

Ligament Injury and Healing: An Overview of Current Clinical Concepts

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ABSTRACT

Ligament injuries are among the most common causes of musculoskeletal joint pain and disability encountered in primary practice today. Ligament injuries create disruptions in the balance between joint mobility and joint stability, causing abnormal force transmission throughout the joint resulting in damage to other structures in and around the joint. Osteoarthritis, the long-term consequence of non-healed ligament injury, continues to be the most common joint disorder in the world.

Ligaments heal through a distinct sequence of cellular events that occur through three consecutive phases: the acute inflammatory phase, the proliferative or regenerative phase, and the tissue remodeling phase. The whole process can occur over months, and despite advances in therapeutics, many ligaments do not regain their normal tensile strength.

Numerous strategies have been employed over the years attempting to improve ligament healing after injury or surgery. One of the most important advances in the treatment of ligament injuries has come from the understanding that controlled early resumption of activity can stimulate repair and restoration of function, and that treatment of ligament injuries with prolonged rest may delay recovery and adversely affect the tissue repair. Likewise, although steroid injections and nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to be effective in decreasing inflammation and pain of ligament injuries for up to six to eight weeks, the histological, biochemical, and biomechanical properties of ligament healing are inhibited. For this reason their use is cautioned in athletes who have ligament injuries. As such, NSAIDs are no longer recommended for chronic soft tissue (ligament) injuries, and for acute ligament injuries should be used for the shortest period of time, if used at all. Regenerative medicine techniques, such as Prolotherapy, have been shown in case series and clinical studies, to resolve ligament injuries of the spine and peripheral joints. More Prolotherapy studies in more controlled settings with larger numbers would further prove the effectiveness of this therapy.

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KEYWORDS: corticosteroids, exercise, immobility, ligament healing, ligament injury, NSAIDs, Prolotherapy.

INTRODUCTION

Ligaments are dense bands of fibrous connective tissue that serve to join two or more bones of the musculoskeletal system. Ligaments cross joints with wide ranges of motion as well as joints with little motion and may appear as long sheets of opaque tissue or short thickened strips in joint capsules. Although they vary in size, shape, orientation, and location, ligaments primarily function to provide stabilization of joints both at rest and during normal range of motion. While ligaments were once thought to be inactive structures, they are, in fact, complex tissues that respond to many local and systemic influences.¹ Ligament injuries are among the most common causes of musculoskeletal joint pain and disability encountered in primary practice today. **Ligament injuries create disruptions in the balance between joint mobility and joint stability, which can lead to abnormal transmission of forces throughout the joint, resulting in damage to other structures in and around the joint.** Knees, hips, shoulders, ankles, elbows, and wrists are among some of the joints most commonly affected by ligament injuries. While there is a vast body of knowledge available regarding the structure and function of normal ligaments, understanding the structure and function of injured ligaments becomes more complicated due to the variability and unpredictable nature of ligament healing. This may be due to the dramatic physiological and structural changes that ligaments sustain as a result of injury, as well as the complex and dynamic cellular processes that occur during healing. These processes create alterations in the biology and biomechanics of the injured ligament, leading to inadequate healing and tissue formation that is inferior to the tissue it replaces. The incomplete healing and persisting differences in the new ligament tissue result in ligament laxity, which predisposes the joint to further injury. Ligament injury and subsequent laxity cause joint instability, which leads to chronic pain, diminished function, and ultimately

osteoarthritis of the affected joint.²⁻⁵ Despite the numerous strategies that have been employed over the years attempting to improve ligament healing after injury, osteoarthritis, the long-term consequence of ligament injury, continues to be the most common joint disorder in the world.⁶ Therefore, understanding the complex cellular processes that occur as a result of ligament injury, along with determining and implementing strategies that optimize ligament restoration are necessary to reduce the enormous individual and public health impacts of osteoarthritis.

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ligaments may be capable of cell-to-cell communication allowing the coordination of cellular and metabolic processes throughout the tissue.^{1, 9, 10} Proteoglycans, also found in the extracellular matrix, store water and contribute to the viscoelastic properties of ligaments. These viscoelastic features allow ligaments to progressively lengthen when under tension and return to their original shape when the tension is removed. Ligaments attach to bones at specific sites on the bone called “insertions.” Both ligaments and their insertion sites can vary in

configuration and their geometric shape appears to relate to the manner in which the fibers within the ligament are engaged as the joint moves. The direction of joint movement determines which fibers within a particular ligament are recruited for the performance of the specific movement. Ligaments are covered by a more vascular and cellular overlying layer called the epiligament, which is often indistinguishable from the actual ligament. The epiligament contains sensory and proprioceptive nerves with more nerves located closer to the bony ligament insertion sites.^{1, 11, 12} When ligaments are strained, the proprioceptive nerves initiate neurological feedback signals that activate muscle contraction around the joint, which allows the body to protect and stabilize the joint after injury.

LIGAMENT STRUCTURE AND FUNCTION

Ligaments are primarily composed of water, collagen, and various amino acids. Approximately two thirds of total ligament mass can be attributed to water and one third can be attributed to solids.¹ Collagen represents approximately 75% of the dry weight of ligaments, while the remaining 25% contains proteoglycans, elastin, and other proteins and glycoproteins. Type I collagen accounts for nearly 85% of the total collagen within ligaments and the remaining balance consists of types III, V, VI, XI, and XIV collagen.^{1, 7} Microscopic studies of ligament tissues have shown that bundles of collagen fibers are composed of smaller fibrils arranged in a parallel fashion along the long axis of the ligament. The collagen fibers appear to have a characteristic, specially designed cross-linked formation, which contributes to the incredible strength of ligaments. Under microscope, the collagen bundles appear undulated or crimped along their length and it is believed that the crimping is present in relation to the loading capacity or tension applied to ligaments. With load-bearing, certain areas of the ligament uncrimp, which allows the ligament to elongate without sustaining structural damage.^{1, 8} It appears that some fibers tighten or loosen depending on musculoskeletal positioning and applied forces, which supports the joint through various tensions and ranges of motion.

