

# Structural Basis of Joint Instability as Cause for Chronic Musculoskeletal Pain and Its Successful Treatment with Regenerative Injection Therapy (Prolotherapy)

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**Abstract:** Joint dysfunctions and associated musculoskeletal pain are among the most common medical complaints presented to clinicians. Ligaments are collagenous fibrous structures that are primarily responsible for maintaining smooth joint motion, restraining excessive joint displacement, and providing stability across the joint. Ligaments also act as sensory organs for the joints and have significant input to pain sensation. When ligaments are subjected to forces beyond their normal range of motion, injury and failure occur, resulting in joint laxity (looseness or instability), and subsequent disruptions in the balance between joint mobility and joint stability. These dysfunctions can result in joint pain and the development of osteoarthritis. Several strategies have been employed over the years in attempts to improve joint instability from ligament injury; however, some of the standard therapeutic approaches (drugs, corticosteroid injections, and surgery) employed to address these problems have not been very effective because they often do not address the underlying cause of the problems, and in fact can inhibit ligament healing and restoration. For these reasons, there is current and growing interest among patients and clinicians in prolotherapy, an alternative therapeutic modality that can reduce or eliminate pain by stimulating the natural regenerative processes in and around the joint to facilitate the restoration of degenerated ligaments and tendons to a healthy state, improving joint support, function and reducing pain. This review presents current evidence from clinical studies demonstrating that prolotherapy is a significant and effective alternative treatment modality for people with ligament-related injuries and resultant joint instability.

**Keywords:** Chronic musculoskeletal pain, drugs, joint instability, ligament, ligament healing, ligament injury, ligamentous laxity, osteoarthritis, prolotherapy, regenerative injection therapy, regenerative medicine, surgery.

## INTRODUCTION

There is a strong and growing support for the idea that chronic pain is a distinct condition that coexists with other health conditions, and as such must be assessed and managed [1, 2]. The most frequently reported chronic pain conditions are associated with musculoskeletal dysfunction and degeneration. New data from a recent study, *Global Burden of Disease 2010*, highlight that increasing pain and disability associated with musculoskeletal disorders affect more than 1.7 billion people worldwide and have the 4<sup>th</sup> greatest impact on the overall health of the world population, considering both death and disability. This burden has increased by 45% during the past 20 years and is predicted to continue to escalate due to aging, increased obesity, and lack of physical activity [3, 4].

In the United States, musculoskeletal disorders and diseases are the leading cause of disability, which account for more than half of all chronic pain conditions in people over 50 years of age. Census data collected in 2008 indicates that musculoskeletal conditions are among the highest prevalence of self-reported primary medical conditions reported by persons aged 18 and older [3, 4]. It is estimated

that musculoskeletal joint conditions, in particular, affect between 100 million and 116 million people in the United States, and more than 1 in 4 persons require medical attention due to associated pain and disability. The impact of chronic musculoskeletal joint pain on individuals, families, and society is monumental; the morbidity costs of these disorders restrict activities of daily living, cause lost work days, and are a major cause of life long pain. Annual direct and indirect costs for joint and bone health are approximately \$849 billion - 7.7% of the gross domestic product. It is estimated that the burden of musculoskeletal conditions is expected to substantially increase in the next 10 to 20 years due to the aging population and sedentary lifestyles [4-6].

## PATHOPHYSIOLOGY OF JOINT PAIN

Any damage to the joints from disease or injury can interfere with movement or range of motion and cause severe pain. The International Association for the Study of Pain defines pain as “. . . an unpleasant sensory and emotional experience that is associated with actual or potential tissue damage or described in such terms” [7]. Joint pain can be acute or chronic and may have multiple causes, including joint injury, crystal deposition, infection, and disease arising from inflammation.

Most often, joint pain occurs when sensory receptors called nociceptors detect signals from damaged tissue,

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including ligaments. Nociceptors are free (bare) nerve endings that mainly originate in the dorsal root and trigeminal ganglia. They are associated with one of the two types of persistent pain (the other is neuropathic pain, occurs when nerves in the central or peripheral nervous system are damaged). When stimulated, nociceptors release a large number of neuromediators, such as substance P and the calcitonin gene-related peptide. Complex neuronal activation occurs, which involves not only local sensitization but also modifications in central pain pathways [8].

Joint pain may arise from structures within or adjacent to the joint. As an example, enthesopathy is a painful disorder at the site of the insertion of ligaments, tendons, fascia, or articular capsule into bone (enthesis) and is the result of an inflammatory rheumatic or non-rheumatic disease process. In enthesopathy, pain develops in the free nerve endings of entheses (enthesalgia), becoming a source of chronic musculoskeletal pain in some individuals. This process also may promote abnormal calcification or ossification of the tendon or ligament at the insertion into the bone.

### Prolotherapy

Prolotherapy is recognized as one of the most effective complementary and alternative medical (CAM) therapies to treat chronic musculoskeletal pain. This therapeutic approach, used in clinical practice for more than 100 years, was formalized by Hackett in the 1950s as a viable therapeutic strategy to treat ligamentous laxity and related musculoskeletal conditions [9, 10]. Prolotherapy entails the *injection of any substance that promotes growth of normal or injured cells or tissues*; it is a non-pharmacotherapeutic and non-surgical alternative that involves injecting small volumes of an irritant solution into painful ligaments and tendon insertions (enthuses, ligament or tendon attachment site to bone at the fibro-osseous junction), joints, and in adjacent joint spaces over several treatment sessions [11-14]. Much of the pain associated with musculoskeletal injuries resulting in laxity and weakness is enthesopathy pain that occurs at the fibro-osseous junction, where ligaments and tendons attach to bone. Prolotherapy injections are done onto the periosteum (except for intraarticular injections) to stimulate the ligaments and tendons to proliferate in the injection area by naturally promoting tissue rejuvenation through the normal inflammatory healing cascade. A major goal of prolotherapy treatment of chronic musculoskeletal conditions is the stimulation of the natural regenerative processes in the joint that will facilitate the restoration of degenerated ligaments and tendons to a healthy state, and thus, improve joint stability, support, function and reduce pain for patient. Prolotherapy is also referred to as proliferation therapy or regenerative injection therapy (RIT). The technique of prolotherapy is founded on the myofascial hypothesis, in which painful muscle spasms are secondary and appear as late sequelae of ligamentous laxity and joint hypermobility or instability. Thus, treatment of the ligament injury-induced myofascial pain should be targeted to restore the ligament and tendon structure. Complete stabilization of the joint and ligament and tendon tissue can then naturally resolve the ligament injury-induced muscle spasms and the trigger points [14-19].

