Orthobiologic Treatment of Ligament Injuries

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KEYWORDS

• Healing cascade • Orthobiologics • Cell-based treatment
• Leukocyte-rich platelet-rich plasma • LR-PRP • Bone marrow concentrate (BMC)
• Tissue healing • Ligaments

INTRODUCTION

Ligament injuries are common causes of joint pain, dysfunction, and disability and result in disruption of joint homeostasis, leading to the imbalance of joint mobility and stability. Ligaments are the most frequently injured tissues within a joint. Ankle sprains and anterior cruciate ligament (ACL) injuries are the leading causes of injury in college athletes.[1] During the past decade, there has also been a significant increase in injuries to the medial elbow, ulnar collateral ligament (UCL) in younger throwing athletes.[1]

Ligament injuries can lead to abnormal force transmission within the joint, resulting in damage to other supporting structures such as articular cartilage, menisci, tendons, and subchondral bone, eventually resulting in arthrosis.

Currently, literature regarding clinical outcomes using orthobiologic or cell-based therapies for ligament injuries is limited. Although clinical results are very promising, variability and conflicting results observed in clinical studies, may be explained by the reporting of inconsistent procedural technique, preparation methods, heterogenicity of the platelet-rich plasma (PRP) or bone marrow concentrate (BMC) compositions and posttreatment rehabilitation.[2] Due to these inconsistencies in the current literature, several orthobiologic reporting guidelines have been created to minimize heterogenicity in reporting and biologic preparation.[3,4] Successful outcomes will depend on developing a better understanding a ligament healing, the anatomy, physiologic differences in healing of specific injury location, for instance intra-articular versus extra-articular ligaments and the biology of the specific cellular therapies used.

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LIGAMENT HEALING

When ligaments are exposed to physiologic loads overtime, they increase in mass, stiffness, and load to failure.[5] However, when ligaments are overloaded or exposed to loads greater than they structurally can sustain, tissue failure occurs, resulting in partial or complete ligament disruption.[1]

A ligament healing response begins when normal healthy tissue sustains an injury. Injuries can occur by different mechanisms and occur in different locations, which may initiate distinct and different healing responses specific to the tissue and location of the injury (intra-articular vs extra-articular environments). Bleeding in vascular tissue initiates the healing cascade.[6–10] In normal circumstances, the healing cascade that ensues is a choreographed, highly regulated series of 4 interdependent phases.[6–9] Depending on the severity and magnitude of the injury, this phase can transpire over weeks to months. The phases of the healing cascade include the following (**Fig. 1** Healing Cascade):

1. Hemostasis—clot formation.
2. Inflammatory phase—platelet activation and immune system mobilization.
3. Proliferative phase—cell multiplication and matrix deposition.
4. Remodeling phase—scar formation and tissue restoration.

Each distinct phase is dominated by a particular cell type, which prepares the injured tissue for the physiologic events that occur in the next phase.[1,6–11] It is extremely important that each phase is executed efficiently to ensure the proper transition between phases. If the phases of healing do not properly transition, the repair process may be disturbed leading to the development of chronic or potentially degenerative pathologic tissue.[10,11]

Hemostasis

Hemostasis is the first and shortest phase of the healing cascade occurring within seconds to minutes, and this is the process of forming a blood clot to stop bleeding. Platelets are vital to hemostasis, also functioning as the physiological trigger to activate acute inflammation and program tissue repair.[8]

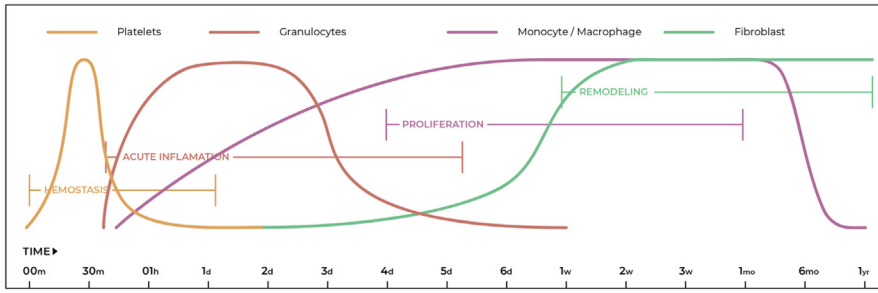

Fig. 1. Healing cascade. The 4 stages of the healing cascade consisting of 4 partially overlapping stages, hemostasis, acute inflammation, proliferation, and remodeling. The particular cell type activity within each phase is crucial for the progression and successful execution of the healing cascade leading to tissue repair. (*Adapted from* Parrish WR, Roides B. Physiology of Blood Components in Wound Healing: an Appreciation of Cellular Co-Operativity in Platelet Rich Plasma Action. J Exec Sports Orthop.4(2):1-14. https://doi.org/10.15226/2374-6904/4/2/00156.)

Platelets contain large numbers of alpha granules that store various growth factors, cytokines, and hormones necessary for wound healing.[8] Platelet activation is a highly regulated process that culminates in degranulation, or the release of granule contents.[10] The process of degranulation is a key step in wound healing because the growth factors and other mediators that platelets release program damaged tissue for repair.[10] In pathological states, such as an injury resulting in bleeding, platelets become activated by contact with components of the extravascular connective tissues including collagen that are exposed at the site of injury.[10] Platelets and leukocytes become activated together in a physiological context for wound repair.[10] Together they have coordinated and cooperative activities in normal would healing that trigger wound repair and limit acute inflammation.[10]

Activated red blood cells (RBCs) influence 3 important actions that contribute critically to the healing cascade: platelet recruitment, thrombin generation, and platelet activation and represents a critical axis between hemostasis and inflammation. The biochemistry and cellular content of the clot is determined by the communication between platelets and red blood cells, to activate thrombin generation during the hemostasis phase.[10,12–14] Activated RBCs play a critical role in amplifying thrombin generation to ensure effective and efficient execution of wound healing.[10,15]