Fibroblasts, which produce and maintain the extracellular matrix, are located between the rows of collagen fibers. Recent studies suggest that fibroblast cells in normal

Ligaments prevent excessive motion of joints by providing passive stabilization and guiding joints through normal range of motion under tensile load. In doing so, ligaments transfer force to and from the skeleton while dynamically distributing the loads applied to them in order to perform specific movement patterns.¹³ Ligaments also function to provide joint homeostasis through their viscoelastic properties that reflect the complex interactions between collagens, proteoglycans, water, and other proteins.^{1, 14} The viscoelastic properties, along with the recruitment of crimped collagen, contribute to the mechanical behavior of the structure under loading conditions. When tension is applied, ligaments deform, or elongate, in a non-linear fashion through the recruitment of crimped collagen fibers. As the tension placed on the ligament increases, the collagen fibers progressively un-crimp, or elongate, until all fibers are nearly linear. (*See Figure 1.*) As the

fibers become increasingly linear, the ligament structure becomes increasingly stiff. Varying degrees of ligament stiffness are necessary for various loads and various ranges of joint motion. Ligaments can lose their ability to retain their original shape when stretched or elongated past a certain point for a prolonged period of time. When this occurs, the ligament becomes lax and unable to properly support the joint, leading to instability, pain, and eventual osteoarthritis of the joint. When an applied load causes all fibers to become nearly linear, the ligament continues to absorb energy until tensile failure or disruption of the tissue. Just as overstretched ligaments cause joint instability, ligament disruptions, or tears, will also create joint instability. In attempt to prevent overstretching and disruption, ligaments utilize their viscoelastic properties to exhibit both creep and relaxation behaviors. Creep and load relaxation behaviors help to prevent fatigue failure of the tissue when ligaments are loaded in tension. Creep is defined as the deformation, or elongation, of a ligament over time under a constant load or stress. Load relaxation refers to a decrease in stress of the tissue over time when the ligament is subjected to a constant elongation.¹⁵⁻¹⁷

LIGAMENT RESPONSE TO INJURY

When ligaments are exposed to loading over an extended period of time, they increase in mass, stiffness, and load to failure.⁷ However, when ligaments are overloaded, or exposed to tensions greater than the structures can sustain, the tissue fails resulting in partial or complete ligament discontinuities. When these discontinuities, also known as disruptions or tears, occur, the body responds by attempting to heal the injury through a specialized sequence of overlapping, but distinct cellular events. These events are the same that occur as part of the body's response to any soft tissue injury and can be categorized by three consecutive phases that occur over time: the acute inflammatory phase, the proliferative or regenerative/repair phase, and the tissue remodeling phase. The acute inflammatory phase begins within in minutes of injury and continues over the next 48-72 hours. During this phase, blood collects at the site of injury and platelet cells interact with certain matrix components to change their shape and initiate clot formation. The platelet-rich fibrin clot releases growth factors that are necessary for healing and provides a platform on which many cellular events occur.

Several growth factors have been identified, each playing a specific role in the inflammatory process. Some of the numerous growth factors which have been identified include Platelet-Derived Growth Factor, Transforming Growth Factor- β , Vascular Endothelial Growth Factor, and Fibroblast Growth Factor. Platelet-Derived Growth Factor and Transforming Growth Factor- β attract immune system cells to the area and stimulate them to proliferate. Vascular Endothelial Growth Factor aids in new blood vessel formation, which increases vascularity in injured areas. Fibroblast Growth Factor promotes the growth of the cells involved in collagen and cartilage formation. When stimulated by growth factors, neutrophils, monocytes, and other immune cells migrate to the injured tissue to initiate matrix turnover by ingesting and removing debris and damaged cells during the inflammatory phase. The proliferative/repair phase begins when immune cells release various growth factors and cytokines, which initiate fibroblast proliferation to rebuild the ligament tissue matrix. The tissue formed initially appears as disorganized scar tissue with more blood vessels, fat cells, fibroblastic and inflammatory cells than normal ligament tissue.^{1, 18} Over the next several weeks, fibroblast cells deposit various types of collagen,

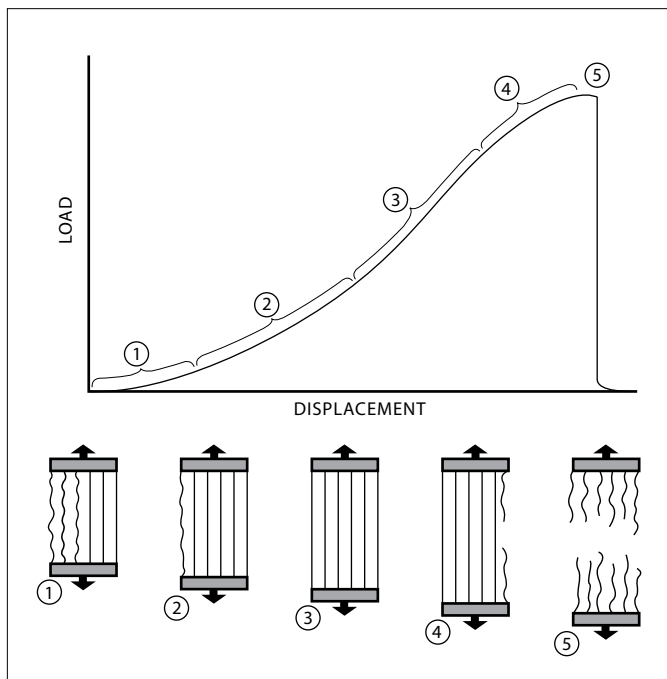


Figure 1. Ligament structural strength graph. As the load is increased, more ligament fibers are recruited (straight lines), and the slack or creep in the fibers is removed until the entire ligament tears. The load at complete failure of the ligament represents its maximum structural strength.

proteoglycans, other proteins and glycoproteins to the matrix. The collagen becomes aligned with the long axis of the ligament during this time, however, the newly formed type of collagen fibrils are abnormal and smaller in diameter than normal ligament tissue. After a few weeks, the proliferative phase merges into the remodeling phase in which collagen maturation occurs for months to years after the initial injury. With time, the tissue matrix starts to resemble normal ligament tissue, however, critical differences in matrix structure and function persist. (See Figure 2.) In fact, evidence suggests that the injured ligament structure is replaced with tissue that is grossly, histologically, biochemically, and biomechanically similar to scar tissue.^{15, 19-21} As Frank et al. note, even fully remodeled scar tissue remains grossly, microscopically, and functionally different from normal tissues.²²

Normal Ligaments	Ligament Scars
<ul style="list-style-type: none"> • Bimodal (large) collagen fibrils • Cell and matrix turnover low • Collagen aligned • Collagen densely packed • High matrix-cell ratio • Low cell density • Mature collagen cross-links • Primarily collagen Type I • Primarily small proteoglycans • Rare cell division 	<ul style="list-style-type: none"> • Smaller collagen fibrils • Cell and matrix turnover high • Collagen disorganized • Flaws between fibers • Lower matrix-cell ratio • Higher cell density • Immature collagen cross-links • More collagen III • Larger proteoglycans • More cell division

Figure 2. Differences between normal ligaments and scars.