### Joints and Ligaments – Anatomy, Function and Biochemical Constituents

Determination of the anatomic part responsible for joint pain is often a difficult task, but it is critical, in that it guides appropriate approach to diagnosis and therapy. Thus, knowledge of the anatomy of complex synovial joints (e.g., the knee, shoulder, and ankle), causes and typical mechanisms of injury/pain and presenting signs/symptoms are an important prerequisite for effective treatment.

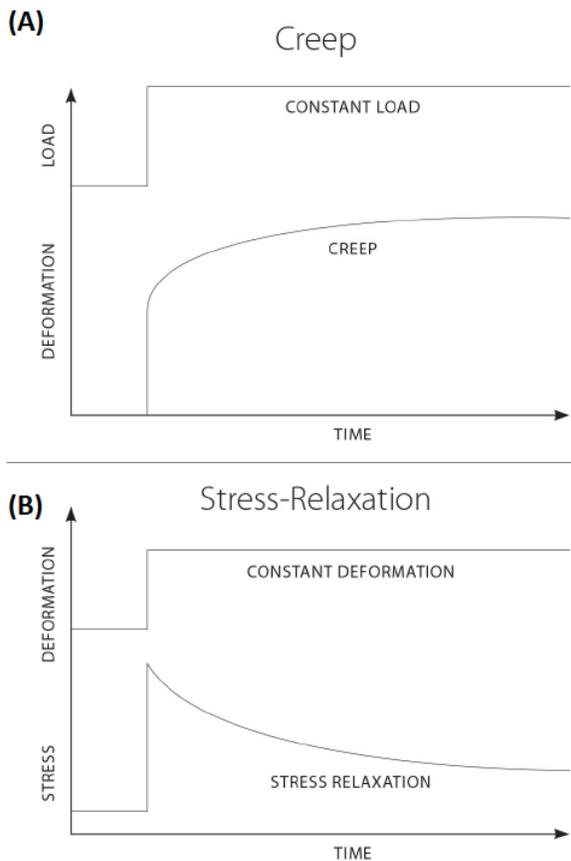
Joints link the bones of the skeletal system into a functional whole - a system that supports the body, permits effective movement, and protects the softer organs. Joints such as the knee, elbow, and shoulder are self-lubricating, almost frictionless, and able to bear heavy loads and withstand compression while executing smooth and precise movements when healthy [20]. However, it is equally important that other joints be less movable or even immobile in order to protect delicate organs. For example, the vertebral column is only moderately movable, which allows for flexibility of the torso and yet protect the delicate spinal cord and support much of the body's weight.

Joints are generally classified into nonsynovial and synovial joints. Nonsynovial joints lack a synovial lining bordering the joint cavity and do not allow for low-friction or large-range movements. Different kinds of nonsynovial joints are found throughout the body, including symphyses, syndesmoses, and synchondroses. Because the most familiar type of joint is the synovial, this review will focus primarily on discussing joint instability and resultant pain arising from synovial joint disorders. Synovial joints are the most structurally complex type of joint and are the most likely to develop uncomfortable and crippling dysfunctions. Examples of synovial joints are knee, hip, shoulder, elbow, ankle, wrist and jaw. There are six basic classes of synovial joints: ball-and-socket, condylar (ellipsoid), saddle, plane (gliding), hinge and pivot joints. Accessory structures associated with a synovial joint include ligaments, tendons, muscles, bursae, labrum (in the shoulder and hip), and menisci (in the knee joint only) [20].

Ligaments play an important role in the function of synovial joints. Ligaments are specialized dense bands of tough, fibrous collagenous connective tissue bundles that attach one bone to another. Ligaments function to hold bones in approximation, assist joint proprioception and provide mechanical support and stability. Ligaments enable smooth joint motion under normal, physiologic circumstances and prevent excessive joint displacement under high loads [20]. Ligaments vary in size, shape, orientation and location [21]. Under polarized light, ligament microstructure shows collagen bundles align along the long axis of the ligament and display an underlying crimp along the length. This crimp may be involved with the biomechanics associated with the ligament's loading state. Increased loading is thought to cause segments of the ligament to uncrimp, allowing the ligament to elongate without withstanding damage [21]. At the microscopic level, ligaments are composed of fibroblasts that are interspersed in the parallel bundles of collagen matrix and responsible for matrix synthesis. Biochemically, two-thirds of a ligament's total weight is composed of water, which contributes to the cellular function and viscoelastic properties. The remaining one-third of the components of

ligaments include proteoglycans, elastin, proteins, glycoproteins and collagen. Type I collagen is the major solid constituent (70-80% dry weight) and is primarily responsible for tensile strength; Types III, VI, V, XI collagen are also present in lesser amounts (8-12% dry weight) [21, 22].

The complex interactions of collagen with elastin, proteoglycans, ground substance, and water results in the time- and history-dependent viscoelastic behaviors of ligaments, helping to provide joint homeostasis. In response to various tensile loading protocols, ligaments exhibit hysteresis (i.e. internal energy dissipation), creep, and stress relaxation. In other words, 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 as excessive creep could result in laxity of the joint thus predisposing it to further injury (Fig. 1). Another function of ligaments is their role in joint proprioception, which is referred to as the conscious perception of limb position in space. When ligaments are strained, they invoke neurological feedback signals that then activate muscular contraction and this appears to play a role in joint stability and position sense [21, 22].



**Fig. (1). Creep and stress relaxation.** (a) When subjected to a constant stress, ligaments display creep behavior: a time-dependent increase in strain. (b) When ligaments are subjected to a long constant strain, they exhibit a decrease in the stresses within the material known as stress-relaxation.

Ligaments are inserted to bone in two ways: indirect fibrous insertion and direct fibrocartilaginous insertion. Ligaments are most often connected to bone indirectly through fibrous insertion. Superficial fibers insert into the periosteum and deep fibers insert directly into bony lamellae via perforating collagen fibers. At insertion, endotenon becomes continuous with periosteum. Through direct fibrocartilaginous insertion, fibers insert directly into the periosteum surrounding the bone [22, 23] (Fig. 2). Transition of ligament to bone occurs in four zones:

Zone 1 – Ligament proper: Consists of well-aligned type I collagen fibers with small amounts of proteoglycan decorin.

Zone 2 – Fibrocartilage: Consists of types II and III collagen, with small amounts of type I, IX and X collagen, and proteoglycans (aggrecan and decorin).

Zone 3 – Mineralized fibrocartilage: consists of type II collagen, with significant amounts of type X collagen and aggrecan.