Thrombin is the most powerful natural platelet activation signal and deficiencies at this stage of the healing cascade can lead to chronic inflammation and prolonged healing. Platelet activation in turn will help determine the biochemistry and cellular content of the fibrin clot establishing the potential for tissue regeneration.[10,15]

INFLAMMATORY PHASE

The *inflammatory phase* begins immediately following the injury and continues for 48 to 72 hours. When platelets aggregate and adhere to the injury site,[1,10,11,16] platelet granules are stimulated to degranulate, releasing inflammatory mediators and growth factors.[6,10,17–20] The largest and most the prevalent of these, the alpha granules, release platelet-derived growth factor-AB (PDGF-AB), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF), among others. Each of which has a specific role in the inflammatory process such as stimulating local tissue growth including angiogenesis and collagen synthesis, initiate cellular differentiation and are crucial to the progression of the healing process[1] [**Table 1** GFs].

Acute inflammation in response to injury is a completely dependent reaction triggered simultaneously with the activation of hemostasis.[10,11,17,21–25] However, unlike hemostasis that occurs to completion within minutes, acute inflammation typically lasts from 4 to 14 days.[7,8,10,11,26] The duration and magnitude of the acute inflammation phase is thought to be dependent on the extent of injury and whether the wound has been significantly contaminated.[26] The physiology of leukocytes is different in the presence of microbial or foreign body contamination than in sterile inflammation such as a contusion, overuse injury, closed traumatic injury, or uncomplicated surgical wound.[10]

The pairing of hemostasis and acute inflammation occurs through the action of thrombin, which helps to ensure the acute inflammatory response is proportional to the magnitude of injury.[10,27,28] A balanced wound healing physiology is important in determining poor or delayed healing.

During the acute inflammatory phase of healing, mobilization of innate immune cells such as granulocytes occurs functioning to debride and decontaminate the wound. Granulocyte activity is tightly regulated, requiring multiple activation steps to drive an inflammatory reaction. Neutrophils require separate priming and activation signals

Table 1
Growth factors in platelet-rich plasma

Growth Factor	Cell Source	Function
PDGF	Platelets, endothelial cells, macrophages, smooth muscle	Stimulates cell proliferation; promotes angiogenesis; promotes epithelialization, potent fibroblast, and immune cell recruitment factor
VEGF	Platelets	Stimulates endothelial cell proliferation, promotes angiogenesis
TGF-β1	Platelets	Promotes extracellular matrix synthesis, potent immune suppressor
FGF	Platelets	Stimulates proliferation of myoblasts, angiogenesis
EGF	Platelets	Proliferation of mesenchymal and epithelial cells, potentiation of other growth factors
Hepatocyte growth factor	Plasma	Angiogenesis, mitogen for endothelial cells, antifibrotic
Insulin-like growth factor	Plasma	Stimulates myoblasts in fibroblasts, mediates growth and repair of skeletal muscle

to elicit an inflammatory response. The first, or "priming signal," wakes up the resting neutrophil. In the case of a sterile injury, activated platelets can provide that priming signal.[10,29] Leukocytes develop the ability to communicate with their environment and gain the ability to generate and release an array of cytokines and growth factors with the ability to modulate the activity of other cells.[6,10,21,30–39] This is of importance because platelet growth factor release is exhausted in the early inflammatory phase, and it is predominantly the activity of white blood cells that guide the healing cascade forward to proliferation and remodeling.[7–11,19,26,40,41] Several authors have also shown that the leukocyte priming reaction is also reversible,[10,42–44] allowing white blood cells greater flexibility responding to signals in the environment driving wound healing. Because priming does not always lead to activation and an inflammatory response, it possibly plays a key role in limiting the nature of platelet-driven acute inflammatory reactions. This may explain the observations that leukocyte-rich PRP (LR-PRP) does not exacerbate inflammatory cytokines in osteoarthritic joints.[10,45]

When leukocytes are primed but not in an activated state, phagocytic activity is enhanced promoting debridement of damaged tissue and the release of anti-inflammatory and immunomodulatory mediators including TGF-b1, IL-1RA, lipoxins (modulators of inflammation) and resolvins, which actively suppress chronic inflammation, preventing the migration and recruitment of new leukocytes into the treated tissue, directing cellular activities toward tissue repair.[10,46–52] Monocytes then differentiate into anti-inflammatory macrophages, specialized for phagocytosis, which in turn helps to guide tissue repair.

PROLIFERATIVE PHASE

Within 2 to 3 days after the injury, the *proliferative phase* begins with the activation of fibroblasts by growth factors and inflammatory mediators released during the acute

inflammatory phase.[7,10,11,26,40,53] The proliferative phase is defined by cell proliferation, neovascularization, and matrix synthesis in addition to other metabolic processes that aid and remodeling and organization of the healing ligament tissue.[54] This phase is initiated by macrophage activity that recruits fibroblasts, endothelial cells, and stem cells into the forming granulation tissue and is primarily driven by macrophage-sustained release of growth factors such as TGF-β, endothelial growth factor (EGF), and VEGF. Growth factors, mitogenic agent, and chemoattractants induce native connective tissue progenitors in locally injured and adjacent tissues to proliferate and differentiate into myoblasts.[55] Various growth factors including PDGF, TGF-b1, and FGF, generated by monocyte-derived macrophages stimulate the process of fibroblast migration from wound margins into the fibrin clot matrix.[8,10,11,40,41] Macrophage-driven fibroblast activity replaces the fibrin matrix with a more durable type 3 collagen matrix, which in turn facilitates the budding and growth of new blood vessels (angiogenesis), that is driven by macrophage factors such as VEGF.