The remodeling phase of ligament repair can continue for months to years, during which time collagen and ligament matrix are continually overturned by processes of tissue synthesis and degradation. This provides ongoing opportunities for the ligament to adapt with functional improvement, or degrade and fail with applied loads. The persisting abnormalities present in the remodeled ligament matrix can have profound implications on joint biomechanics depending on the functional demands placed on the tissue. Because remodeled ligament tissue is morphologically and biomechanically inferior to normal ligament tissue, ligament laxity results, causing functional disability of the affected joint and predisposing other soft tissues in and around the joint to further damage. Some of the identifiable differences in remodeled matrix versus normal ligament matrix include altered proteoglycan and collagen types,^{23, 24} failure of collagen crosslinks to mature,^{7, 25} persistence of small collagen fibril diameters,^{22, 26}

altered cell connections,²⁸ increased vascularity,^{22, 25} abnormal innervation, increased cellularity and the incomplete resolution of matrix flaws.^{1, 22} Research suggests that persisting collagen abnormalities may be the most critical to ligament tissue function, however, virtually all tissue components other than collagen likely play equally important direct and indirect roles in tissue function.^{22, 29-31} Normal ligament tissue is primarily composed of type I collagen, which is responsible for the stiffness and strength of the tissue. After injury, fibroblasts primarily synthesize type III collagen and to a much lesser extent Type I collagen.^{32, 33} The densely packed cross-linked formation of type I collagen fibrils in normal ligaments accounts for stability, strength, and stiffness of the ligament. The abnormal collagen cross-linking and smaller collagen fibril sizes of the repaired ligament create weaknesses in tissue strength and stiffness which remain for months to years after initial injury.^{22, 25, 29, 30, 34-36} In addition, evidence suggest that remodeled collagen fibrils are not packed as densely as in normal ligaments and the remodeled tissue contains materials other than collagen, such as blood vessels, fat cells, and inflammatory cell pockets which contribute to weakness.^{1, 18, 22}

In order to understand ligament healing, many studies use the medial collateral ligaments (MCLs) of rabbits as experimental models. Studies on rabbit MCLs have shown that healing or remodeled MCLs are ultimately weaker, less stiff, and absorb less energy before failure than normal MCLs.^{34, 37, 38} Several studies have documented that conservatively treated injured MCLs typically regain only 40% to 80% of their structural stiffness and strength compared to normal MCLs.^{15, 17, 22} On the other hand, the viscoelastic characteristics of the injured MCL have a somewhat better recovery, as these properties return to within 10-20% of normal MCL behavior.²² This results in greater stress relaxation, which indicates that the ligament which sustained the injury maintains loads less efficiently than the normal ligament. Remodeled MCLs also exhibit inferior creep properties, elongating more than twice as much as normal MCLs, even at low tensions.^{1, 22, 39, 40} In addition, remodeled MCLs are at risk for permanent elongation because after loading they do not appear to return to their original length as quickly or as completely as normal MCLs.²² The laxity of the healing MCL leads to mechanical instability of the knee joint, which alters the contact mechanics of the joint. When the knee or any joint is unstable, sliding between joint surfaces increases, and the efficiency of muscles surrounding the joint decreases.

This creates alterations in the load distribution of the joint, which disrupts the underlying cartilage and bone, causing wear and increasing shear, eventually leading to osteochondral degeneration or osteoarthritis.⁴¹

Animal studies have shown that different ligaments heal at different rates^{15, 42-47} and combined ligament injuries heal with inferior rate and quality than isolated injuries.^{15, 42, 43, 48-52} Most animal studies focus on the ACL and MCL of the knee joint and while these structures may heal at varying rates comparatively and among different animal species, the quality of the remodeled tissue remains inferior to that of normal ligaments.^{26, 30, 32, 35, 42, 54, 55-57} In fact, studies of healing ligaments have consistently revealed that following rupture, certain ligaments do not heal independently, while others do heal, but with inferior compositional properties compared to normal tissue.^{37, 48, 58, 59} It is not uncommon for individuals to experience more than one ligament injury during a single traumatic event. Rabbit models have demonstrated that combined ACL/MCL injuries result in inferior structural and material properties of the healing MCL compared with those of the isolated MCL model.^{42, 43, 49-52} Some researchers believe that this may be related to the immobility of animals with painfully unstable knees or the excessive forces placed on the healing MCL tissue when there is damage to the ACL.¹⁵ As previously mentioned, while some ligaments heal spontaneously, be it with inadequate tissue configuration, other ligaments exhibit very poor intrinsic healing ability. This may be related to the specific properties of the particular ligament that was injured, the type of ligament injury (partial or full disruption), or interventions employed after ligament injury.

CURRENT STRATEGIES FOR OPTIMIZING LIGAMENT REPAIR

As discussed earlier, ligament healing is slow and often incomplete. Joint laxity caused by ligament injury improves slowly over a period of six weeks to a year. However, at six weeks to one year after injury, a large percentage of patients still have objective mechanical laxity and subjective joint instability.^{60, 61} In ligament injuries to the ankle, up to 31% exhibit a positive anterior drawer sign six months after injury. Additionally, feelings of instability affected 7% to 42% of participants up to one year after injury.⁶¹ Several strategies have been implemented over the years attempting to restore the properties of the injured ligament to pre-injury status including rest,

mobilization, non-steroidal anti-inflammatory drugs, corticosteroid injections, and Prolotherapy, among others. While each of these therapies can help with the subjective symptom of pain following ligament injury, they do not all contribute to the cellular repair and healing of ligament tissue. In fact, some of these therapies have been shown to be detrimental to the ligament healing process by suppressing and inhibiting certain cellular processes that are required for ligament tissue repair. Other therapies have been shown to contribute to healing through their stimulation of certain cellular processes involved in the regeneration of ligament tissue.