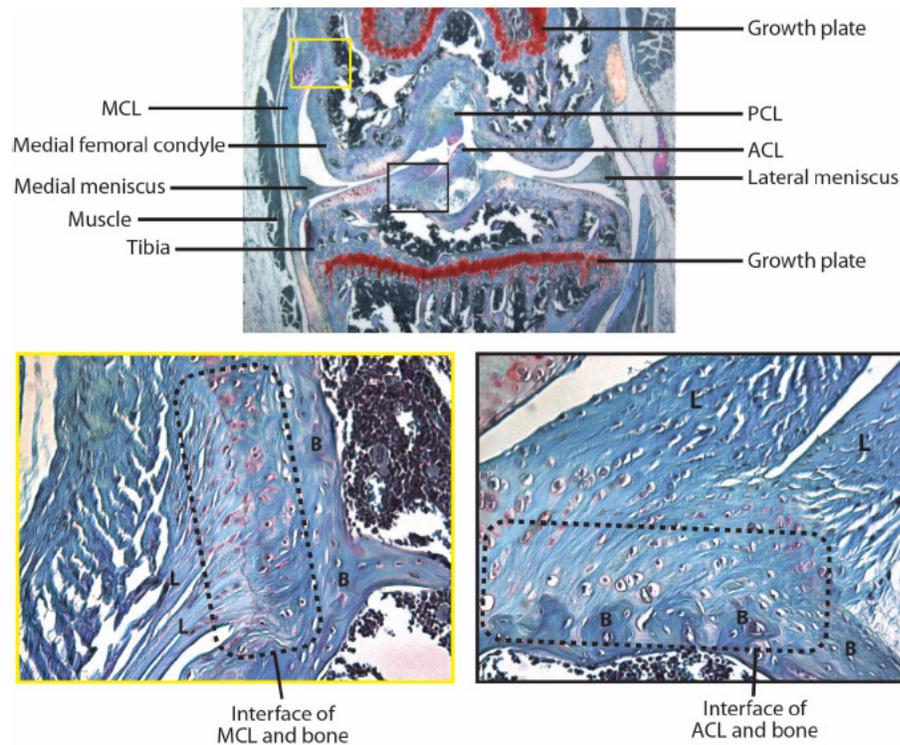
Zone 4 – Bone: Consists of type I collagen, with high mineral content [22, 23].

#### Ligament Injury, Joint Laxity and Instability

Ligaments function primarily to maintain smooth joint motion, restrain excessive joint displacement, and provide stability across the joint. For example, ligaments of the knee provide passive stability, guide the motion of the femur and tibia, define contact mechanics between the femur and tibia, and restrain excessive motion to prevent dislocation [24]. When the forces to which ligaments are subjected are too great, failure occurs, resulting in drastic changes in the structure and physiology of the joint. Ligamentous injuries can result in joint laxity, which presents as looseness or instability of the joint; this can occur at almost every joint in the body, particularly synovial joints, which are highly dependent on the ligaments for stability.

Ligaments are the primary passive joint stabilizers whereas muscles are the active joint stabilizers. Mechanical laxity refers to an excess in the range of motion in the joint due to loss of integrity of the ligaments and other soft tissues which contribute to joint stability. Functional instability refers to a sense of instability or giving out of the joint experienced in the course of daily activities or strenuous exercise. It is possible to have functional joint instability from muscle weakness alone; thus functional instability may or may not be associated with mechanical laxity [25].

Ligament injuries create disruptions in the balance between joint mobility and joint stability, causing abnormal force transmission through the joint, which results in damage to other structures in and around the joint, including the articular cartilage. Ligament injuries are among the most common causes of musculoskeletal joint laxity, and the resultant pain and disability encountered in primary care practice today. Medial collateral ligaments (MCL) and anterior cruciate ligaments (ACL) are the most frequently injured tissues within the knee joint. For example, about 150,000 ACL injuries occur annually in the United States, and more than 4 million knee arthroscopies are performed worldwide each year. ACL tears rank second to ankle sprains (torn ligaments and tendons) as the leading cause of injury in



**Fig. (2). Microstructures of ligament-bone interfaces. Upper panel:** A photomicrograph (25x) of a mouse knee joint at the age of 8 months shows intraarticular and peri-articular ligaments as well as other joint tissues. MCL = medial collateral ligament (tibial collateral ligament), PCL = posterior cruciate ligament, ACL = anterior cruciate ligament. Note, PCL and ACL are not intact at this sectioning plane; cartilage cells and their matrices are stained in red by Safranin-O; normal growth plate is still observable at this age in rodents. **Lower left panel:** A photomicrograph (200x) from the area outlined with a yellow box in the upper panel shows the MCL-bone interface (dotted rectangular box). **Lower right panel:** A photomicrograph (200x) from the area outlined with a black box in the upper panel shows the ACL-bone interface (dotted rectangular box). Both lower panels demonstrate interdigitation of ligament and bone tissues in the interface areas. Cartilage cells (stained in red) within fibrocartilage is observed in and adjacent to the interface areas. L = ligament, B = bone. Safranin-O and fast green staining.

college athletes, and are reported to be the leading cause of knee injuries in children. Damage to the ACL causes the highest incidence of pathologic joint instability [26, 27]. ACL ruptures, in some cases, are associated with marked short term morbidity and long-term consequences, such as degeneration of the joint surfaces, development of osteoarthritis, moderate to severe disabilities, joint instability and chronic pain [24, 28, 29]. ACL damage typically occurs in the younger population and, as such, leads to prolonged disability and economic cost largely due to work loss [30].

### Ligament Response to Injury and Healing Process

When ligaments are injured, a healing response is initiated in an attempt to repair the damage. The degree of healing and repair is dependent on the ligament's location and the amount of damage that has occurred. Ligaments with greater vascularity (i.e., MCL) have the ability to undergo substantial repair, whereas other ligaments (i.e., ACL) are more limited in their ability to heal, and restore joint strength and stability. Once an ACL is injured, natural healing can, at best, restore it to 50-70% of its pre-injury tensile strength [31]. As a full recovery to its pre-injury abilities does not occur, the joint is subjected to instability. This inevitably leads to biomechanical changes across joint surfaces, increasing the risk for degenerative changes and the development of osteoarthritis [24, 28, 32, 33]. In few cases,

through a three-stage overlapping healing process (hemorrhage with inflammation, cellular and matrix proliferation and finally, remodeling and maturation), the body may be able to repair the injury enough for a full *clinical* recovery of the initial structural/functional abilities (i.e., strength and ability to stabilize the joint), but this healing process can take months to resolve itself and underlying instability may still be present. If the injury is severe or if multiple injuries have taken place at a joint, the damage to the surrounding ligamentous, tendinous and cartilaginous tissues and other structures of the joint can reach a state that is beyond the body's ability to fully repair and restore.

While there is a vast body of knowledge available regarding the structure and function of normal ligaments, there is less literature addressing the effects of injury on ligament structure and function in terms of the variability and unpredictable nature of ligament healing. Ligament injuries result in significant physiological and structural changes and lead to complex and dynamic cellular processes during healing. Depending on the functional demands placed on the ligament in question, these healing processes cause profound alterations in the biology and biomechanics of the injured ligament, resulting in inadequate healing and tissue formation that is inferior (morphologically, biochemically and biomechanically) to the original tissue (Table 1) [34].

