effect of orthobiologics will provide a clearer understanding of this mechanism. This will enable specialized treatment based upon the different characteristics of the structure injured and its response to the different biologic factors available to augment healing.

Acknowledgements The authors wish to acknowledge the University of California San Diego Orthopaedic Connective Tissue Biochemistry Laboratories; the Musculoskeletal and Joint Research Foundation, San Diego; Ms Fran Shepherd for her word processing assistance; and Ms Divyani Patel for her editing assistance.

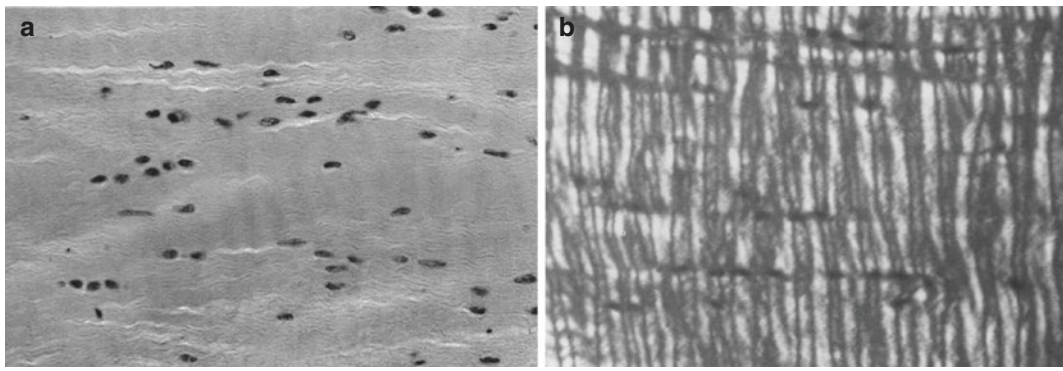

Fig. 23.17 (**a**) Normal ACL, hematoxylin and eosin stain. (**b**) Normal ACL, polarized microscopy. (From: Lane JG McFadden P, Bowden K and Amiel D: Arthroscopy 9(2):151, 1993)

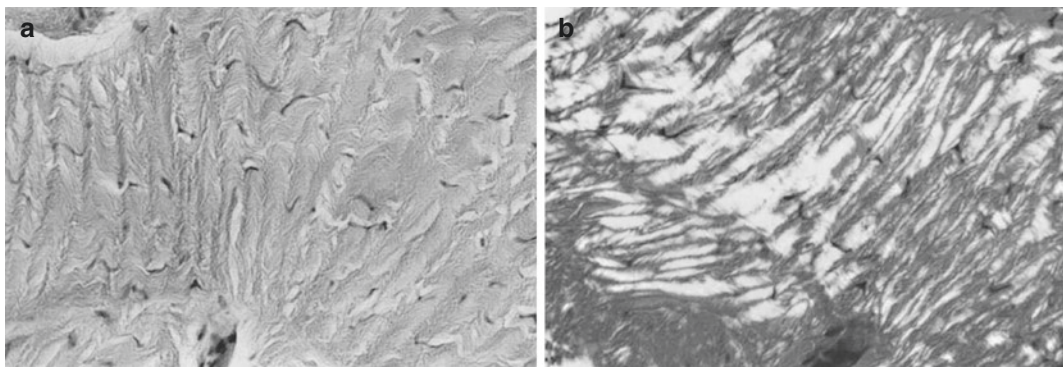

Fig. 23.18 Histology and polarized microscopy from human hamstring tendon, human semitendinosis autograft, and human ACL (×100). (**a**) Normal hamstring tendon, hematoxylin and eosin stain. (**b**) Normal hamstring tendon, polarized microscopy. (From: Lane JG McFadden P, Bowden K and Amiel D: Arthroscopy 9(2):151, 1993)