IMMOBILIZATION AND REST

Injured limbs are traditionally rested by splinting or casting. While immobilization of the affected joint has long been prescribed following ligament injury, it has since been discovered that healing ligaments are dramatically affected by the presence or absence of joint motion. The theory is that rest or immobilization will prevent further tissue damage in the joint by limiting movement, thereby decreasing pain and swelling. It is also thought that rest may improve recovery time, decrease functional problems, and reduce long-term pain. However, immobilizing a joint with a ligament injury can cause detrimental side effects, such as synovial adhesions,⁶² increasing collagen degradation with decreasing collagen synthesis,⁷ and a greater percentage of disorganized collagen fibrils.^{34, 38} Despite this evidence, rest and the RICE (Rest, Ice, Compression, Elevation) protocol continue to be commonly prescribed as the first line treatment for ligament, tendon, and other soft tissue injuries. Immobilization causes ligament physiology to progressively switch from an anabolic to a more catabolic state. One study that measured collagen fiber bundle diameters in the normal and repaired ligaments of dogs, clearly documented that increased or decreased levels of exercise will greatly influence the strength of ligaments. The study showed that the amount of exercise performed by the animal was directly correlated with the number of collagen fibrils, their arrangement, and their average thickness within the ligament.⁶³ Decreased loading of ligament tissue alters matrix turnover so that with time, matrix degradation exceeds formation and the newly synthesized matrix is less well organized, and the tissue stiffness and strength declines. Prolonged limb immobilization decreases the glycosaminoglycan and water content and the degree of orientation of the matrix collagen fibrils within the ligaments. Ultimately this causes

the ligaments to have less mass and strength. (See Figure 3.) Decreased ligament loading has a profound effect on decreasing the strength of the ligament-bone junction (fibro-osseous junction) because immobilization causes subperiosteal osteoclasts to resorb much of the bony inserts of the ligaments. This causes a substantial decline in the tensile strength at the bone-ligament interface.⁶⁴ According to the most recent systematic reviews of research on soft tissue injuries in humans, there appears to be no controlled study that favors immobilization for the treatment of ligament injuries.^{65, 66}

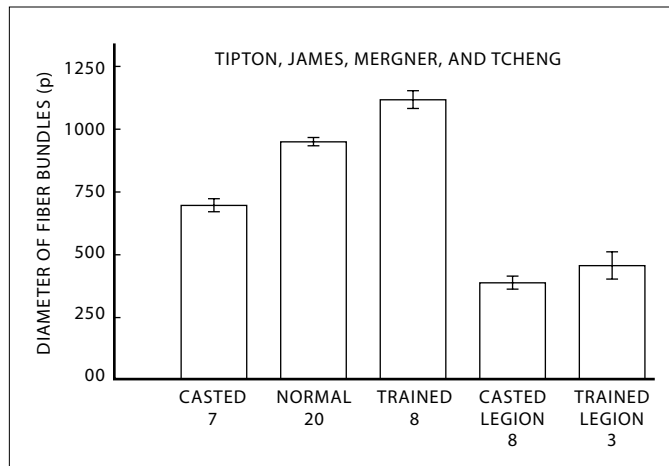


Figure 3. Ligament fiber bundle diameters. Ligament collagen fiber diameters are increased with exercise and diminished significantly when limbs are immobilized.

MOBILIZATION AND EXERCISE

Early controlled resumption of activity after injury, including repetitive loading on injured soft tissue structures such as ligaments and tendons has profoundly beneficial effects including enhanced cellular synthetic and proliferative effects, increased strength, size, matrix organization and collagen content of ligaments and tendons.⁶⁷ Mobilization has been shown to benefit the injured ligament by causing it to form more connective tissue, resulting in tissue that is stronger and stiffer than an immobilized counterpart.^{15, 42-44, 68} Motion causes an increase of blood flow to the affected joint, providing the damaged ligament tissue with nutrients and metabolites necessary for tissue repair and healing. Under loading conditions, cells within the ligament detect tissue strains and respond by modifying the tissue. Results of numerous animal studies have shown that the strength of repaired ligaments is greater in animals which were allowed to continue to exercise, rather than to rest.⁶⁹⁻⁷² According to

Kerkhoff et al., in a systematic review of research on ankle ligament injuries in 2,184 adults, functional treatment involving motion of the affected joint was a statistically significant strategy for healing the injured ligament, compared with immobilization. Patients who treated their ligament injuries with motion, versus immobilization, were able to return to work quicker, return to sport quicker, and demonstrated less objective instability as tested by stress X-ray.⁶⁵ In another systematic review, early mobilization was found to decrease pain, swelling and stiffness, and allowed a greater preservation of range of motion and return to work.⁶⁶ Mobilization for the treatment of soft tissue damage has also been found to decrease muscle atrophy, disuse osteoporosis, adhesions, and joint stiffness following injury.⁷³⁻⁷⁹ Overall, carefully controlled exercise plans promote healing of injured ligaments.

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS)

NSAIDs have been a mainstay treatment of ligament injuries for many years, especially for acute sports injuries, but new research has shown that NSAIDs are only mildly effective in relieving the symptoms of most muscle, ligament, and tendon injuries and are potentially deleterious to soft tissue healing.^{80, 81} There are reasons to expect that NSAIDs might have an adverse effect on healing as prostaglandin-induced inflammation is an early sequel of injury and results in the recruitment of cells into the area of injury for the removal of necrotic debris and the initiation of the healing process. NSAIDs specifically block the cyclooxygenase enzymes which catalyze the conversion of arachidonic acid to prostaglandins which play a significant role in ligament healing.⁸² Furthermore, the analgesic effect of NSAIDs may permit patients to ignore early symptoms of ligament injury, further damaging ligaments, and thus, delay definitive healing. One study looked at the use of Piroxicam in the treatment of acute ankle sprains in the Australian military. While the recruits were able to resume training more rapidly, in the long-term, an increase in ankle instability was evidenced by a positive anterior drawer sign in the Piroxicam group.⁸³ Multiple studies on the use of NSAIDs of the cyclooxygenase-2 (COX-2) inhibitor class have shown these medications inhibit ligament healing, leading to impaired mechanical strength.⁸⁴⁻⁸⁶ Their use is cautioned in athletes who have ligament injuries. As such, NSAIDs are no longer recommended for chronic soft tissue (ligament) injuries, and for acute ligament injuries should be used for the shortest period of time, if used at all.⁸⁷⁻⁸⁹

CORTICOSTEROID INJECTIONS

Corticosteroid injections have long been used to treat musculoskeletal disorders including ligament injuries. Although steroid injections have been shown to be effective in decreasing inflammation and pain of ligament injuries for up to six to eight weeks, the histological, biochemical, and biomechanical properties of ligament healing are inhibited.^{90,91} Their anti-inflammatory actions stem from their ability to prevent lysosomal enzyme release and to inhibit the accumulation of neutrophils and other inflammatory cells and the synthesis of inflammatory mediators, including cytokines, at the injury site.⁹²

Corticosteroid injections into injured ligaments have an adverse effect on healing. Corticosteroid injections into ligaments and tendons have been known to inhibit fibroblast function and thus collagen synthesis⁹³⁻⁹⁵ even causing collagen necrosis at the injection site.^{96,97} The steroid-injected ligaments have smaller cross sectional areas^{91,98,100} and are weaker with decreased peak tensile strength^{99,100} and decreased load (energy) to ligament failure.^{99,100} Because of these inhibitory effects on ligament healing, several extensive reviews have cautioned against their use to treat ligament injuries especially in athletes.¹⁰¹⁻¹⁰³