**Table 1. Biochemical differences between normal and scarred ligaments.**

Normal Ligaments	Ligament Scars
Bimodal (large) collagen fibrils	Smaller collagen fibrils
Cell and matrix turnover low	Cell and matrix turnover high
Collagen aligned	Collagen disorganized
Collagen densely packed	Flaws between fibers
High matrix-cell ratio	Lower matrix cell-ratio
Low cell density	Higher cell density
Mature collagen cross-links	Immature collagen cross-links
Primary collagen type 1	More collagen type 3
Primary small proteoglycans	Larger proteoglycans
Rare cell division	More cell division

**Biomechanical Consequences of Ligament Injury on Joint Instability and Chronic Pain**

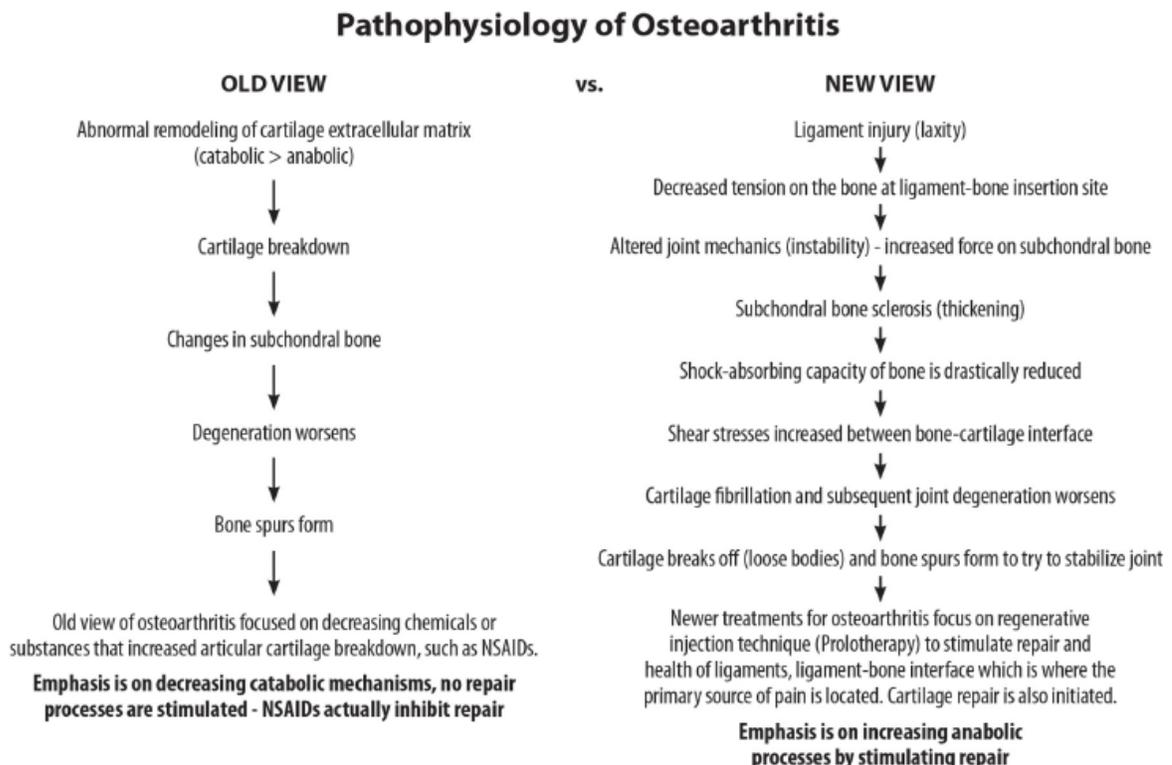
Incomplete healing and lower functional integrity of the new ligament tissue may result in ligament laxity, joint instability, and secondary muscle weakness, which predispose the joint to osteoarthritis (OA). Studies on athletes who were followed for 5 to 12 years after a ligament injury have revealed an early onset of OA of the joint and an inability to return to their pre-injury level of activity [28, 35-38]. At 10 years 21%-48% of these athletes were found to

have OA. A separate study on female athletes for 12 years after an ACL injury reported that 50% of the females had radiographic OA and approximately 80% had other features of OA [39]. Thus, sports trauma, which begins with ligament injury and subsequent laxity, can cause joint instability, which then leads to chronic pain, diminished function, and ultimately to OA of the affected joint [28, 35, 38]. Despite the use of numerous strategies over the years, attempts to improve ligament healing after injury have not been entirely successful. OA remains one of the long-term consequences of ligament injury and continues to be the most common joint disorder of the world [28, 40, 41]. Therefore, understanding the complex cellular processes that occur after ligament injury, as well as determining and implementing those strategies that optimize ligament restoration, are necessary steps in reducing the enormous individual and public health burden of ligament injury-associated OA and its related chronic musculoskeletal pain.

**Ligament Laxity – Pathway to Chronic Joint Pain and Osteoarthritis**

OA is one of the most common consequences of ligament damage, and subsequent laxity. Traditionally, the pathophysiology of OA was thought to be due to aging and wear and tear on a joint, but more recent studies have shown that ligament injury is one of the initial causes for the development of OA [28, 42, 43]. The possible mechanisms of ligament injury-associated development of OA and proposed prolotherapy healing are illustrated in (Fig. 3). [24, 28, 35].

It is evident from published scientific and medical literature that enormous efforts and resources have been devoted over the years to characterizing and discussing



**Fig. (3). A new view on osteoarthritis.**

osteoarthritic articular cartilage degeneration or loss. Consequently, the structural or anatomical basis for disease and its molecular pathogenesis has been viewed mostly in terms of cartilage [44, 45]. Although it is not disputed that changes in articular cartilage play a crucial role in the pathophysiology of OA, it has been suggested that considering OA as primarily a disease of articular cartilage is too simplistic [46]. Indeed, emerging evidence from the application of magnetic resonance imaging (MRI) in early OA has confirmed several different anatomical or structural abnormalities within diseased joints [47-50]. Furthermore, the earliest structural changes seen in some models of spontaneous OA in the knee joint occur first in the cruciate ligaments, while the subchondral bone and articular cartilage are secondarily affected. The subchondral bone sclerosis, often being the first radiographic evidence of OA, is thought to occur because of increased pressure on the joint secondary to poor joint mechanics [35, 45, 51, 52]. Thus, key emerging research findings demonstrate that several types of primary OA show ligament-related pathology at the time of clinical presentation [52]. Although there is also emerging evidence for OA initiation in other structures of the joint, [45] we shall focus on ligament injury initiated or derived OA in the section below. There are several reasons for this focus:

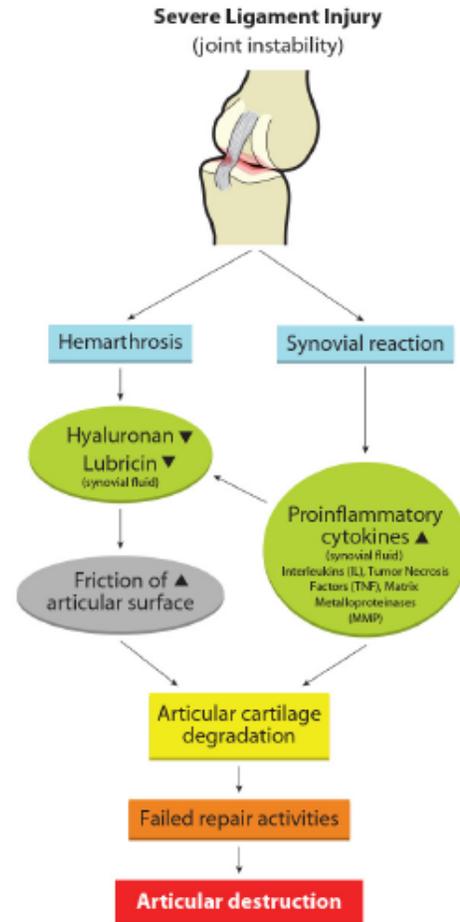
(1) Chronic musculoskeletal pain can be caused primarily by ligamentous laxity, or enthesopathy; the painful muscle spasms and trigger points associated with them are secondary and appear as late sequelae of ligamentous laxity and resultant joint instability [15].

(2) There is emerging evidence from recent scientific findings that the mechanism of ligament injury-derived OA begins with ligament injury and the subsequent changes in ligament mechanics and biochemistry that renders the joint unstable [35, 52, 53].

(3) It has been reported that OA is best modeled as a disease of organ failure, in which injury to one joint constituent leads to damage of other components, and collectively results in joint failure and the clinical manifestations of OA [54, 55]. Indeed, published reports indicate that ligament injury induces damage to the entire joint; synovitis, effusions and hematoma are associated with ligament injury and are known to affect articular cartilage, the subchondral bone, the injured ligament, and other soft tissue structures in the given joint [35] (See Fig. 4). Recently, Andriacchi *et al.* described a possible mechanical mechanism for the onset and progression of osteoarthritis based on ligament injury [56].

(4) Joint laxity and instability as a result of ligament deterioration or rupture have long been considered to be a strong contributor to the development of post-traumatic osteoarthritis [57-61].

(5) A structural model of OA provides a useful reference framework for a focused understanding of the magnitude of disease in the same way that tumors can be classified and staged according to their tissue of origin and extent of involvement. Moreover, this approach has implications for therapeutic strategies (for example, regenerative medicine therapy schemes targeted to specific structural locations of the joint) [44, 62].



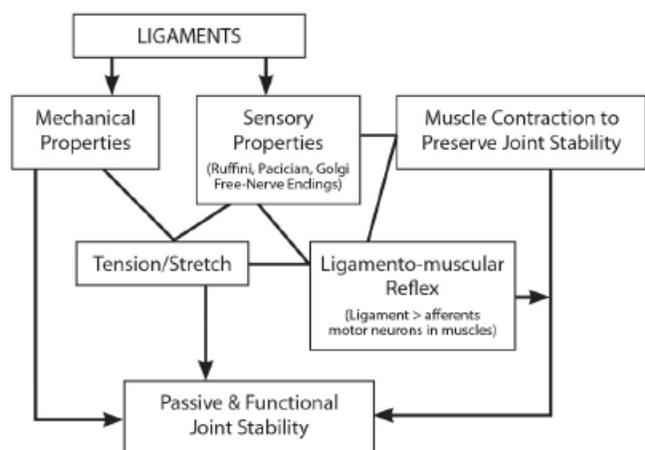
**Fig. (4). Mechanisms underlying the development of posttraumatic osteoarthritis after ligament injury.** Although the initial pathological changes may vary depending on the damage to specific joint tissues, these changes eventually lead to articular cartilage degradation and joint destruction. The synovium and articular cartilage can interact with each other through specific mediators in synovial fluid, which are secreted by either chondrocytes or synoviocytes. The acute hemarthrosis after injury eventually resolves, but the synovial reaction continues indefinitely until joint stability is restored. Modified and used with permission from: Kramer WC, *et al.* Pathogenetic mechanisms of posttraumatic osteoarthritis: opportunities for early intervention. *Int J Clin Exp Med* 2011; 4(4): 285-98.

### Ligament Mechanoreceptors Involvement in Inflammation, Osteoarthritis and Pain Sensing

Ligaments in joints and the spine are endowed with mechanoreceptors, including pacinian, golgi, ruffini and bare nerve endings. (Fig. 5). By virtue of their innervation, ligaments also play an important role in proprioception and kinesthesia, and have a direct role in reflex activation or inhibition of muscular activities to preserve joint stability [63]. There is increasing evidence that some ligaments and/or the joint capsules that reinforce joints can have complex functional interactions with adjacent bone surfaces and with other joint tissues near their entheses, forming part of an enthesis organ and synovio-entheseal complex [44, 64-66]. The enthesis has been shown to be a dynamic area - metabolically active, endowed with a rich vascular supply,

and highly innervated, particularly with C and A  $\delta$  pain fibers [23]. Thus, where a tendon/ligament contacts a bone immediately adjacent to its enthesis, the intermittent compressive loading may be sufficiently high to provoke a chondrogenic metaplasia near the interface of the two contacting tissues.

### Ligaments as a Sensory Organ



**Fig. (5). Ligaments as a sensory organ.** Basic organizational plan adapted from: Johansson H, Sojka P. A sensory role for the cruciate ligaments. *Clinical Orthopaedics and Related Research* 1991; 268: 161-78.

In view of the structural and physiological characteristics of the ligaments and their entheses, an exaggerated response to an insult can occur. This anatomical arrangement may be key to the development of the OA phenotype [67, 68]. The imbalance between the breakdown and repair of joint tissues in OA is the result of the activation of joint cells by inflammatory mediators, matrix components and mechanical stress [69]. A number of putative mediators have been implicated in the catabolic process, including matrix-degrading proteases, superoxide radicals and pro-inflammatory cytokines [70]. Counterbalancing this, insulin growth factor (IGF-1), transforming growth factor (TGF)- $\beta$  and bone morphogenetic proteins (BMPs) are endogenous anabolic factors that stimulate bone and cartilage regeneration and remodeling [71, 72]. Thus, it is now becoming clear that primary changes in ligaments and their insertions can profoundly affect the adjacent bone and synovial tissues [44]. At the macroscopic level, inflammation is directly linked to clinical symptoms such as joint swelling, synovitis and inflammatory pain. Structural joint instability from injuries to ligaments results in an inflammatory reaction that initiates a chemical cascade in and around the joint characteristic of most injured tissues. [23]. The collateral ligaments appear to be at the epicenter of the inflammatory process and their involvement explains the periarticular pattern of inflammation evident in hand OA [47, 48, 73].

