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Review Article

Hylonome

Ligament structure, physiology and function

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Abstract

Ligaments are specialized connective tissues with very interesting biomechanical properties. They have the ability to adapt to the complex functions that each are required to perform. While ligaments were once thought to be inert, they are in fact responsive to many local and systemic factors that influence their function within the organism. Injury to a ligament results in a drastic change in its structure and physiology and creates a situation where ligament function is restored by the formation of scar tissue that is biologically and biomechanically inferior to the tissue it replaces. This article will briefly review the basic structure, physiology and function of normal versus healing knee ligaments, referring specifically to what is known about two of the most extensively studied and clinically relevant knee ligaments, the anterior cruciate (ACL) and medial collateral (MCL) ligaments of the knee. Those readers wishing for more comprehensive sources of information on ligament biology and biomechanics are referred to many excellent reviews on these topics[1-5].

Keywords: Ligament, Structure, Function, Healing, Biomechanics

Normal ligament structure and physiology

Skeletal ligaments are defined as dense bands of collagenous tissue (fibres) that span a joint and then become anchored to the bone at either end. They vary in size, shape, orientation and location. Their unique and complex bony attachments are called insertions and they often involve unusual shapes on the bone that are likely critical to how the fibres within the ligament are recruited as the joint moves. Ligaments often have a more vascular overlying layer termed the "epiligament" covering their surface[6] and this layer is often indistinguishable from the actual ligament and merges into the periosteum of the bone around the attachment sites of the ligament. Removal of the epiligament exposes the fibrous architecture of the ligament which is further organized hierarchically into groups of parallel fibres known as bundles that are difficult to separate suggesting that they are interconnected in some fashion. While the ligament appears as a single structure, with joint movement, some fibres appear to tighten or loosen depending on the bone positions and the forces that are applied confirming that these structures are more complex than originally thought. The epiligament is more vascular[7], receiving its blood supply from a branch of the superior medial geniculate artery, than the ligament and more cellular with more sensory and proprioceptive nerves. These nerves travel in close proximity to the blood vessels with more nerves nearer to the bony ligament insertions. Recent reports[8,9] have suggested that disruptions in joint innervation combined with injury and ageing may play a role in the pathogenesis of osteoarthritis although its pathogenesis is likely multifactorial thus requiring further investigation to make firm conclusions. At the microscopic level, ligaments are much more complex, being composed of cells called fibroblasts which are surrounded by matrix. The cells are responsible for matrix synthesis and they are relatively few in number and represent a small percentage of the total ligament volume. Although these cells may appear physically and functionally isolated, recent studies have indicated that normal ligament cells may communicate by means of prominent cytoplasmic extensions that extend for long distances and connect to cytoplasmic extensions from adjacent cells, thus forming an elaborate 3-dimensional architecture[10,11]. Gap junctions have also been detected in association with these cell connections raising the possibility of cell-to-cell communication and the potential to coordinate cellular and metabolic responses throughout the tissue. Ligament microstructure can be visualized using polarized light that reveals collagen bundles aligned along the long axis of the ligament and displaying an underlying "waviness" or crimp along the length. Crimp is thought to

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play a biomechanical role, possibly relating to the ligaments loading state with increased loading likely resulting in some areas of the ligament uncrimping, allowing the ligament to elongate without sustaining damage[12]. Biochemically, ligaments are approximately two-thirds water and one-third solid with the water likely responsible for contributing to cellular function and viscoelastic behaviour. The solid components of ligaments are principally collagen (type I collagen accounting for 85% of the collagen and the rest made up of types III, VI, V, XI and XIV) which accounts for approximately 75% of the dry weight with the balance being made up by proteoglycans (<1%), elastin and finally other proteins and glycoproteins such as actin, laminin and the integrins. Not all of the dry weight components have been characterized and this remains an area of continuing research focus. Ultrastructural studies have revealed that collagen fibres are actually composed of smaller fibrils. At the molecular level, collagen is synthesized as procollagen molecules and these are secreted into the extracellular space through structures in the cell called microtubules. Once outside the cell, a post-transitional modification takes place and the triple helical collagen molecules line up and begin to form fibrils and then fibres. This step is promoted by a specialized enzyme called lysyl oxidase, which promotes crosslink formation – a process involving placement of stable crosslinks within and between the molecules. Crosslink formation is the critical step that gives collagen fibres such incredible strength. During growth and development, crosslinks are relatively immature and soluble but with age they mature and become insoluble and increase in strength. This process will become much more relevant during the discussion on healing ligaments.