PROLOTHERAPY

Prolotherapy has emerged as an injection therapy treatment option for musculoskeletal and arthritic pain. It involves the injection of a small amount of various proliferant solutions (such as hypertonic dextrose, sodium morrhuate, platelet rich plasma) at the painful entheses of ligaments and tendons, as well as trigger points and adjacent joint spaces to induce healing of the injured structures.¹⁰⁴ Histologic studies of ligaments and tendons following Prolotherapy injections have shown an enhanced inflammatory healing reaction involving fibroblastic and capillary proliferation, along with growth factor stimulation.¹⁰⁵⁻¹⁰⁷ Growth factors, including basic fibroblast growth factor and platelet-derived growth factor, mediate the biological processes necessary for soft tissue repair in muscles, tendons, and ligaments after acute, traumatic or overuse injury.^{108,109} Prolotherapy injection therapy is known by various names including proliferative therapy, regenerative injection therapy and platelet rich plasma.¹¹⁰ Animal research has documented that Prolotherapy-injected ligaments have an increased ligament mass, extracellular matrix, thickness and junction strength with bone.¹¹¹⁻¹¹⁵

Prolotherapy is given to the articular ligaments of the entire spine, pelvis and peripheral joints to tighten unstable joints. Case series have documented the efficacy of Prolotherapy for ligament injuries of the sacroiliac joint,¹¹⁶⁻¹¹⁸ low back,^{119,120} neck,^{121,122} shoulder,¹²³ elbow,¹²⁴ knee,^{125,126} temporomandibular joint,^{127,128} and other articulations.^{129,130}

CONCLUSION

Ligament injuries are among the most common causes of musculoskeletal joint pain and disability encountered in primary practice today. Ligament injuries create disruptions in the balance between joint mobility and joint stability, causing abnormal force transmission throughout the joint resulting in damage to other structures in and around the joint. Osteoarthritis, the long-term consequence of non-healed ligament injury, continues to be the most common joint disorder in the world.

Ligaments heal through a distinct sequence of cellular events that occur through three consecutive phases: the acute inflammatory phase, the proliferative or regenerative phase, and the tissue remodeling phase. Ligament healing is often slow and incomplete. Joint laxity caused by ligament injury improves slowly over a period of six week to a year. However, at six weeks to one year after injury, a large percentage of patients still possess objective mechanical laxity and subjective joint instability. In ligament injuries to the ankle, up to 31% who experience positive anterior drawer signs six months after surgery. Additionally, feelings of instability affected 7% to 42% of participants up to one year after injury.

Numerous strategies have been employed over the year attempting to improve ligament healing after injury or surgery. One of the most important advances in the treatment of ligament injuries has come from the understanding that controlled early resumption of activity can stimulate repair and restoration of function, and that treatment of ligament injuries with prolonged rest may delay recovery and adversely affect the tissue to repair. Likewise, although steroid injections and nonsteroidal anti-inflammatory medications have been shown to be effective in decreasing inflammation and pain of ligament injuries for up to six to eight weeks, the histological, biochemical, and biomechanical properties of ligament healing are inhibited. For this reason their use is cautioned in athletes who have ligament injuries. As

such, NSAIDs are no longer recommended for chronic soft tissue (ligament) injuries, and for acute ligament injuries should be used for the shortest period of time, if used at all. Regenerative medicine techniques, such as Prolotherapy, have shown success in case series involving ligament injuries of the spine and peripheral joints, but studies in more controlled settings and with large numbers are needed in the future. ■

REFERENCES

- Frank C. Ligament structure, physiology and function. *Journal of Musculoskeletal and Neuronal Interactions*. 2004;4(2):199-201.
- Fleming B, et al. Ligament injury, reconstruction, and osteoarthritis. *Current Opinion in Orthopedics*. 2005;16(5):354-362.
- Koh J, et al. Osteoarthritis in other joints (hip, elbow, foot, toes, wrist) after sports injuries. *Clinical Sports Medicine*. 2005;24:57-70.
- Connell D, et al. MR imaging of thumb carpometacarpal joint ligament injuries. *Journal of Hand Surgery*. 2004;29:46-54.
- Martou G, et al. Surgical treatment of osteoarthritis of the carpometacarpal joint of the thumb: a systematic review. *Plastic and Reconstructive Surgery*. 2004;114:1-32.
- Arden N, et al. Osteoarthritis: epidemiology. *Best Practice & Research Clinical Rheumatology*. 2006;20(1):3-25.
- Verecke, et al. Soft-tissue physiology and repair. In: Vaccaro A, ed. *Orthopaedic Knowledge Update 8*. Rosemont, IL: American Academy of Orthopaedic Surgeons; 2005:15-27.
- Amiel D, et al. Repetitive motion disorders of the upper extremity: effect of loading on metabolism and repair of tendons and ligaments. *American Academy of Orthopaedic Surgery*. 1995;217-213.
- Benjamin M, et al. The cell and developmental biology of tendons and ligaments. *International Review of Cytology*. 2000;196:85-130.
- Lo I, et al. The cellular matrix: a feature of tensile bearing dense soft connective tissues. *Histology and Histopathology*. 2002;17:523-537.
- Chowdhury P, et al. The "epiligament" of the rabbit medial collateral ligament: a quantitative morphological study. *Connective Tissue Research*. 1991;27:33-50.
- Bray R. Blood supply of ligaments: a brief overview. *Orthopaedics*. 1995;3:39-48.
- Benjamin, M, et al. Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. *Journal of Anatomy*. 2006;208:471-490.
- Jung H, et al. Understanding normal, injured and healing ligaments and tendons: the role of biomechanics. Available at: <http://www.gustrength.com/injury:understanding-ligaments-and-tendons>. Accessed December 30, 2011.
- Frank C, et al. Ligament healing a review of some current clinical and experimental concepts. *The Iowa Orthopaedic Journal*. 1992;12:21-28.
- Akeson W, et al. Ligament biology and biomechanics. In: Finerman G, ed. *American Academy of Orthopaedic Surgeons Symposium on Sports Medicine: The Knee*. St. Louis, MO: CV Mosby Co; 1985:111.
- Andriacchi T, et al. Ligament injury and repair. In: Woo SL-Y, Buckwalter JA, eds. *Injury and Repair of the Musculoskeletal Soft Tissues*. Park Ridge, IL: Am Acad Orthop Surg; 1988:103.
- Shrive N, et al. Soft-tissue "flaws" are associated with material properties of the healing rabbit medial collateral ligament. *Journal of Orthopaedic Research*. 1995;13:923-929.
- Jack E. Experimental rupture of the medial collateral ligament of the knee. *Journal of Bone and Joint Surgery*. 1950;32(B):306.
- Miltner J, et al. Experimental reproduction of joint sprains. *Proceedings of the Society for Experimental Biology and Medicine*. 1933;30:883.
- Miltner J, et al. Experimental joint sprain pathologic study. *Archives of Surgery*. 1937;35:234.
- Frank C, et al. Optimization of the biology of soft tissue repair. *Journal of Science and Medicine in Sport*. 1999;2(3):190-210.
- Plaas A, et al. Proteoglycan metabolism during repair of the ruptured medial collateral ligament in skeletally mature rabbits. *Archives of Biochemistry and Biophysics*. 2000;374:35-41.
- Amiel D, et al. Collagen alteration in medial collateral ligament healing in a rabbit model. *Connective Tissue Research*. 1987;16:357-366.
- Frank C, et al. Rabbit medial collateral ligament scar weakness is associated with decreased collagen pyridinoline crosslink density. *Journal of Orthopaedic Research*. 1995;13:157-165.
- Frank C, et al. Collagen fibril diameters in the healing adult rabbit medial collateral ligament. *Connective Tissue Research*. 1992;27:251-263.
- Lo I, et al. The cellular networks of normal ovine medial collateral and anterior cruciate ligaments are not accurately recapitulated in scar tissue. *Journal of Anatomy*. 2002;200:283-296.
- Bray R, et al. Normal and healing ligament vascularity: a quantitative histological assessment in the adult rabbit medial collateral ligament. *Journal of Anatomy*. 1996;188:87-95.
- Shrive N, et al. Soft tissue "flaws" are associated with the material properties of the healing rabbit medial collateral ligament. *Journal of Orthopaedic Research*. 1995;13:923-929.
- Frank C, et al. Collagen fibril diameters in the rabbit medial collateral ligament scar: a longer term assessment. *Connective Tissue Research*. 1997;36:261-269.
- Hildebrand K, et al. Scar formation and ligament healing. *Canadian Journal of Surgery*. 1998;41:425-429.
- Hsu S, et al. Functional tissue engineering of ligament healing. *Sports Medicine, Arthroscopy, Rehabilitation, Therapy & Technology*. 2010;2:2-10.
- Liu S, et al. Collagen in tendon, ligament, and bone healing: A current review. *Clinical Orthopedics and Related Research*. 1995;318:265-278.
- Woo S, et al. Biomechanics of knee ligaments: injury, healing, and repair. *Journal of Biomechanics*. 2006;39:1-20.