### Current Standards of Treatment and Management for Optimizing Ligament Repair and Healing

Ligament healing is generally slow and often incomplete. Joint laxity caused by ligament injury improves slowly, often

over several weeks to a year or more, after which a large percentage of patients still have objective mechanical laxity and subjective joint instability [28, 74]. Several standard, novel/biotechnological and complementary alternative medicine (CAM) treatment and management strategies have been implemented over the years in attempt to heal and restore the structural and functional properties of injured ligaments to pre-injury status. Available treatments include, over the counter (OTC) medications [analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroid and hyaluronate (viscosupplementation) injections, narcotics/opioids, anti-depressants, muscle relaxants, sedatives, physical therapy and exercise, rest and mobilization, diet and nutrition, surgery (arthroscopy, ligament reconstruction, debridement, fusion, arthroplasty), acupuncture, and chiropractic manipulation [28, 75-86].

While each of these therapies may help to temporarily alleviate the subjective symptom of acute or chronic joint pain following ligament injury, they do not all address damage to the ligaments, resultant instability, of the joint or contribute to the actual cellular repair and healing processes of ligament tissue. In fact, some of these therapies (NSAIDs and corticosteroids) have been shown to be detrimental to the ligament healing process because these drugs suppress and inhibit certain cellular processes that are required for ligament tissue repair and healing. Analgesics can also have some very serious adverse side effects on the patient (such as hepato-toxicity and potential renal damage). Narcotics not only alter the neuropsychological and pathophysiological responses of the body, but also suppress both innate and adaptive immune function [24, 26, 87]. Other therapies (e.g., prolotherapy) have been shown to contribute to repair and healing through stimulation of certain cellular processes involved in the regeneration of ligament tissue. In the following subsections, we shall briefly discuss the effectiveness of two of the main therapies used for chronic musculoskeletal pain: NSAIDs and corticosteroids, as well as prolotherapy in repairing and healing injured ligaments.

### Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been a mainstay treatment in ligament injuries for several years, especially in the case of acute sports injuries. However, new research has shown that these anti-inflammatory drugs are only mildly effective in relieving the symptoms of most ligament, tendon and muscle injuries and are potentially deleterious to soft tissue healing [88, 89]. There are valid reasons to expect that NSAIDs might have an adverse effect on healing, since prostaglandin-induced inflammation is an early sequel in the cascade of the healing of injury induced events. This response normally results in the recruitment of cells into the injured area where they remove necrotic debris and initiate the healing process. However, NSAIDs are known to specifically block the cyclooxygenase enzymes, which catalyze the conversion of arachidonic acid to prostaglandins that would otherwise play a significant role in ligament healing [90]. Additionally, because of the analgesic effect of NSAIDs, patients may feel no discomfort while doing strenuous activities and ignore early symptoms of ligament injury, which could cause further damage to the ligament, and thus, delay definitive healing.

Multiple studies have been conducted on the cyclooxygenase-2 (COX-2) inhibitor class of NSAIDs, and researchers have concluded that the use of these medications inhibits ligament healing and leads to impaired mechanical strength [91, 92]. Therefore, NSAIDs are no longer recommended for chronic soft tissue (ligament) injuries. In the case of acute ligament injuries, NSAIDs should be used for the shortest period of time possible, if used at all [93, 94].

**Cortisone Injections**

Corticosteroid injections have also been a long-standing treatment regimen for musculoskeletal disorders, including ligament injuries. Although steroid injections have been shown to be effective in decreasing inflammation and pain in ligament injuries for up to six to eight weeks, they inhibit the histological, biochemical, and biomechanical properties of ligament healing. While the anti-inflammatory actions of corticosteroids stem from their ability to prevent lysosomal enzyme release, this also inhibits neutrophils and other inflammatory cells from accumulating at the injury site, as well as disrupts the synthesis of cytokines and other inflammatory mediators [95]. This impedes the normal repair processes that are stimulated after normal joint trauma and injury [95, 96].

Further evidence that corticosteroid injections into injured ligaments have an adverse effect on healing has become known. Corticosteroid injections into ligaments and tendons can inhibit fibroblast function and thus collagen

synthesis [96, 97], even to the extent of causing collagen necrosis at the injection site [98]. Given the inhibitory effects corticosteroid injections have on ligament healing, reviews have cautioned against their use for treating ligament injuries, especially in athletes [28, 99].

**Application of Prolotherapy Biology for the Healing of Injured Ligaments**

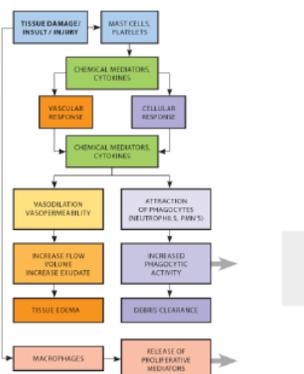
The healing phases and the biomechanical consequences of ligament injury have been previously discussed in brief. Fig. (6) is a schematic depiction of the application of the therapeutic principle of prolotherapy – encompassing the inflammatory, proliferation and tissue remodeling phases of the healing and restoration processes of injured ligaments/tendons. The mechanism of action behind prolotherapy is not completely understood; however, modern theory suggests the injected proliferants mimic the natural healing process by initiating a local inflammatory response, which triggers or signals a healing cascade that releases growth factors and collagen deposition. This process leads to proliferation, ligament tissue remodeling, strengthening of new tissue, joint stability and reduction in pain and dysfunction [100, 101].

Most human cells contain only 0.1% dextrose. However, *in vitro* studies on human fibroblasts and chondrocytes demonstrate stimulation of growth factors when cells are exposed to dextrose concentrations of only 0.5% [102, 103]. These growth factors include platelet-derived growth factor,

# The Biology of Prolotherapy

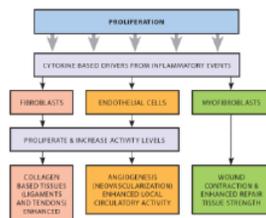
**Inflammatory Cascade.**

After tissue damage by injury, the body attempts to heal the area by mediating this cascade. When the body is unable to heal itself, which is often the case when avascular (no or little blood supply) tissues such as ligaments, tendons, cartilage and fibrocartilage (meniscus and labrum) are injured, Prolotherapy is utilized to stimulate healing.