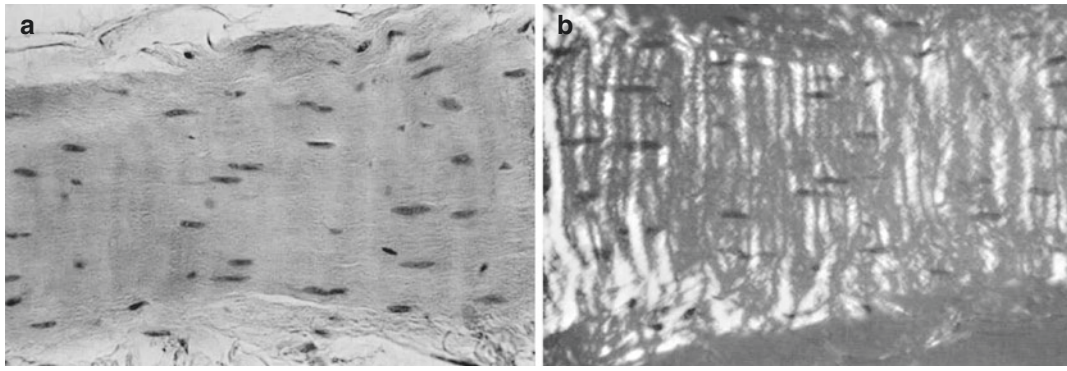

Fig. 23.19 (**a**) Semitendinosis autograft, hematoxylin and eosin stain. (**b**) Semitendinosis autograft, polarized microscopy. (From: Lane JG McFadden P, Bowden K and Amiel D: Arthroscopy 9(2):151, 1993)

References

1. Noyes FR, DeLucas JL, Torvik PJ. Biomechanics of anterior cruciate ligament failure: an analysis of strain rate sensitivity and mechanisms of failure in primates. J Bone Joint Surg. 1974;56A:236–53.
2. Noyes FR, Grood ES. The strength of the anterior cruciate ligament in humans and the rhesus monkeys: age-related and species-related changes. J Bone Joint Surg. 1976;58A:1074–82.
3. Noyes FR, Grood ES, Butler DL, et al. Clinical biomechanics of the knee-ligament restraints and functional stability. In: Funk Jr FJ, editor. Surgical repair and reconstruction. Am Acad Orthop Surg Symp on the Athlete's Knee. St Louis: CV Mosby; 1980. p. 1–55.
4. Bloom W, Fawcett DW, editors. A textbook of histology. 8th ed. Philadelphia: WB Saunders; 1962.
5. Copenhaver WM, Bunge RP, Bune MP, editors. Bailey's textbook of histology. 16th ed. Baltimore: Williams and Wilkins; 1971.
6. Han AW, editor. Histology. 6th ed. Philadelphia: JB Lippincott; 1974.
7. Amiel D, Nimni ME. The collagen in normal ligaments. Iowa Orthop J. 1993;13:49–55.
8. Nimni ME, Harkness RD. Molecular structures and functions of collagen. In: Nimni ME, editor. Collagen: biochemistry. Boca Raton: CRC Press; 1988. p. 1–77.
9. Amenta P, editor. Histology. 3rd ed. New Hyde Park: New York Medical Examination Publishing Company; 1983.
10. Bailey FR [AS8]. In: Kely DE, Wook RL, Enders AC, editors. Bailey's textbook of microscopic anatomy. 18th ed. Baltimore: Williams and Wilkins; 1984.
11. Leeson CR, Leeson TS, editors. Textbook of histology. 5th ed. Philadelphia: WB Saunders; 1985.
12. Snell RS, editor. Clinical and functional histology for medical students. 1st ed. Boston: Little Brown; 1984.
13. Amiel D, Frank CB, Harwood FL, et al. Tendons and ligaments: a morphological and biochemical comparison. J Orthop Res. 1984;1(3):257–65.
14. Viidick A. Simultaneous mechanical and light microscopic studies of collagen fibers. Z Anat Entwicklungsgesh. 1972;136:204.
15. Clore JN, Cohen K, Diegelmann RF. Quantitation of collagen types I and III during wound healing in rat skin. Proc Soc Exp Biol Med. 1979;161:337.
16. Dunphy JE. Wound healing. New York: Medcom Press; 1974.
17. Gay S, Viljanto J, Rackallio J, et al. Collagen types in early phases of wound healing in children. Acta Chir Scand. 1978;144:205.
18. Amiel D, Billings E, Akeson WH. Ligament structures, chemistry, and physiology. In: Daniel D, Akeson W, O'Connor J, editors. Knee ligaments: structure, function, injury and repair. New York: Raven Press; 1990. p. 77–91.
19. Frank CB, Woo SL-Y, Andriacchi T, et al. Normal ligament: structure, function and composition. In: Woo SL-Y, Buckwalter JA, editors. Injury and repair of the musculoskeletal soft tissues. Park Ridge: American Academy of Orthopaedic Surgeons; 1988.
20. Bray DF, Frank CB, Bray RD. Cytochemical evidence for a proteoglycan-associated filamentous network in the ligament extracellular matrix. J Orthop Res. 1990;8:1–12.
21. Buckwalter JA. Cartilage. In: Delbecco R, editor. Encyclopedia of human biology. 2nd ed. San Diego: Academic Press; 1991. p. 201–15.
22. Buckwalter JA, Cooper RR. The cells and matrices of skeletal connective tissues. In: Albright JA, Brand RA, editors. The scientific basis of orthopaedics. Norwalk: Appleton and Lange; 1987. p. 1–29.
23. Buckwalter JA, Cruess R. Healing of musculoskeletal tissues. In: Rockwood CA, Green DP, editors. Fractures in adults. Philadelphia: Lippincott; 1991. p. 181–222.
24. Buckwalter JA, Maynard JA, Vailas AC. Skeletal fibrous tissues: tendon, joint capsule, and ligament. In: Albright JA, Brand RA, editors. The scientific basis of orthopaedics. Norwalk: Appleton and Lange; 1987.