35. Niyibizi C, et al. Type V collagen is increased during rabbit medial collateral ligament healing. *Knee Surgery and Sports Traumatology Arthroscopy*. 2000;8(5):281-285.
36. Plaas A, et al. Proteoglycan metabolism during repair of the ruptured medial collateral ligament in skeletally mature rabbits. *Archives of Biochemistry and Biophysics*. 2000;374(1):35-41.
37. Weiss J, et al. Evaluation of a new injury model to study medial collateral ligament healing: primary repair versus nonoperative treatment. *Journal of Orthopaedic Research*. 1991;9(4):516-528.
38. Woo S, et al. The biomechanical and morphological changes in the medial collateral ligament of the rabbit after immobilization and remobilization. *Journal of Bone and Joint Surgery—American Volume*. 1987;69(8):1200-1211.
39. Thornton G, et al. Early medial collateral ligament scars have inferior creep behavior. *Journal of Orthopaedic Research*. 2000;18:238-246.
40. Newton P, et al. Ultrastructural changes in knee ligaments following immobilization. *Matrix*. 1990;10(5):314-319.
41. Fleming B, et al. Ligament injury, reconstruction and osteoarthritis. *Current Opinion in Orthopedics*. 2005;16(5):354-362.
42. Bray R, et al. Joint instability alters scar quantity and quality and quality in healing a rabbit ligament. *Orthopedic Transactions*. 1990;14:322.
43. Bray R, et al. The early effects of joint immobilization on medial collateral ligament healing in an ACL-deficient knee: a gross anatomic and biomechanical investigation in the adult rabbit model. *Journal of Orthopaedic Research*. 1991.
44. Hart D, et al. Healing of the medial collateral ligament in rats. The effects of repair, motion and secondary stabilizing ligaments. *Journal of Bone and Joint Surgery*. 1987;69(A):1194.
45. Inoue M, et al. Treatment of the medial collateral ligament injury. The importance of anterior cruciate ligament on the varus-valgus knee laxity. *American Journal of Sports Medicine*. 1987;15:15.
46. Ogata K, et al. The intra-articular effect of various post-operative managements following knee ligament repair. An experimental study in dogs. *Clinical Orthopedics*. 1980;150:271.
47. Piper T, et al. Early mobilization after knee ligament repair in dogs: an experimental study. *Clinical Orthopedics*. 1980;150:277.
48. Woo S, et al. Injury and repair of ligaments and tendons. *Annual Review of Biomedical Engineering*. 2000;2:83-118.
49. Fetto J, et al. Medial collateral ligament injuries of the knee: a rationale for treatment. *Clinical Orthopedics*. 1978;132:206-218.
50. Warren R, et al. Injuries of the anterior cruciate and medial collateral ligaments of the knee. A long term follow-up of 86 cases. Part II. *Clinical Orthopedics*. 1978;136:198-211.
51. Yamaji T, et al. Medial collateral ligament healing one year after a concurrent medial collateral ligament and anterior cruciate ligament injury: an interdisciplinary study in rabbits. *Journal of Orthopaedic Research*. 1996;14:223-227.
52. Ohno K, et al. Healing of the medial collateral ligament after a combined medial collateral and anterior cruciate ligament: comparison of repair and non-repair of medial collateral ligament tears in rabbits. *Journal of Orthopaedic Research*. 1995;13:442-449.
53. Scheffler S, et al. Structure and function of the healing medial collateral ligament in a goat model. *Annals of Biomedical Engineering*. 2001;29(2):173-180.
54. Frank C, et al. Medial collateral ligament healing. A multidisciplinary assessment in rabbits. *American Journal of Sports Medicine*. 1983;11(6):379-389.
55. Abramowitch S, et al. A biomechanical and histological evaluation of the structure and function of the healing medial collateral ligament in a goat model. *Knee Surgery, Sports Traumatology, Arthroscopy*. 2003;11(3):155-162.
56. Marshall J, et al. Instability of the knee. A long-term experimental study in dogs. *Journal of Bone and Joint Surgery*. 1971;53(A):1561.
57. O'Donoghue D, et al. Repair and reconstruction of the anterior cruciate ligament in dogs. Factors influencing long-term results. *Journal of Bone and Joint Surgery*. 1971;53(A):710.
58. Woo S, et al. Treatment of the medial collateral ligament injury. II: Structure and function of canine knees in response to differing treatment regimens. *American Journal of Sports Medicine*. 1987;15:22-29.
59. Frank C, et al. Molecular biology and biomechanics of normal and healing ligaments: a review. *Osteoarthritis and Cartilage*. 1999;7:130-140.
60. Hintermann B. Biomechanics of the ligaments of the unstable ankle joint. *Sportverletz Sportschaden*. 1996;10(3):48-54.
61. Hubbard T. Ankle ligament healing after an acute ankle sprain: an evidence-based approach. *Journal of Athletic Training*. 2008;43(5):523-529.
62. Woo S, et al. Connective tissue response to immobility. Correlative study of biomechanical and biochemical measurements of normal and immobilized rabbit knees. *Arthritis & Rheumatism*. 1975;18:257-264.
63. Tipton C, et al. Influence of exercise on strength of medial collateral knee ligaments of dogs. *American Journal of Physiology*. 1970;216(3):894-902.
64. Buckwalter J. Activity vs. rest in the treatment of bone, soft tissue and joint injuries. *Iowa Orthopaedic Journal*. 1995;15:29-42.
65. Kerkhoffs G, et al. Immobilisation and functional treatment for acute lateral ankle ligament injuries in adults. *Cochrane Database Systematic Review*. 2002;(3). Retrieved online on 12/23/11 from <http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD003762/abstract>.
66. Nash C. Resting injured limbs delays recovery: a systematic review. *The Journal of Family Practice*. 2004;53(9). Retrieved online on 12/23/11 from <http://www.jfponline.com/Pages.asp?AID=1772>.
67. Buckwalter J. Activity vs. rest in the treatment of bone, soft tissue and joint injuries. *Iowa Orthopaedic Journal*. 1995;15:29-42.
68. Vilas A. Physical activity and its influence on the repair process of medial collateral ligaments. *Connective Tissue Research*. 1981;9:25.
69. Goldstein W, et al. Early mobilization of rabbit medial collateral ligament repairs: biomechanical and histologic study. *Archives of Physical Medicine and Rehabilitation*. 1984;65(5):239-242.