The ability of leukocytes to generate new growth factors overtime becomes critical at this point in the healing cascade to replenish the pool of growth factors that were originally released during the inflammatory phase.[7,10,19]

The establishment of healthy granulation tissue marks the culmination of the proliferation phase. Granulation tissue is made up of primarily fibroblasts and new blood vessels.[7–11,40] Provisional matrix deposition working in parallel with the formation of new blood vessels (angiogenesis), drive tissue repair during the proliferation phase.[10,56] Oxygen is critical in the formation of granulation tissue[10,57] and is a key rate-limiting step in the healing cascade. Macrophage-derived growth factors TGF-b1, PDGF, VEGF, and FGF have all been shown to influence developing capillaries and angiogenesis.[7–9,20,40,41,56,57]

Within the wound, type III collagen is produced from fibroblasts, providing a weaker and less extensively cross-linked tissue matrix, then type I collagen is found in uninjured or mature repaired tissue.[10,56] Type III collagen will be replaced in the matrix by type I collagen as healing progresses from proliferation to the remodeling phase.[7,9,20,26,40,56]

Fibroblasts are driven by macrophage signals (TGF-b1) at the end of the proliferation phase to transdifferentiate into myofibroblasts,[10,58–61] which are specialized cells that generate new matrix and become contractile through the expression of smooth muscle actin. Contraction is important because it provides mechanical strength to the granulation tissue reducing wound size.[59–61] The TGF-b1 autocrine signaling augments collagen I production from the myofibroblast. New collagen I fibers are deposited in bundles aligning with the direction of myofibroblast contractile forces, strengthening and reinforcing the tissue to resist mechanical shear stress.[10,59–61] The proliferation phase transitions to the remodeling phase when granulation tissue matures into a scar[59,62,63] after myofibroblasts degrade the provisional type 3 collagen matrix.[10,59,62,63]

REMODELING PHASE

The *remodeling phase* is the longest phase of the healing cascade, beginning several weeks after the initial injury and may last months to more than a year depending on the severity of the initial injury.[7,9,10,41,43,55]

Fibroblasts are responsible for remodeling, replacing the type 3 collagen matrix with the stronger type 1 collagen matrix. Fibroblasts either die through apoptosis or differentiate into myofibroblasts that align to the direction of force within the tissue. Failure to properly transition from the proliferation phase may lead to excessive or hypertrophic scarring.[10,11,54,58,62]

During the modeling phase, collagen is refined, and its associated extracellular matrix is refined. Healing collagen synthesis and destruction both occur at a greater rate compared with normal tissue.[10,59–61,64] Collagen fibers and ligament matrix components undergo nearly continuous remodeling to promote strong ligamentous growth. Ultimately, ligaments heal with fibrovascular scar, which possess inferior biomechanical and mechanical properties compared with native structures.

Ultimately, collagen realignment restores strength and function to the repair tissue, which evolves into a mature and relatively acellular and avascular scar.[10,64] Overall, the normal outcome of the wound healing cascade is a mature scar and functional tissue, with around 80% of the strength of the original tissue.[10,11,58,59,64]

Cellular cooperativity is important for the execution of each phase of the healing cascade. None of the components of whole blood functions alone in the normal physiology of wound healing.[10]

INTRA-ARTICULAR AND EXTRA-ARTICULAR LIGAMENTS

Studies have shown variability in the potential for healing capabilities between intra-articular and extra-articular structures. The differences in repair potential may be related to the differences in the mechanical stabilization and microenvironment surrounding each ligament. In the knee for example, the ACL is surrounded by synovial fluid, whereas the medial collateral ligament (MCL) is an extra-articular structure and not necessarily influenced directly by synovial fluid.[55,65] Synovial fluid has been shown to prevent clot formation at the injury site of ACL injury, restricts the release of growth factors, limiting its ability to form a provisional scaffold to initiate self-repair.[66–68]

Elbow–Ulnar Collateral Ligament

Injury to the UCL in throwing athletes, particular baseball pitchers, are potentially career ending. At one time, injury of the UCL was predominantly diagnosed in high-level collegiate or professional throwing athletes; however, there has been a dramatic increase in the number of elbow UCL injuries diagnosed in younger adolescent, high school and collegiate athletes. In professional baseball, an estimated one-third of professional baseball pitchers have undergone surgical reconstruction of the UCL.[69,70] With modern surgical treatment, athletes can return to play at their previous level of competition or higher with a low rate of complications but it is a season-ending procedure requiring a prolonged period of rehabilitation, with an estimated return to play of 1 to 2 years.[71–74]

The literature has almost exclusively addressed surgical treatment techniques for UCL injuries such as the Tommy John procedure first performed by Dr Frank Jobe in 1974. Since Jobe and colleagues[75] published their original article describing UCL reconstruction in 1986, many surgical techniques for reconstructing, repairing, and now repairing with augmentation have been described.[71,76,77] The UCL reconstruction has been considered the gold standard of surgical repair but unfortunately, requires a prolonged rehabilitation and recovery period ranging from 1 to 2 years with reported return to play rates between 53% and 90%.[78–85] In general, UCL surgery, reconstruction, repair or repair with augmentation has been reserved for patients with complete or partial UCL tears that have failed nonoperative treatment.[86] However, disparity in the literature exists regarding postoperative UCL reconstruction outcomes. It has been reported that 3% to 40% of surgical reconstructions result in complications.[72,78,84]

Nonoperative treatment of UCL injuries historically has not shown optimal long-term outcome. Rettig and colleagues[87] reported on the 31 athletes with UCL insufficiency, treated nonoperatively with 3 months of rest and exercise with 42% returned to throwing. Classically, nonoperative treatment emphasizes activity modification, correcting ROM deficits, muscle imbalances, scapula mobility and stability, and kinetic chain strengthening.