Normal ligament function

One of the main functions of ligaments is mechanical as they passively stabilize joints and help in guiding those joints through their normal range of motion when a tensile load is applied. Ligaments exhibit nonlinear anisotropic mechanical behaviour and under low loading conditions they are relatively compliant, perhaps due to recruitment of "crimped" collagen fibres as well as to viscoelastic behaviours and interactions of collagen and other matrix materials. Continued ligament-loading results in increasing stiffness until a stage is reached where they exhibit nearly linear stiffness and beyond this, then, ligaments continue to absorb energy until tensile failure (disruption). Another ligament function relates to its viscoelastic behaviour in helping to provide joint homeostasis. Ligaments "load relax" which means that loads/stresses decrease within the ligament if they are pulled to constant deformations. Ligaments also "creep" which is defined as the deformation (or elongation) under a constant or cyclically repetitive load. Creep is particularly important when considering joint injury or reconstructive surgery as excessive creep could result in laxity of the joint thus predisposing it to further injury. A third function of ligaments is their role in joint proprioception, which is referred to as the conscious perception of limb position in space. In joints such as the knee, proprioception is provided principally by joint, muscle and cutaneous receptors. When ligaments are strained, they invoke neurological feedback signals that then activate muscular contraction and this appears to play a role in joint position sense. Although progress continues to be made to elucidate the role of proprioception in normal ligament function and during injury, more precise quantification is the subject of ongoing analysis.

Ligament response to injury

Ligaments are most often torn in traumatic joint injuries that can result in either partial or complete ligament discontinuities. Due to the difficulty in studying partial disruptions, most of the discussion here will be focused on complete disruptions. Referring to the rabbit model, the medial collateral ligament (MCL) heals by a process which includes three phases: hemorrhage with inflammation, matrix and cellular proliferation and finally, remodeling and maturation. The first phase involves retraction of the disrupted ligament ends, formation of a blood clot, which is subsequently resorbed, and replaced with a heavy cellular infiltrate. Subsequently, a considerable hypertrophic vascular response takes place in the gap between the disrupted ends and results in an increase in both vascularity and blood flow, both of which decrease with time. The proliferative phase is defined as the production of "scar tissue" (dense, cellular, collagenous connective tissue matrix bridging the torn MCL ends) by hypertrophic fibroblastic cells. This scar tissue is initially quite disorganized with more defects (more blood vessels, fat cells, fibroblastic and inflammatory cells and loose connective tissue) identified histologically than normal ligament matrix[13]. After a few weeks of healing, the collagen becomes quite well aligned with the long axis of the ligament despite the fact that the types of collagen are abnormal (more type III in relation to type I and an increase in type V) and the collagen fibrils have smaller diameters in the proliferating tissue. The third phase of ligament healing is matrix remodeling. Defects in the scar become filled in but although the matrix becomes more ligament-like with time, some major differences in composition, architecture and function persist. Differences which persist include altered proteoglycan[14] (increased biglycan and decreased decorin protein and mRNA levels) and collagen types[15], failure of collagen crosslinks to mature[16], persistence of small collagen fibril diameters[17], altered cell connections[18], increased vascularity[19], abnormal innervation, increased cellularity and the incomplete resolution of matrix "flaws". The functional recovery of MCL scars demonstrates a slow recovery of many properties. During the remodeling phase, viscoelastic properties recover to within 10-20% of normal, implying that scars tend to stress-relax to a greater extent, therefore maintaining a load less efficiently than normal ligament. Ligament scars also have inferior creep properties, creeping twice as much as normal MCLs during cyclic and static loads that are only a fraction of their failure loads[20]. Failure behaviours of healing MCLs do not recover with injured complexes being weaker (50% of normal

failure loads), less stiff and absorbing less energy before failure than normal MCLs. Biomechanically, ligament recovery or healing in the long term may be dependent on a number of variables including the size of the initial gap, whether contact exists between the torn ligament ends and to what degree of joint movement they are subjected. Many different strategies have been employed to "heal" ligaments back to their original properties and functions; some such as controlled joint motion[21], biochemical modulation, surgical repair[22], grafting[23], gene therapy[24] and tissue engineering[25] have assisted in our understanding of ligament healing, however, complete ligament healing continues to be elusive and will remain the continuing focus of future investigations.