25. Hardingham TE. Proteoglycans: their structure, interactions, and molecular organization on cartilage. Biochem Soc Trans. 1981;9:489–97.
26. Hascall VC. Interactions of cartilage proteoglycans with hyaluronic acid. J Supramol Struct. 1977;7:101–20.
27. Muir H. Proteoglycans as organizers of the extracellular matrix. Biochem Soc Trans. 1983;11:613–22.
28. Kowalewski K, Yong S. Effect of growth hormone and an anabolic steroid on hydroxyproline in healing dermal wounds in rats. Acta Endocrinol. 1968;59:53.
29. Laato M, Niinikoski J, Lebel L, et al. Stimulation of wound healing by epidermal growth factor. Ann Surg. 1986;203:379–81.
30. Prudden JF, Nishihara G, O'Campo L. Studies on growth hormone. III. The effect on wound tensile strength of marked postoperative anabolism induced with growth hormone. Surg Gynecol Obstet. 1958;107:481.
31. Deuel TF, Senior M, Huang JS, et al. Chemotaxis of monocytes and neutrophils to platelet-derived growth factor. J Clin Invest. 1982;69:1046–9.
32. Roberts AB, Anzano MA, Lamb LC, et al. New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues. Proc Natl Acad Sci U S A. 1981;78:5339–43.
33. Roberts AB, Sporn MB, Assoian RK, et al. Transforming growth factor type β. Rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc Natl Acad Sci. 1986;83:4167–71.
34. Seppa H, Grotendorst G, Seppa S, et al. Platelet-derived growth factor is chemotactic for fibroblasts. J Cell Biol. 1982;92:584.
35. Sporn MB, Roberts AB, Wakefield LM, et al. Transforming growth factor β: biological function and chemical structure. Science. 1986;233:532–4.
36. Molloy T, Wang Y, Murrell G. The roles of growth factors in tendon and ligament healing. Sports Med. 2003;33:381–94.
37. Montesano R, Vassalli JD, Baird A, et al. Basic fibroblast growth factor induces angiogenesis *in vitro*. Proc Natl Acad Sci. 1986;83:7297.
38. Batten ML, Hansen JC, Dahners LE. Influence of dosage and timing of application of platelet-derived growth factor on early healing of the rat medial collateral ligament. J Orthop Res. 1996;14(5):736–41.
39. Lane JG, Healey RM, Chase DC, Amiel D. Use of platelet-rich plasma to enhance tendon function and cellularity. Am J Orthop. 2013;42(5):209–14.
40. Murray MM, Spindler KP, Ballard P, Welch TP, Zurakowski D, Nanney LB. Enhanced histologic repair in a central wound in the anterior cruciate ligament with a collagen-platelet-rich plasma scaffold. J Orthop Rep. 2007;25:1007–17.
41. Phillips GD, Stone AM, Jones BD, Schultz JC, Whitehead RA, Knighton DR. Vascular endothelial growth factor (rhVEGF165) stimulates direct angiogenesis in the rabbit cornea. In Vivo. 1994;8:961–5.
42. Corral CJ, Siddiqui A, Wu L, Farrell CL, Lyons D, Mustoe TA. Vascular endothelial growth factor is more important than basic fibroblastic growth factor during ischemic wound healing. Arch Surg. 1999;134:200–5.
43. Lyon RM, Akeson WH, Amiel D, et al. Ultrastructural differences between the cells of the medial collateral and the anterior cruciate ligaments. Clin Orthop. 1991;272:279–86.
44. Murray MM, Spector M. Fibroblast distribution in the anteromedial bundle of the human anterior cruciate ligament: the presence of alpha-smooth muscle actin positive cells. J Orthop Res. 1999;17:18–27.
45. Nagineni CN, Amiel D, Green MH, et al. Characterization of the intrinsic properties of the anterior cruciate and medial collateral ligament cells: an in vitro cell culture study. J Orthop Res. 1992;10:465–75.
46. Burridge K, Chrzanowska-Wodnicka M. Focal adhesions, contractility, and signaling. Annu Rev Cell Dev Biol. 1996;12:463–518.
47. Sung PK-L, Kwan MK, Maldanado F, et al. Adhesion strength of human ligament fibroblasts. J Biomech Eng. 1994;116:237–42.
48. Sung PK-L, Steele LL, Whittermore D, et al. Adhesiveness of human ligament fibroblasts to laminin. J Orthop Res. 1995;13:166–73.
49. Hannafin JA, Attia ET, Warren RF, et al. Characterization of chemotactic migration and growth kinetics of canine knee ligament fibroblasts. J Orthop Res. 1999;17:398–404.
50. Amiel D, Foulk RA, Harwood FL, et al. Quantitative assessment by competitive ELISA of fibronectin (Fn) in tendons and ligaments. Matrix. 1989;9:421–7.
51. Fetto JF, Marshal JL. The natural history and diagnosis of anterior cruciate ligament insufficiency. Clin Orthop. 1978;132:206–18.
52. Kennedy JC, Fowler PJ. Medial and anterior instability of the knee: an anatomic and clinical study using stress machines. J Bone Joint Surg Am. 1971;53:1257–70.
53. Kennedy JC, Weinberg HQ, Wilson AS. The anatomy and function of the anterior cruciate ligament, as determined by clinical and morphological studies. J Bone Joint Surg Am. 1974;56:223–5.
54. Norwood LA, Cross MJ. Anterior cruciate ligament: functional anatomy of its bundles in rotary instabilities. Am J Sports Med. 1979;7:23–6.
55. Odensten M, Gillquist J. Functional anatomy of the anterior cruciate ligament and a rationale for reconstruction. J Bone Joint Surg Am. 1985;67:257–62.
56. Scuderi GR, Scott WN, Insall JN. Injuries of the knee. In: Rockwood Jr CA, Green DP, Bucholz RW, Heckman JD, editors. Fractures in adults. 4th ed. New York: Lippincott-Raven; 1996.
57. Arnold JA, Coker TP, Heaton LM, et al. Natural history of anterior cruciate tears. Am J Sports Med. 1979;7:305–13.