Treatment of UCL injuries with orthobiologic or cell-based regimens that are capable of reliably returning athletes to play, quickly, and without resorting to season-ending reconstruction have become increasingly desirable.

Improvements in diagnostic imaging has led to improved ability to diagnose partial UCL injuries, thus improving our ability to determine who might better respond to nonoperative treatments. With appropriate patient selection in addition to potentially improving the biological characteristics of the injured tissue, cell-based therapies have gained interest by clinicians and investigators. Cell-based therapies have demonstrated promising healing benefits for the treatment of ligament and various other musculoskeletal injuries.[2,86] PRP has been used as a biologic treatment to enhance tissue and ligament repair, with evidence suggesting pain reduction, accelerated ligament repair and quicker return to function.[2,86,88–91] However, other investigators have found minimal to no benefit using PRP specifically to treat ligament healing after ACL reconstruction (ACL-R).[2,92–94]

Understanding the UCL anatomy, vascular supply, kinematics, and location of the UCL injury, assists in predicting outcomes, for example, why proximal injuries heal better and why distal lesions are more likely to do poorly with nonoperative care.[95,96]

Before Podesta and colleagues[86] 2013 publication, there was no literature regarding the application of orthobiologics therapies for the treatment of UCL injuries in throwing athletes. Before 2010, there was significant variability reported in the literature regarding treatment techniques and orthobiologic content used to treat soft tissue injuries including tendons and ligaments.

Podesta and colleagues[86] performed a prospective study evaluating 34 overhead athletes with partial UCL tears diagnosed by physical examination and confirmed by MRI, and dynamic ultrasound examination. They hypothesized that a single treatment with LR-PRP with a higher platelet and leukocyte concentration would be sufficient to treat the UCL in the thrower, stimulate adequate ligament healing to allow safe return to the same level of function and competition or higher. All patients had failed at least 2 months of conservative treatment and failed an attempt to return to play. Each patient underwent a single intraligamentous UCL LR-PRP injection under ultrasound guidance at the site of injury. LR-PRP was chosen for its increased platelet concentration of 5 to 6 times baseline and increased leukocyte and growth factor concentrations. They successfully determined that a single intraligamentous LR-PRP injection (88%) was sufficient to heal the UCL in the thrower, allowing them to return to the same level of competition in 12 to 15 weeks after injection and at 70-week follow-up (**Box 1**).

Several other clinical studies using biologic adjunct therapies for the nonoperative treatment of UCL injuries have been reported with promising but variable results. Dines and colleagues[69] reported on 44 competitive baseball players treated with 1 to 3 autologous conditioned plasma (ACP; Arthrex, Inc.), injections with lower reported platelet and leukocyte concentration. They reported excellent results in 15 of the 44 players (34%), 17 good, 2 fair, and 10 poor outcomes.

Another study by Deal and colleagues[71] performed a series of 2 LR-PRP injections on 23 UCL injured patients, spaced 2 weeks apart, followed by posttreatment unloader bracing, structured entire body kinetic chain physical therapy, and a structured

Box 1
Ten-year platelet-rich plasma follow-up Podesta[86,97–99]

Long-Term Follow-up-UCL Injuries Treated with LR-PRP 2010-2020

- Since the 2013 AJSM publication, questions remain regarding:
  - Treatment efficacy, long-term viability

Reviewed long-term outcomes of our original 30 patients.
- From 2010 to 2020
- No one lost to follow-up

Series of questions:
1. Where you able to continue to play competitively?
   a. For how long?
   b. What level?
2. Any recurrences of medial elbow pain or UCL injury?
3. When did you stop playing or retire from baseball?
   a. For what reason?

94% 29/30 continued to play for a minimum of 4 y.

34.5% 10/29 continued to play for 8 y.

No new UCL tears or pain recurrence

100% continued to play recreationally

1 player sustained a shoulder injury

None stopped playing or retired due to UCL injury

return-to-throwing program. Ninety-six percent of patients were able to return to play and demonstrated MRI evidence of healing at 6 weeks, 2 of 3 patients that failed PRP therapy had previous UCL surgery.

Questions regarding treatment efficacy and viability over time continue to exist regarding the treatment of UCL injuries with cell-based therapies (**Table 2**).

In 2020, Podesta[97–99] reviewed the long-term outcomes of his original 30 patients who had returned to throw competitively following a single, LR-PRP injection from 2010 through 2020, reporting that 94% of patients were able to continue to throw at the same level of play competitively for more than 4 years, without recurrence of medial elbow pain or UCL injury; 38% continued to play for more than 8 years, at the same level of competition or better, none were forced to retire from baseball as a result of their UCL injuries, and 100% continued to play recreationally. One patient sustained a shoulder injury, after returning to play, forcing him to retire from baseball prematurely and concluded these results confirm that a single ultrasound-guided LR-PRP was a viable treatment and will hold up over time in the thrower (**Fig. 2**).

COMBINED THERAPIES

Can we improve nonoperative therapies in higher demand athletes with more severe UCL injuries, complete tears, or distal injuries? Unfortunately, there are no clinical studies published evaluating combined therapies including the addition of medicinal signaling cells (MSC) or autologous scaffolds in addition to high-platelet concentration LR-PRP for these more severe injuries.