References

1. Akeson WH, Woo SL-Y, Amiel D, Frank CB. The biology of ligaments. In: Funk FJ, Hunter LY (eds) Rehabilitation of the Injured Knee. Mosby, St Louis; 1988:93-148.
2. Frank C, Woo S, Andriacchi T, Brand R, Oakes B, Dahners L, DeHaven K, Lewis J, Sabiston P. Normal ligament: structure, function and composition. In: Woo SL-Y, Buckwalter JA (eds) Injury and Repair of the Musculoskeletal Soft Tissues. Am Acad Orthop Surg, Park Ridge; 1988:45-101.
3. Woo SL, Young EP. Structure and function of tendons and ligaments. In: Mow VC, Hayes WC (eds) Basic Orthopaedic Biomechanics. Raven Press, New York; 1991:199-243.
4. Lo IKY, Thornton G, Miniaci N, Frank CB, Rattner JB, Bray RC. Structure and function of diarthrodial joints. In: McGinty JB (ed) Operative Arthroscopy 3rd Edition. Lippincott Williams and Wilkins, Philadelphia; 2003:41-126.
5. Frank CB, Shrive NG, Lo IKY, Hart DA. Form and function of tendon and ligament. In: Buckwalter JA, Einhorn TA, Simon SR (eds) Orthopaedic Basic Science: Biology and Biomechanics of the Musculoskeletal System. Am Acad Orthop Surg, Rosemont; 2004: (in press).
6. Chowdhury P, Matyas JR, Frank CB. The "epiligament" of the rabbit medial collateral ligament: a quantitative morphological study. Connect Tissue Res 1991; 27:33-50.
7. Bray RC. Blood supply of ligaments: a brief overview. Orthopaedics 1995; 3:39-48.
8. Salo P. The role of joint innervation in the pathogenesis of arthritis. Can J Surg 1999; 42:91-100.
9. Johansson H, Sjolander P, Sojka P. A sensory role for the cruciate ligaments. Clin Orthop Relat Res 1991; 268:161-178.
10. Benjamin M, Ralphs JR. The cell and developmental biology of tendons and ligaments. Int Rev Cytol 2000; 196:85-130.
11. Lo IK, Chi S, Ivie T, Frank CB, Rattner JB. The cellular matrix: a feature of tensile bearing dense soft connective tissues. Histol Histopathol 2002; 17:523-537.
12. Amiel D, Chu CR, Lee J. Effect of loading on metabolism and repair of tendons and ligaments. In: Funk FJ, Hunter LY (eds) Repetitive Motion Disorders of the Upper Extremity. Am Acad Orthop Surg, Rosemont; 1995:217-230.
13. Shrive N, Chimich D, Marchuk L, Wilson J, Brant R, Frank C. Soft-tissue "flaws" are associated with the material properties of the healing rabbit medial collateral ligament. J Orthop Res 1995; 13:923-929.
14. Plaas AH, Wong-Palms S, Koob T, Hernandez D, Marchuk L, Frank CB. Proteoglycan metabolism during repair of the ruptured medial collateral ligament in skeletally mature rabbits. Arch Biochem Biophys 2000; 374:35-41.
15. Amiel D, Frank CB, Harwood FL, Akeson WH, Kleiner JB. Collagen alteration in medial collateral ligament healing in a rabbit model. Connect Tissue Res 1987; 16:357-366.
16. Frank C, McDonald D, Wilson J, Eyre D, Shrive N. Rabbit medial collateral ligament scar weakness is associated with decreased collagen pyridinoline crosslink density. J Orthop Res 1995; 13:157-165.
17. Frank C, McDonald D, Bray D, Bray R, Rangayyan R, Chimich D, Shrive N. Collagen fibril diameters in the healing adult rabbit medial collateral ligament. Connect Tissue Res 1992; 27:251-263.
18. Lo IK, Ou Y, Rattner JP, Hart DA, Marchuk LL, Frank CB, Rattner JB. J Anat 2002; 200:283-296.
19. Bray RC, Rangayyan RM, Frank CB. Normal and healing ligament vascularity: a quantitative histological assessment in the adult rabbit medial collateral ligament. J Anat 1996; 188:87-95.
20. Thornton GM, Leask GP, Shrive NG, Frank CB. Early medial collateral ligament scars have inferior creep behaviour. J Orthop Res 2000; 18:238-246.
21. Buckwalter JA. Activity vs. rest in the treatment of bone, soft tissue and joint injuries. Iowa Orthop J 1995; 15:29-42.
22. Chimich D, Frank C, Shrive N, Dougall H, Bray R. The effects of initial end contact on medial collateral ligament healing: a morphological and biomechanical study in a rabbit model. J Orthop Res 1991; 9:37-47.
23. Boorman RS, Thornton GM, Shrive NG, Frank CB. Ligament grafts become more susceptible to creep within days after surgery: evidence for early enzymatic degradation of a ligament graft in a rabbit model. Acta Orthop Scand 2002; 73:568-574.
24. Nakamura N, Hart DA, Boorman RS, Kaneda Y, Shrive NG, Marchuk LL, Shino K, Ochi T, Frank CB. Decorin antisense gene therapy improves functional healing of early rabbit ligament scar with enhanced collagen fibillogenesis *in vivo*. J Orthop Res 2000; 18:517-523.
25. Huard J, Li Y, Peng H, Fu FH. Gene therapy and tissue engineering for sports medicine. J Gene Med 2003; 5:93-108.