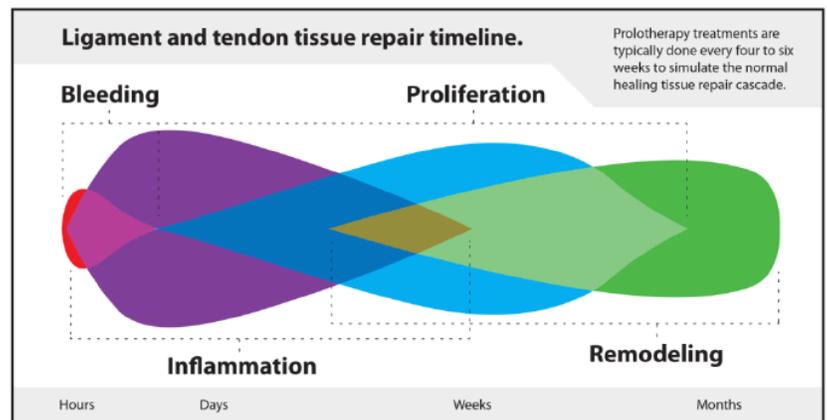
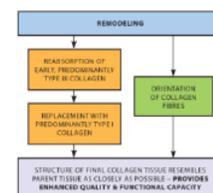
**Proliferation Cascade.**

Prolotherapy stimulates healing via inflammation. After Prolotherapy solutions are injected into the injury site, a cellular reaction takes place in which various cells including fibroblasts, endothelial cells and myofibroblasts form new blood vessels and ultimately lay down collagen which enhances tissue repair and strength.



**Tissue Remodeling Cascade.**

The final phase of healing is tissue remodeling. For many months after an injury or Prolotherapy, tissue continues to remodel. The new tissue that results looks and functions very closely to the original tissue before the injury. Once the tissue strength approaches that of the normal parent tissue, pain resolves.



**Fig. (6). The biology of prolotherapy.** Prolotherapy induces the three stages of healing and restoration: inflammatory, proliferation, and tissue remodeling.

transforming growth factor- $\beta$ , epidermal growth factor, basic fibroblast growth factor, insulin-like growth factor, and connective tissue growth factor—having been found *in vitro* to promote the expression of type 1 and 3 collagen in tenocytes, and are pertinent to the formation and growth of ligament, tendon and cartilage [104-107]. Prolotherapy solutions that contain concentrations less than 10% dextrose are known to be non-inflammatory, while those greater than 10% are inflammatory [19, 100]. In summary, an increase of glucose concentration (dextrose) with Prolotherapy causes an increase in cell protein synthesis, DNA synthesis, cell volume, and proliferation [108]. Thus, simple dextrose solution is an inexpensive method of growth stimulation that may prove to be cost-effective for the treatment and long-term management of diverse musculoskeletal joint-related pain [106].

Several molecules have been recognized as potential proliferants and it has been suggested that the mechanism of action by which they induce local, controlled inflammation might vary depending on the type of substance. Theoretically, the main proliferants have been classified into three groups based on their possible biochemical mechanisms as irritants, osmotics, and chemotactics [10, 109]. The osmotic shock agents, such as concentrated dextrose, zinc and glycerin solutions, are believed to act by dehydrating cells at the injection site. This leads to local tissue trauma, which, in turn, attracts granulocytes and macrophages. The most common prolotherapy agents used in clinical practice are dextrose solutions ranging from 12.5%-25% [107]. Dextrose proliferant has been approved for injection by FDA, but not for prolotherapy; thus, it is currently used in prolotherapy as an off-label substance [11]. Phenol, guaiacol, and punic acid belong to the second class known as irritants and are thought to act directly damaging cell membranes. Chemotactic agents, such as polidocanol and sodium morrhuate are purported to be direct chemotactic agents to inflammatory cells. Newer forms of prolotherapy termed “cellular prolotherapy” involve using a person’s own cells as the proliferant and include whole blood, platelet rich plasma, bone marrow and adipose tissue [109-111].

### Effective Applications of Prolotherapy in the Treatment of Chronic Musculoskeletal Conditions

Prolotherapy injections stimulate ligament size and mass, tendon hypertrophy, extracellular matrix, fibroblastic proliferation, increased ligament-bone junction strength and repair of articular cartilage defects [19, 108, 112]. Prolotherapy has been utilized for the following groups of conditions including: degenerative arthritis; enthesopathies; ligament injury; tendinopathy, including tendinosis and tendinitis; joint instability from a myriad of causes including ligament, labrum or meniscus injury and a host of other conditions with a few of them discussed in the following sections [108, 113-116].

**Osteoarthritis** - Rabago *et al.* conducted a randomized controlled trial of dextrose prolotherapy in the treatment of symptomatic chronic knee OA [13, 117, 118]. The 3-arm, blinded (injector, assessor, injection group participants) were randomized to blinded injection with dextrose or saline which were compared, along with a control group that did at-home exercises. Outcomes were assessed by the validated

Western Ontario McMaster University Osteoarthritis Index (WOMAC; 100-point scale) at 52 weeks. In this trial, WOMAC scores among dextrose prolotherapy recipients were more improved at 52 weeks than scores among saline control and at-home exercise participants (mean [SD] score change, 15.3 [3.5] vs 7.6 [3.4] and 8.2 [3.3], respectively;  $P < .05$ ) [118].

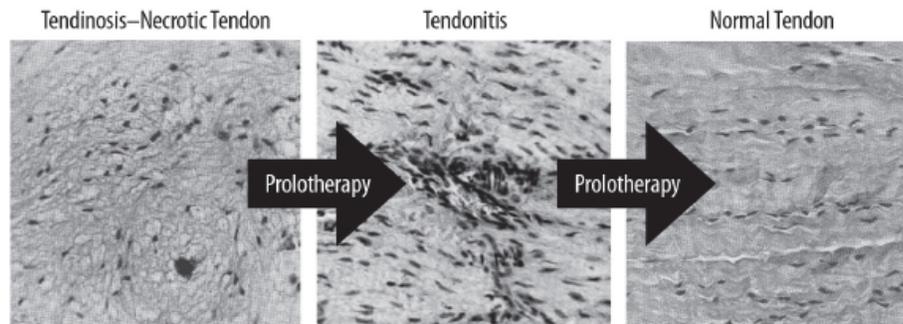
Additional evidence of the efficacy of dextrose prolotherapy in the treatment of osteoarthritis was demonstrated by Reeves *et al.* The authors conducted two studies that examined the treatment of knee, and finger and thumb osteoarthritis in patients with at least 6 months of associated pain and radiographic evidence of significant joint space narrowing, a moderate sized osteophyte, or both in at least one compartment of the affected joint space. Participants in both studies were randomized to receive either dextrose and lidocaine, or lidocaine and bacteriostatic water injections, at 0, 2, and 4 months. Compared to control groups, subjects in the experimental groups in both studies showed positive outcomes, with improvement in pain at rest and with activity, joint stabilization, and improved range of motion. However, neither study’s results achieved statistical significance [119]. Interestingly, blinded radiographic readings at 0 and 12 months revealed improvement in lateral patellofemoral cartilage thickness and distal femur width. Three-year follow up data also showed improvements in pain during walking, subjective reports of decreased swelling and increased range of motion in the group treated with dextrose.