We are currently studying the treatment of higher grade and distal UCL injuries in higher demand elite pitchers with a combination of a single high-platelet concentration LR-PRP combined with bone marrow-derived progenitor cells, with and without

Table 2
Key observations important to improve outcomes

LR-PRP vs LP-PRP	• Cellular cooperativity is important for execution of each phase of the healing cascade. (Plasma, plts, RBCs, WBCs all have important roles in tissue repair)
Deliverable platelets	• 1.5 to 4.0 $\times$ 10^5 platelets/μL
Treat the entire ligament	• Treat flexor-pronator tendon/flexor, pronator musculature if necessary
Protect the ligament early	• First 2 wk critical • Bracing for grade 2 lesions
Dynamic US examination	• Ligament heals in ~6 wk, joint space closes narrows[71,86]
Posttreatment rehab progression	• Rehab progression based on dynamic US measurements • Begin valgus loaded exercise after humeral-ulnar jt. space narrows and ligament heals

activation with autologous thrombin with greater than 5-year preliminary data showing promising clinical outcomes regarding ligament healing, elbow stabilization and return to sports[98–100] (**Box 2**).

Understanding the patient's specific circumstances and demands allows us to tailor an appropriate biologic graft specific for that injury and situation. Knowing the patient's age, severity, and chronicity of injury and applied demands are important and needs to be considered. **Box 3** describes the authors recommendations for success compiled during the past 22 years treating UCL injuries in overhand throwing athletes. In our opinion, they are also applicable to treatment of most other ligament injuries encountered as well.

KNEE

Anterior Cruciate Ligament and Medial Collateral Ligament

Knee ligament injuries frequently occur in both athletic and nonathletic populations. Injuries to both the anterior cruciate ligament and collateral ligaments of the knee remain a frequent occurrence and burden to the health-care system.[2] Comparisons between the MCL and ACL have revealed differences in healing capabilities and have been reported by several authors. The ACL is an intra-articular, extrasynovial ligament consisting of 2 separate bundles that serve to resist anterior translation of the tibia relative to the femur, it consists primarily of type 1 collagen, has elastic characteristics that assist maintaining its stability, has a poor blood supply, and is covered with synovial tissue and surrounded by synovial fluid.[54]

There has been significant interest in the use of orthobiologic therapies such as PRP, BMC, and adipose tissue to enhance tissue healing and ligament repair. Investigators have reported early evidence suggesting the combination of cellular molecular components of PRP may reduce pain, accelerate tissue repair, and expedite return the function. However, other investigators have found minimal to no benefit, particularly with applications to enhance surgical reconstruction due to the heterogeneity in clinical studies published.[2]

Data on the use of BMC in the treatment of ACL injuries in humans are limited; however, several case series have shown clinical evidence of improvements in ACL integrity and increased function in patients treated with percutaneous PRP and or BMC injection to the ACL to augment surgical repair or as a nonsurgical therapy.[99,101–107]

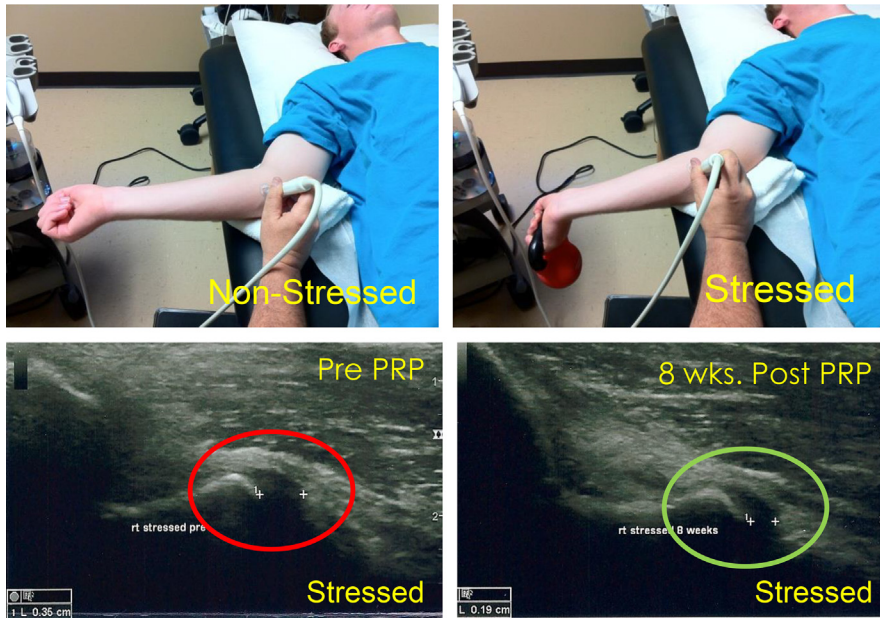

Fig. 2. Dynamic ultrasound imaging. The authors technique of dynamic ultrasound evaluation of the UCL with and without applied valgus stress. Ultrasound images of pretreatment and 8 weeks posttreatment showing normalization of the humeral-ulnar joint space distance secondary to UCL healing and tightening resulting in joint restabilization. (*From* Podesta L, Crow SA, Volkmer D, et al. Treatment of partial ulnar collateral ligament tears in the elbow with platelet-rich plasma. Am J Sports Med 2013;41(7): 1689–94.)