58. Cabaud JE, Rodkey WG, Feagin JA. Experimental studies of acute anterior cruciate ligament injury and repair. Am J Sports Med. 1979;7:18–22.
59. Clayton ML, Miles JS, Abdulla M. Experimental investigations of ligamentous healing. Clin Orthop. 1968;61:146–53.
60. O'Donoghue DH. An analysis of end results of surgical treatment of major injuries to the ligaments of the knee. J Bone Joint Surg Am. 1955;37:1–13.
61. Wiig ME, Amiel D, Vandeberg J, et al. The early effect of high molecular weight hyaluronan (hyaluronic acid) on anterior cruciate ligament healing: and experimental study in rabbits. J Orthop Res. 1990;8:425–34.
62. Frank C, Woo SL-Y, Amiel D, et al. Medial collateral ligament healing: a multidisciplinary assessment in rabbits. Am J Sports Med. 1983;11:379–89.
63. Urban JP. Diffusion of small solutes into the intervertebral disc: an in vivo study. In: Goodman CC, Fuller KS, editors. Pathology for the physical therapist assistant. St. Louis: Saunders, 2011. ISBN 1437708935.
64. Sherman MF, Bonamo JR. Primary repair of the anterior cruciate ligament. Clin Sports Med. 1988;7:739–50.
65. Lo IK, de Naat GH, Valk JW, et al. The gross morphology of torn human anterior cruciate ligaments in unstable knees. Arthroscopy. 1999;15: 301–6.
66. Woo SL, Young EP, Ohland KJ, et al. The effects of transection of the anterior cruciate ligament on healing of the medial collateral ligament: a biomechanical study of the knee in dogs. J Bone Joint Surg Am. 1990;72:382–92.
67. Loitz-Ramage BJ, Frank CB, Shrive NG. Injury size affects long-term strength of the rabbit medial collateral ligament. Clin Orthop. 1997;337:272–80.
68. Kleiner JB, Roux RD, Amiel D, et al. Primary healing of the ACL. Trans Orthop Res Soc. 1986;11:131.
69. Marshall JL, Rubin RM, Wang JB, et al. The anterior cruciate ligament: the diagnosis and treatment of its injuries and their serious prognostic implication. Orthop Rev. 1978;7:35–46.
70. Alm A, Stromberg B. Vascular assessment of the periarticular ligaments. A microangiographic and histologic investigation in the god. Acta Chir Scand. 1974;445(Suppl):25–35.
71. Arnocsky SP, Rubin RM, Marshall JL. Microvasculature of the cruciate ligaments and its response to injury. J Bone Joint Surg Am. 1979;61:1221–9.
72. Wallace CD, Amiel D. Vascular assessment of the periarticular ligaments of the rabbit knee. J Orthop Res. 1991;9:787–91.
73. O'Donoghue DH, Rockwood Jr CA, Frank GR, et al. Repair of the ACL in dogs. J Bone Joint Surg Am. 1966;48:503–19.
74. Ropes MW, Bennett GA, Bauer W. The origin and nature of normal synovial fluid. J Clin Invest. 1939;18:351–72.