70. Walsh S, et al. Knee immobilization inhibits biomechanical maturation of the rabbit medial collateral ligament. *Clinical Orthopedics and Related Research*. 1993;297:253-261.
71. Thornton G, et al. Strength of medial structures of the knee joint are decreased by isolated injury to the medial collateral ligament and subsequent joint immobilization. *Journal of Orthopedic Research*. 2005;23(5):1191-8.
72. Thornton G, et al. Healing ligaments have decreased cyclic modulus compared to normal ligaments and immobilization further compromises healing ligament response to cyclic loading. *Journal of Orthopedic Research*. 2003;21(4):716-722.
73. Pneumáticos S, et al. The effects of early mobilization in the healing of Achilles tendon repair. *Foot & Ankle International*. 2000;21:551-557.
74. Gelberman R, et al. The effects of mobilization on the vascularization of healing flexor tendons in dogs. *Clinical Orthopedics*. 1980;153:283-289.
75. Kannus P. Immobilisation or early mobilization after an acute soft tissue injury. *The Physician and Sportsmedicine*. 2000;28:58-63.
76. Halikis M, et al. Effect of immobilization, immediate mobilization and delayed mobilization of the resistance to digital flexion using a tendon injury model. *American Journal of Hand Surgery*. 1997;22(A):464-472.
77. Romanelli D, et al. Achilles rupture in the athlete: current science and treatment. *Sports Medicine and Arthroscopy Review*. 2000;8:377-386.
78. Viidik A, et al. The effect of training on the tensile strength of isolated rabbit tendons. *Scandinavian Journal of Plastic and Reconstructive Surgery*. 1967;1:141-147.
79. Peacock E, et al. Biological principles in the healing of long tendons. *Surgical Clinics of North America*. 1965;45:461-476.
80. Mehallo C, et al. Practical management: Nonsteroidal anti-inflammatory drug use in athletic injuries. *Clinical Journal of Sports Medicine*. 2006;16:170-174.
81. Dhners L, et al. Effects of nonsteroidal anti-inflammatory drugs on bone formation and soft-tissue healing. *Journal of the American Academy of Orthopedic Surgery*. 2004;12:139-143.
82. Radi Z, et al. Effects of cyclooxygenase inhibition on bone, tendon, and ligament healing. *Inflammation Research*. 2005;54:358-366.
83. Slatyer M. A randomized controlled trial of Piroxicam in the management of acute ankle sprain in Australian regular army recruits. *American Journal of Sports Medicine*. 1997;25:544-553.
84. Elder C, et al. A cyclooxygenase-2 inhibitor impairs ligament healing in the rat. *American Journal of Sports Medicine*. 2001;29:801-810.
85. Warden S, et al. Low-intensity pulsed ultrasound accelerates and a nonsteroidal anti-inflammatory drug delays knee ligament healing. *American Journal of Sports Medicine*. 2006;34:1094-1102.
86. Warden S. Cyclo-oxygenase-2 inhibitors: beneficial or detrimental for athletes with acute musculoskeletal injuries? *Sports Medicine*. 2005;35:271-283.
87. Ziltener J, et al. Non-steroidal anti-inflammatory drugs for athletes: an update. *Annals of Physical Medicine and Rehabilitation*. 2010;53:278-282.
88. Fournier P, et al. Sports injuries and NSAID. *Rev Med Suisse*. 2008;6:1702-1705.
89. Paoloni J, et al. Non-steroidal anti-inflammatory drugs in sports medicine: guidelines for practical but sensible use. *British Journal of Sports Medicine*. 2009;43:863-865.
90. Mackie J, et al. Mechanical properties of rabbit tendons after repeated anti-inflammatory steroid injections. *Medicine and Science in Sports*. 1974;6:198-202.
91. Walsh W, et al. Effects of a delayed steroid injection on ligament healing using a rabbit medial collateral ligament model. *Biomaterials*. 1995;16:905-910.
92. Shapiro P, et al. The effect of local corticosteroid or Ketorolac exposure on hilologic and biomechanical properties of rabbit tendon and cartilage. *Hand*. 2007;2:165-172.
93. Berliner D, et al. Effects of corticosteroids on fibroblast functions. *Research Journal of the Reticuloendothelial Society*. 1967;4:284-313.
94. Kapetanos G. The effect of the local corticosteroids on the healing and biomechanical properties of the partially injured tendon. *Clinical Orthopedics*. 1982;163:170-179.
95. Oxlund H. The influence of a local injection of cortisol on the mechanical properties of tendons and ligaments and the indirect effect on skin. *Acta Orthopedica Scandinavica*. 1980;51:231-238.
96. Balasubramaniam P, et al. The effect of injection of hydrocortisone into rabbit calcaneal tendons. *The Journal of Bone and Joint Surgery*. 1972;54B: 729-734.
97. Scutt N, et al. Glucocorticoids inhibit tenocyte proliferation and tendon progenitor cell recruitment. *Journal of Orthopedic Research*. 2006;24:173-182.
98. Wiggins M, et al. Effects of local injection of corticosteroids on the healing of ligaments. A follow-up report. *Journal of Bone and Joint Surgery*. 1995;77A:1682-1691.
99. Noyes F, et al. Effect of intra-articular corticosteroids on ligament properties. A biomechanical and histological study in rhesus knees. *Clinical Orthopedics*. 1977;123:197-209.
100. Wiggins M. Healing characteristics of a type-1 collagenous structure treated with corticosteroids. *American Journal of Sports Medicine*. 1994;22:279-288.
101. Nichols A. Complications associated with the use of corticosteroids in the treatment of athletic injuries. *Clinical Journal of Sports Medicine*. 2005;15:370-375.
102. Fredberg U. Local corticosteroid injection in sport: review of literature and guidelines for treatment. *Scandinavian Journal of Medical Science and Sports*. 1997;7:131-139.
103. Fadale P, et al. Corticosteroid injections: Their use and abuse. *Journal of the American Academy of Orthopedic Surgery*. 1994;2:133-140.
104. Kim S, et al. Critical review of Prolotherapy for osteoarthritis, low back pain and other musculoskeletal conditions: A physiatric perspective. *American Journal of Physical Medicine and Rehabilitation*. 2004;83:379-389.
105. Maynard J. Morphological and biomechanical effects of sodium morrhuate on tendons. *Journal of Orthopaedic Research*. 1985;3:236-248.