**Chronic Tendinopathies** - Tendinopathy refers to a painful clinical condition that occurs often as a result of overuse. Prolotherapy has been used clinically for multiple types of tendinopathy (Fig. 7) and has been studied for the treatment of lateral epicondylitis, Achilles tendinopathy, plantar fasciitis and hip adductor tendinopathies [100]. In a single-blind randomized controlled trial comparing dextrose and dextrose-sodium morrhuate prolotherapy to a control group with chronic lateral epicondylitis; both prolotherapy groups showed improved grip strength compared to the wait and see group at 16 weeks ( $P < 0.05$ ). Both prolotherapy groups reported improved composite patient-rated tennis elbow evaluation scores [120]. In a double-blind randomized control trial of 20 adults with refractory lateral epicondylitis, the dextrose-sodium morrhuate group, compared to controls, reported statistically significant improvements in pain scores and grip strength that persisted at 52 weeks [121].

Maxwell *et al.* used ultrasound guidance to provide intratendinous injections of a dextrose and anesthetic solution to 36 adults with chronic, refractory Achilles tendinopathy at 6-week intervals. The researchers reported statistically significant reductions in pain scores at 6 weeks as well as decreased neovascularity as measured by ultrasound in 55% of the tendons [113]. Another study showed that dextrose prolotherapy in combination with eccentric loading exercises provided the most relief in the first 6 weeks in the management of Achilles tendinopathy compared with prolotherapy or exercises alone. However, the study yielded no significant difference between the treatment groups at 12 months [122].

A case series examined the efficacy of prolotherapy on hip adductor tendinopathy in male athletes engaged in

## The Histology of How Prolotherapy Heals



**Fig. (7). Prolotherapy for tendinosis: histology samples.** Used with permission from *Overuse Injuries of the Musculoskeletal System* - Marko M. Peling, CRC Press, 1993, Boca Raton, FL.

kicking sports. Subjects with groin pain for a mean of 15.5 months that was unresponsive to specified physical therapy were injected monthly with dextrose and lidocaine into the areas of maximal tenderness. The average number of injections given was 2.8. Twenty of 24 athletes had complete resolution of painful symptoms, and nearly all the participants were able to return to their sport without restrictions as measured by pain and functional scales [123].

A pilot study by Ryan *et al.* examined the effects of prolotherapy on chronic plantar fasciopathy in patients who had failed conservative treatments. The researchers injected 36 symptomatic adults with hyperosmolar dextrose and lidocaine solution under ultrasound guidance. They then used visual analog scales for pain at rest, during activities of daily living, and during or after physical activity. Researchers reported significantly decreased mean scores in all areas at the final treatment consultation [124].

**Dysfunctional Sacroiliac Joint** - Use of prolotherapy for treatment of sacroiliac joint dysfunction has also been demonstrated to be effective. Cusi *et al.* used computed tomography to guide injections of hyperosmolar dextrose into painful, dysfunctional sacroiliac joints in 25 patients. The authors reported significant improvement in pain and disability scores compared to baseline scores; however, there were no control subjects used in this study [114]. A similar study compared the effects of hyperosmolar dextrose versus triamcinolone acetonide fluoroscopically guided intraarticular injections into painful sacroiliac joints. Results demonstrated improvements in pain and disability scores from baseline in both groups; however, the effects of the dextrose group lasted longer than the steroid group [125]. In another study, intraarticular dextrose prolotherapy to the sacroiliac joint gave significant improvements in the numeric rating scale and Oswestry Disability Index compared to baseline ( $p < 0.01$ ) [126].

**Chronic Coccygodynia and Other Chronic Musculoskeletal Conditions** - Khan and his group studied 37 patients with chronic coccygodynia. The patients received up to three dextrose-lidocaine injections into the coccyx. Thirty of the 37 patients had improved visual analog scores [127]. Numerous other studies including case reports have documented success in utilizing prolotherapy in the treatment of chronic musculoskeletal pain [107, 115, 116, 128-136]. These studies offer promise for prolotherapy for a

host of conditions and support the need for further RCTs aimed at more precise indications with this approach.

### CONCLUDING REMARKS

The most frequently reported chronic pain conditions are associated with musculoskeletal dysfunction and degeneration. One primary cause of this is joint instability from ligament injury, which is hampered by standard therapeutics such as NSAIDs and corticosteroid injections. The degenerative process associated with weak and unstable joints can be slowed and potentially prevented by treatment with prolotherapy. If treated in the early stages, the proliferation of new ligament tissue strengthens the joint and helps restore proper joint mechanics and smooth/frictionless joint motion. By decreasing laxity of the ligaments and instability of the joint, contact forces can be redistributed back onto the areas of thickest cartilage that are designed to handle high loads and reduce the stress at thinner, weaker points, preventing deleterious biochemical and biomechanical events in the joint and allow healing to take place. Even in later stages of degeneration and OA, improvements in pain, instability and function are possible as amply described in the published literature. For some patients, prolotherapy is the treatment of choice for ligament injuries (sprains, tears, instability, and benign hypermobility syndrome) and the resultant cartilage degeneration they cause [24].

Prolotherapy is an old and respected technique of alternative musculoskeletal pain treatment that has its place in comprehensive musculoskeletal joint pain and joint instability management. Careful patient selection with attention to individual anatomy, course of disability and pain, level of functional impairment, and identification of patient treatment goals are the best starting points for determining the appropriateness of prolotherapy. In some cases, prolotherapy as a solo treatment modality might not be sufficiently effective to completely alleviate chronic musculoskeletal pain, ligament/tendon injury, or joint osteoarthritis. However, when combined with a carefully planned and individualized, integrative pain management program, prolotherapy is a very valuable addition to standard or CAM methods.

Although the results of the cited studies indicate good promise for dextrose prolotherapy in the treatment of

musculoskeletal pain and joint instability, additional comparative effectiveness studies need to be conducted which include biomechanical and imaging outcome measures to assess potential disease modification in order to definitively determine the clinical utility of prolotherapy. As more research into joint instability and effective healing is obtained and well-designed clinical studies are performed confirming current understanding of the beneficial effects of prolotherapy, it will likely become more acceptable by mainstream medical practitioners, and available to vast numbers of people who suffer from the disabling effects of chronic musculoskeletal pain [24, 69].

### CONFLICT OF INTEREST

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: RH, PB, and DS perform prolotherapy in their practices.

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### PATIENT'S CONSENT

Declared none.

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