Gobbi and colleagues[101] evaluated 5-year clinical results of 58 athletes treated with ACL suture repair and PRP injection in addition to microfracture of the intercondylar notch. They reported 70% of patients return to sport activities, with a significant difference in anterior translation, in which side-to-side difference decreased from 4.1 mm (SD = 1.6) preoperatively to 1.4 mm (SD = 0.8) postoperatively ($P < .5$). Four players had sports-related retears, undergoing ACL-R within 2 years from the primary surgical repair, concluding that a PRP injection was effective in restoring knee stability and function in young athletes with acute partial ACL tears.[78]

Figueroa and colleagues[108] performed a systematic review of the literature on the use of PRP and ACL-R, finding only 11 clinical articles (516 patients) for inclusion observing different clinical studies showed an enhanced effect over the ligamentization (remodeling) of the intra-articular component of the ACL-R graft, with only 1 study showing improved integration.[104] Most of the investigations reported different PRP volumes and concentrations and failed to have validated methods and scores for measuring graft maturation. Only one study demonstrated a positive correlation with clinical evaluation, showing the PRP-treated patients had significantly better anterior posterior knee stability than patients without PRP.[105]

ACL-R remains the gold standard for treatment of ACL injuries particularly complete tears. There is emerging evidence supporting alternative treatments utilizing orthobiologic therapies alone or while augmenting surgical repairs of partial ACL lesions.[104] Preservation of the native ACL insertion site fibers and proprioceptive function may lead to better physiologic knee biomechanics.[105] Seijas and colleagues[107] reported

Box 2
Combined therapy platelet-rich plasma + bone marrow concentrate

Higher grade tears, higher demand and loads
- Acute and chronic
- Grade 2 partial tears (prox, mid, and distal)
- Calcified ligaments

31 pitchers (HS and College) 2015–present
- Average Age: 18.5 y (16–24 y)

LR-PRP (7% Hct) + BMC (7% Hct)
- ~2 mL, entire ligament, flex pronator fascia
- Single US-guided injection, UCL, FPT

Distal tears, Grade 2+, Grade 3 braced

Same rehabilitation progression

Preliminary results
Subjective outcome studies
- KJOC score, DASH, Dash Sports Module

Objective evaluation
- Dynamic US–Documented UCL healing and joint stability at 6 wk

31/31 Return to play 12 to 15 wk
- Return to play same level of play

Continue to pitch >5 y s/p tx
- Same or higher level of play

1 Failure (2 y, 4 mo out)
- Grew 6 in in height, increased fastball velocity 10 mph, increased repetitions, and work load

on 19 professional soccer players with partial ACL tears treated with intraligamentous and intra-articular PRP injections under direct arthroscopic guidance, with high return to sports and no complications. MRI evaluation showed complete ligamentization and satisfactory anatomic arrangement of all ACL remnant bundles at 1-year follow-up.

Kunze and colleagues[2] reported a systematic review of the basic science literature with protocol assessment reviewing the efficacy of PRP treatment of ligament injuries. They reviewed 43 articles (31 in vivo and 12 in vitro studies) investigating ACL/cranial cruciate ligament, MCL, suspensory ligament, patella ligament, and Hook ligament. They noted significant reporting variability in PRP regarding platelet concentrations, leukocyte composition, and red blood cell counts. With PRP treatments, 5 of 12 studies demonstrated significant increase in cell viability, 6 of 12 in gene expression, 14 of 32 in vivo studies reported superior ligament repair via histological evaluation, and 13 in vivo studies reported superior mechanical properties. In all articles reviewed, variability in PRP preparation methods was noted. Only 1 study reported all the necessary information to be classified by the 4 schemes used to evaluate reporting. Detection and performance biases were consistently high, although selection, attrition, reporting, and other biases were consistently low in in vivo studies. Concluding the observed conflicting data on the cellular and molecular effects of PRP for ligament injuries was secondary to study heterogenicity, limiting study interpretation and ability to draw meaningful conclusions.

Centeno and colleagues[109] have recently reported on the midterm analysis of their randomized controlled cross-over trial evaluating percutaneous, image-guided injection using a specific protocol of autologous BMC and platelets into partial or full-thickness, nonretracted ACL tears. The results suggest that autologous BMC injection

Box 3
Pearls for success in treating ligament injuries with orthobiologics

Tailor orthobiologic to patients' circumstances, injury severity, chronicity, loads
- Growth factors (GFs) or GF + Progenitor cells (MSCs),
  - Younger, lower demand, small, proximal-mid lig. Lesions–*LR-PRP (5–6+ x plt. conc.),*
  - Older, more demanding, larger or chronic tears–*LR-PRP (incr. plt. conc.); LR-PRP + BMC ± Autologous thrombin.*

Understand the anatomic location of the injury (proximal, mid, distal)?
- Distal lesions do worse, But! – LR-PRP (incr. plt. conc.), (LR-PRP + BMC)
  - Multiple injections
  - + autologous thrombin.

Protect the treated ligament
- Functional/unloader bracing (With ability to control ROM).

Guidance is extremely important!
- Needle guidance is critical,
- Orthobiologic placement needs to be precise for optimal results.

Injection technique
- Treat entire ligament, fenestrate, intraligamentous injection,
  - Flexor/Pronator Fascia-UCL when Perry ligamentous edema is present on imaging or in chronic cases,
  - Multiple treatments if there is no evidence of healing at 6 wk.

Activation with autologous thrombin
- Create a biologic scaffold,
- Stimulates the healing cascade, angiogenesis,
- Prevents run off.

Posttreatment rehabilitation progressions
- Dynamic imaging, MSK US, assists in determining safe rehab progression,
- Guides appropriate valgus exercise progression,
- Safe progression to throwing and athletic activity.

under fluoroscopic guidance into partial or full-thickness, nonretracted ACL tears resulted in improved patient function at 3 months when compared with exercise alone, and this treatment effect was sustained through 24 months across multiple functional outcome measures. MRI analysis was suggestive of interval ligament healing and maturation at 6 months.

Medial Collateral Ligament

The use of PRP for the treatment of knee MCL injuries has focused on enhancing nonoperative management, promoting healing with the goal to obtain a faster rehabilitation, and enabling quicker return to sports. Da Costa and colleagues,[110] and Yoshioka and colleagues,[111] studied the application of PRP to treat MCL injuries in rabbits, reporting accelerated ligament healing and improved structural properties after application of PRP.