75. Andrish J, Holmes R. Effects of synovial fluid on fibroblasts in tissue culture. Clin Orthop. 1979;138:279–83.
76. Nickerson DA, Joshi R, Williams S, et al. Synovial fluid stimulates proliferation of rabbit ligament. Clin Orthop. 1992;274:294–9.
77. Amiel D, Abel MF, Kleiner JB, et al. Synovial fluid nutrient delivery in the diarthrial joint: an analysis of rabbit knee ligaments. J Orthop Res. 1986;4:90–5.
78. Warren RF. Primary repair of the ACL. Clin Orthop. 1983;172:65–70.
79. Kohn D. Arthroscopy in acute injuries of anterior cruciate-deficient knees: fresh and old intraarticular lesions. Arthroscopy. 1986;2:98–102.
80. Amiel D, Ishizue KK, Harwood FL, et al. Injury of the ACL: the role of collagenase in ligament degeneration. J Orthop Res. 1989;7:486–93.
81. Cheung HS, Halverson PB, McCarty DJ. Release of collagenase, neutral protease, and prostaglandins from cultured mammalian synovial cells by hydroxyapatite and calcium pyrophosphate dehydrate crystals. Arthritis Rheum. 1981;24:1338–44.
82. Werb Z, Reynolds JJ. Stimulation of endocytosis of the secretion of collagenase and neutral proteinase from rabbit synovial fibroblasts. J Exp Med. 1974;140:1482–97.
83. Ehrlich MG, Mankin HJ, Jones H, et al. Collagenase and collagenase inhibitors in osteoarthritic and normal cartilage. J Clin Invest. 1977;59:226–33.
84. Ridge SC, Oransky AL, Kerwar SS. Induction of the synthesis of latent collagenase and latent neutral protease in chondrocytes by a factor synthesized by activated macrophages. Arthritis Rheum. 1980;23: 448–54.
85. Lindy S, Turto H, Sorsa T, et al. Increased collagenase activity in human rheumatoid meniscus. Scand J Rheumatol. 1986;15:237–42.
86. Andriacchi T, Sabiston P, DeHaven K. Ligament: injury and repair. In: Woo SL-Y, Buckwater JA, editors. Injury and repair of the musculoskeletal soft tissues. Chicago: American Academy of Orthopedic Surgeons; 1988.
87. Arnoczsky SP. Physiologic principles of ligament injuries and healing. In: Scott NA, editor. Ligament and extensor mechanism injuries of the knee: diagnosis and treatment. St. Louis: CV Mosby; 1991. p. 67–81.
88. Neurath MF, Printz H, Stofft E. Cellular ultrastructure of the ruptured anterior cruciate ligament. Acta Orthop Scand. 1994;651:71–6.
89. Liu SH, Yang RS, al-Shaikh R, et al. Collagen in tendon, ligament, and bone healing: a current review. Clin Orthop. 1995;318:265–78.
90. Lane JG, McFadden P, Bowden K, Amiel D. The ligamentization process: a 4-year case study following ACL reconstruction with a semi-tendinosis graft. Arthroscopy. 1993;9(2):149–53.