106. Hackett G. Joint stabilization: an experimental, histologic study with comments on the clinical application in ligament proliferation. *American Journal of Surgery*. 1955;89:968-973.
107. Kim H, et al. The effects of anti-inflammatory drugs on histologic findings of the experimental Prolotherapy model. *Journal of the Korean Academy of Rehabilitation Medicine*. 2006;30:378-384.
108. Reeves K. Prolotherapy: injection of growth factors or growth factor production stimulants to growth normal cells or tissue. In Waldman SD (ed): *Pain Management*. Philadelphia, PA: Elsevier; 2006;1106-1127.
109. Creaney L, et al. Growth factor delivery methods of sports injuries: the state of play. *British Journal of Sports Medicine*. 2008;42:314-320.
110. Sanchez M, et al. Platelet-rich therapies in treatment of orthopaedic sport injuries. *Sports Medicine*. 2009;39:345-354.
111. Liu Y. An in situ of the influence of a sclerosing solution in rabbit medial collateral ligaments and its junction strength. *Connective Tissue Research*. 1983;2:95-102.
112. Jensen K, et al. Response of knee ligaments to Prolotherapy in a rat injury model. *American Journal of Sports Medicine*. 2008;36:1347-1357.
113. Klein R. Proliferant injections for low back pain: histologic changes of injected ligaments and objective measures of lumbar spine mobility before and after treatment. *Journal of Neurology, Orthopedic Medicine and Surgery*. 1989;10:141-144.
114. Harman R, et al. A retrospective review of 62 cases of suspensory ligament injury in sport horses treated with adipose-derived stem and regenerative cell therapy. *Proceedings of the Veterinarian Orthopedic Society*, 2006.
115. Dahlgren L. Use of adipose derived stem cells in tendon and ligament injuries. *American College of Veterinarian Surgery Symposium on Equine Small Animal Proceedings*. 2006;150-151.
116. Hackett G. Shearing injury to the sacroiliac joint. *Journal of the International College of Surgeons*. 1954;22:631-642.
117. Lee J, et al. Effects of intraarticular Prolotherapy on sacroiliac joint pain. *Korean Journal of Pain*. 2009;229-233.
118. Cusi M, et al. The use of Prolotherapy in the sacro-iliac joint. *British Journal of Sports Medicine*. 2010;44:100-104.
119. Hackett G. Back pain following trauma and disease-Prolotherapy. *Military Medicine*. 1961;July:517-525.
120. Hackett, G. Low back pain. *The British Journal of Physical Medicine*. 1956;19:25-35.
121. Hooper R, et al. Case series on chronic whiplash related neck pain treated with intraarticular zygapophysial joint regeneration injection therapy. *Pain Physician*. 2007;10:313-318.
122. Centeno C, et al. Fluoroscopically guided cervical Prolotherapy for instability with blinded pre and post radiographic reading. *Pain Physician*. 2005;8:67-72.
123. Jo D, et al. The effects of Prolotherapy on shoulder pain. *Korean Journal of Anesthesiology*. 2004;46:589-592.
124. Hauser R, et al. Hackett-Hemwall dextrose Prolotherapy for unresolved elbow pain. *Practical Pain Management*. 2009;October:14-26.
125. Kim J. The effect of Prolotherapy for osteoarthritis of the knee. *Journal of the Korean Academy of Rehabilitation Medicine*. 2002;26:445-448.
126. Reeves K, et al. Randomized prospective double-blind placebo-controlled study of dextrose Prolotherapy for knee osteoarthritis with or without ACL laxity. *Alternative Therapies*. 2000;6:68-79.
127. Hakala R. Prolotherapy in the treatment of TMD. *The Journal of Craniomandibular Practice*. 2005;23:1-6.
128. Schultz L. A treatment of subluxation of the temporomandibular joint. *Journal of the American Medical Association*. September 25, 1937.
129. Reeves K, et al. Evidence-based regenerative injection therapy (Prolotherapy) in sports medicine. In Seidenberg PH, Beutler PI. (Eds). *The Sports Medicine Resource Manual*. Saunders (Elsevier); 2008;611-619.
130. Hauser R, et al. *Prolo Your Sports Injuries Away!* Oak Park, IL: Beulah Land Press; 2001.