The evidence for PRP to treatment of MCL tears is limited mostly to case reports. Zou[112] treated 52 patients with chronic pain for 3 months or greater after low-grade MCL injuries (6.5 ± 1.11 months) with 3 intra-articular PRP injections spaced 1 week apart. They reported superior Visual Analog Scores and International Knee Documentation Committee scores between pretreatment and posttreatment. Posttreatment MRI showed complete healing of proximal ligament injuries. Eirale and colleagues[113] reported on a case study of a professional soccer player with a grade II

Table 3
Ligament injury- exercise progression, goal, clinical rationale

Rehabilitation Phase	Criteria to Progress to This Phase	Anticipated Impairments and Functional Limitations	Intervention	Goal	Rationale
Phase I-II Hemostasis/ Inflammatory Phase Post Injection (0–7 d)	• Post Injection with no signs of infection	• Day 1–2: painful in the tissue/joint • Day 3–6: Diminishing pain and improving significantly • Day 7: Minimal pain, improved quality of ROM	Restrictions: ***Avoid all varus, valgus, A-P & rotational loads or ligament stressing activities/exercises x 7 wk*** • Tissue/joint specific protected bracing & weight bearing • No exercise except for rehab program • UE injections - no lifting > body wt. • Tylenol for pain • Heat pack for 15 min, 4x/day for 1–2 wk. • Avoid ice over treatment site • Shower ok 24 h after procedure • No submersion in water, bath, pool, hot tub or ocean for 1 wk. PT Progression: (Home Based) • Progress PROM to AROM, to point of	• Protect tissue • Allow biologic to absorb • Daily activity as tolerated within provided brace • Avoid excess loading or stress to treated area • Improve tissue vascularity and joint synovialization via gentle movement of extremity to improve • Avoid tissue overload or exercise unless approved by doctor	• Minimizes stress on injection site • Cross link initiation and homeostasis occurring as biologic activating to preparing for cross bridging

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Rehabilitation Phase	Criteria to Progress to This Phase	Anticipated Impairments and Functional Limitations	Intervention	Goal	Rationale
			initial resistance, within brace restraints, and only within physician ROM restraints • Gentle sub maximum Isometrics (lower to mid-range sub maximal holds) twice a daily		
Phase II Inflammatory Phase (8–14 d)	• No signs of infection **2–4 wk delay/slower progression with ligament injections due to decreased vascularization*	• Pain • Limited ROM ○ Pain with light UCL stress tests and ADL's ○ Limited UE strength	Restrictions: • ***Avoid all varus, valgus, A-P & rotational loads or ligament stressing activities/exercises x 7 wk*** • Tissue/joint specific protected bracing & weight bearing • No exercise except for rehab program • No concentric contractions or exercises to affected tissue except for unloaded ADLs and/or ambulation • For UE procedures, no lifting more than a dinner plate.	• Facilitate collagen deposition • Avoid homeostasis • Avoid disruption of collagen crosslink • Continue Phase 1 Rehabilitation recommendations • Consult physician regarding cross-training and return to exercise options • Improve tissue vascularity and joint synovialization by initiating upper body exercise if you had lower body procedure or LB exercise for UB	• Minimizes stress on injection site • Allow the PRP to absorb at the location • Prepare for cross bridging

			PT Progression: (Home Based) • Gradually progress AROM to point of initial resistance ○ Obtain > 90% full ROM by end of week 2 ○ Shoulder-AAROM to point of resistance • Continue Phase 1 exercises, (gentle submax. isometrics) • Gradually progress to full weight bearing with protective brace if applicable • Continue Heat pack as in Phase I		
Phase III Proliferative Phase (3–6 wk)	• Full pain-free ROM • No pain within sagittal plane functional mobility (Flexion/Extension, Dorsiflexion/ Plantarflexion)	• Limited UE/LE strength and cardiovascular endurance • Limited tissue tolerance to tensile loading exercises or functional activities • Pain (diminishing) • Limited tolerance with heavier lifting, pushing, pulling functional activities	Restrictions: ***Avoid all valgus loads or ligament stressing activities/exercises x 7 wk*** • Improve tissue vascularity and joint synovialization by initiating upper body cardiovascular exercise if you had lower body procedure or LB exercise for UB	• Protect tissue • Facilitate collagen deposition • Avoid disruption of collagen cross-link • Minimize deconditioning • *Communication among physician, physical therapist & patient is essential during this key transitional phase*	• Pain threshold significantly reduced • Collagen synthesis occurring, aligning in the longitudinal axis • Cross bridging occurring and matrix integrity improving • Tissue beginning to withstand tensile forces and loads • Use modalities to facilitation collagen formation & remodeling

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Rehabilitation Phase	Criteria to Progress to This Phase	Anticipated Impairments and Functional Limitations	Intervention	Goal	Rationale
			• Continue use of assisted devices as instructed by physician procedures • No over stressing of tissue through exercise or impact activity • No exercises except for supervised rehab program • *(3–6 wk)* PT Progression Overview: • Pain should not increase > 2 points on 10-point VAS Modalities for symptom control: • Moist heat, non-thermal ultrasound, cold laser, Russian stim, ES, Shock Wave Therapy (ESWT) Manual Therapy Techniques: • Gentle Soft Tissue Mobilization along the line of tissue fibers		• Soft tissue mobilization techniques have mechanical, physiological, histological, and neurological effects on the tissue which facilitate the healing mechanism and fiber alignment • Progress toward light ligamentous loading by end of phase III • Ligament tensile strength should be strong enough to initiate stress loading exercises • BFR enables strengthening utilizing a light load and a relatively low volume of work • Cardiovascular training to improve endurance & tissue repair

- Joint Mobilizations to maintain arthrokinematics

Therapeutic Exercises:

- AROM to point of initial resistance sub maximum to
- Max Isometrics
- Emphasize proper postural alignment, distal joint position

Adjust exercise progression based on severity of injury

- Initiate low resistance, high repetition, concentric, open chain exercise
- Initiate Blood Flow Restriction (BFR) exercises
- Initiation and progression of eccentric exercises as concentric strength increases

Neuromuscular Re-education:

- PNF & Rhythmic Stabilization exercises
- Proprioceptive training

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Rehabilitation Phase	Criteria to Progress to This Phase	Anticipated Impairments and Functional Limitations	Intervention	Goal	Rationale
			• Use of taping techniques as indicated for facilitation/ inhibition		
Phase IV Remodeling Phase (6–15 wk)	*Overlap of timelines is based on the patient's condition and severity of injury* • Pain-free ligament provocation & joint stability with stress testing • Demonstrate tissue integrity & joint stability with dynamic imaging • Subjective Functional Index Tool indicates patient is ready to progress through Phase IV to return to play status • Functional Testing performed to determine return to activity	• Limited UE strength • Limited ligament tensile strength during early phase IV • Limited joint proprioception • Altered timing and mechanics with sports specific & functional activities	Diagnostic imaging: • Diagnostic Ultrasound (~6–8 wk) to determine extent of healing and exercise progression and return to activity or sports status PT Progression: (Physical Therapy) Modalities: • Continue as needed Manual Therapy; • Continue Deep transverse friction mobilization/ massage to increase tissue vascularization and break up tissue adhesions Therapeutic Exercise: • Progress exercise and functional mobility integrating UE/LE CKC exercises as appriopriate	• Restore normal tissue integrity & fiber alignment • Maximize tissue vascularity and joint synovialization • Increase tissue tensile strength • Improve joint proprioception • Improve force production, tissue elasticity and ability to withstand tensile stretching ***Critical Decision Making Period- determine if tissue has sufficiently healed via dynamic imaging or if a second injection and/or surgical intervention is warranted* • Prepare for return to activity, sports	• Increased tensile strength of repaired tissue • Improved ability to produce force, withstand tensile stretching and increased elasticity • *Reassess Functional Index Score and dynamic imaging to correlate with objective exam findings to determine ligament healing, joint stability, and if exercise progression can continue or if a second injection and/ or surgical intervention is warranted*

- Progress eccentric exercise
- Progressive plyometric loading from <body weight to full body weight-bilateral to single
- Progress to ballistic, explosive training
- Sport specific training
  - ≤50% effort up to week 8
  - ≤Below 75% effort up to week 10
  - ≤Below 90% effort up to week 12
- Initiate Interval Sport Programs (Throwing, running, on field drills) pending results of Diagnostic US
- Return to sports 10–15 wk depending on the sport/ activity

Neuromuscular Reeducation:

- Light concentric resistance pulley or tubing patterns with controlled speed emphasis

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Rehabilitation Phase	Criteria to Progress to This Phase	Anticipated Impairments and Functional Limitations	Intervention	Goal	Rationale
			• Light Resistance PNF exercises performed manually using distal hand placements and initiating joint specific motions and adding pulleys or tubing/bands • Progress proprioception exercises to unstable surfaces **Week 6–7 Critical Decision Making Period** ***Dynamic imaging (MSK ultrasound) is utilized to confirm ligament healing, joint stability, and load progression.* • Initiate ligament and joint loading when healing and joint stability are determined, exercise progression is initiated.		

If sufficient healing and stability has not occurred at 6–7 wk, a second injection vs surgical stabilization may be warranted

Week 8–10: Progress to fast twitch and dynamic exercises

- Increase speed, resistance, and functional strengthening
- Add kinetic chain functional and sport specific loading progressions

**Pending repeated US imaging findings progress to return to play phase 4

Week 10–12:

Reassess Objective Exam results, Functional Testing, and Subjective Functional Tool Scores to determine return to higher level activity and/or sport-specific play

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Rehabilitation Phase	Criteria to Progress to This Phase	Anticipated Impairments and Functional Limitations	Intervention	Goal	Rationale
			• Begin interval Return to Sport program. Start interval throwing, batting, tennis serves, volleyball hitting programs pending repeat US imaging findings, *Weeks 12–16*: Progress from 75-90+ % in controlled setting. Gradual return to sport at 12–15 wk		

MCL tear treated conservatively with multiple PRP injections and rehabilitation. They reported symptom free return to play after 18 days with excellent functional outcomes. Radiographic imaging showed incomplete healing of the ligament; however, the athlete had no recurrence of injury or further complications at 16 months follow-up.

SUMMARY

Ligament integrity is extremely important in maintaining joint stability and homeostasis. Chronic ligament instability can lead to chronic pain, osteochondral injury, eventually leading to osteoarthritis. Ligament injuries have historically been treated surgically. However, for more than a decade, there has been increased interest in orthobiologic and cell-based therapies such as PRP and bone marrow–derived progenitor cells to treat ligament injuries particularly in the athlete, supported by promising preclinical and clinical data. Unfortunately, due to lack of reporting standardization in the current literature, conflicting data on the cellular and molecular effects of orthobiologic therapies for the treatment of ligament injuries exists, making it extremely difficult interpret or compare findings. The autologous orthobiologic or cell-based preparation used for treatment can influence the varying results reported in the literature. Therefore, to truly understand and compare results of these powerful therapies, reporting standardization, such as harvesting techniques, concentration techniques, a quantification of the delivered product (platelets, progenitor cells), formulations (leukocyte content), number of injections performed, activation, how injections are performed (guided vs unguided), in addition to the posttreatment rehabilitation process, are all important and necessary to evaluate and compare efficacy of future studies (**Table 3**).

DISCLOSURE

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