Platelet Rich Plasma (PRP) Matrix Grafts

PRP application techniques in musculoskeletal medicine utilize the concentrated healing components of a patient’s own blood—reintroduced into a specific site—to regenerate tissue and speed the healing process.

By David Crane, MD and Peter A.M. Everts, PhD

Platelet Rich Plasma (PRP) grafting techniques are now being utilized in musculoskeletal medicine with increasing frequency and effectiveness. Soft tissue injuries treated with PRP include tendonopathy, tendinosis, acute and chronic muscle strain, muscle fibrosis, ligamentous sprains, and joint capsular laxity. PRP has also been utilized to treat intra-articular injuries. Examples include arthritis, arthrosebrosis, articular cartilage defects, meniscal injury, and chronic synovitis or joint inflammation.

Platelet Rich Plasma was first used in cardiac surgery by Ferrari et al. in 1987 as an autologous transfusion component after an open heart operation to avoid homologous blood product transfusion.1 It is now being utilized by musculoskeletal (MSK) providers following the effective use in multiple specialties. PRP has also been successfully used in various specialties such as maxillofacial, cosmetic, spine, orthopedic, podiatric and for general wound healing.2,3

MSK practitioners began using PRP for tendonosis and tendinosis in the early 1990s.4 PRP techniques have most commonly been applied by MSK practitioners previously trained in the use of—and on the knowledge backbone of—prolotherapy. Although there is a paucity of well designed, randomized trials for its use in MSK medicine, animal studies, case reports, and anecdotal evidence suggests that this technique will continue to develop as a way to regenerate tissue that has lost its inherent homeostasis and thereby relieve associated pain and dysfunction.

Constituents and Properties of an Effective Regenerative Graft

Normal tissue homeostasis is maintained in a prescribed physiologic manner. These stages will be reviewed from a hypothetical time of injury through the healing phase to understand how to maximize PRP graft matrix preparation. Platelets contain two unique types of granules—the alpha-granules and dense granules.

Alpha-granules contain a variety of hemostatic proteins (coagulation proteins), as well as growth factors, cytokines,
chemokines (pro-inflammatory activation-inducible cytokines) and other proteins such as adhesion proteins. Of primary interest to the clinician are the three adhesion molecules and seven growth factors present in the alpha granule. Dense granules contain factors that promote platelet aggregation (ADP calcium, serotonin). Cell activation of platelets causes the discharge of granule contents. In other words, platelets require activation in order to begin the cascade of events that lead to collagen restoration and growth. This activation must occur at the tissue level (where the platelets aggregate and adhere to collagen at the site of grafting).

A synopsis of the various growth factors in PRP, together with their source and function, is presented in Table 1. A PRP Matrix Graft is made in a clinical or operative setting by using one of the several available table-top machines on the market. Several authors offer reviews of available graft preparation centrifuges and their ability to concentrate growth factors. Each machine has a separate, disposable unit that concentrates platelets in a small amount of plasma. A thin layer of platelets is found immediately above the leukocytes in the buffy coat of centrifuged blood. When a concentrated platelet portion is made, theuffy coat containing elevated levels of leukocytes—along with concentrated platelets—are suspended in a small amount of plasma for subsequent grafting. The clinician hopes that the platelets are not activated and remain suspended until grafting and contact with thrombin or collagen occurs.

### Necessary Stages of Healing
Normal platelet activation leads to three necessary stages of healing: Inflammation, Proliferation, and Remodeling. The cellular components involved in the three phases of healing are depicted in Figure 1. If any of these stages are incomplete—or if they proceed unabated—tissue homeostasis is lost and pain and loss of function may result. Most reviews on this topic focus on only the growth factors contained within the alpha granule of the platelet which is released upon platelet activation. It is important to understand, however, that if the platelets aren’t suspended with biologic levels of other constituents of plasma—such as leukocytes, cytokines, and fibrin (the matrix)—the graft is either not effective or less effective. If fibrin levels are too high, or platelet activation occurs prior to collagen binding, the graft is also inhibited. Other functions of platelet activation and the subsequent cascade of events that occur include cytokine signaling, chemokine release, and mitogenesis.

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<td>Platelets, extracellular matrix of bone, cartilage matrix, activated TH1 cells and natural killer cells, macrophages/macrophages/neutrophils</td>
<td>Stimulates undifferentiated mesenchymal cell proliferation; regulates endothelial, fibroblastic and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits macrophage and lymphocyte proliferation</td>
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<td>Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells</td>
<td>Mitogenic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/endothelial/smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis</td>
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**Figure 1.** The physiology of healing of the chronic wound. From: emedicine.com. Used with permission.

**TABLE 1. SYNOPSIS OF GROWTH FACTORS PRESENT IN PRP**

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**TABLE 1. Synopsis of growth factors present in PRP** From Peter A.M. Everts et al. Platelet-Rich Plasma and Platelet Gel: A Review.
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Inflammatory Phase
During the inflammatory phase, the functions of activated platelets include:
- Anti-microbial
- Adhesion
- Aggregation
- Clot retraction
- Pro-coagulation
- Cytokine signaling
- Chemokine release
- Growth factor release

There is now evidence to suggest that at certain concentrations, or dose response curves, platelet rich plasma grafts may be anti-inflammatory or pro-inflammatory in certain tissues. A dose response relationship exists to a currently unknown level of PRP concentration and ensuing migration and proliferation of progenitor stem cells at the tissue injury site (see Figure 2).

There is emerging evidence to suggest that PRP grafts in the four- to six-fold range (10^6 platelets) have more anti-inflammatory mediators and effects and are clinically relevant and useful for most situations. PRP grafts in the eight- to thirteen-fold range may be pro-inflammatory in nature. Further elucidation of this effect is required, however, as some studies showed beneficial effects of higher concentrations of PRP.

Hesham El-Sharkawy et al. evaluated this effect in periodontal tissue. The conclusions were that PRP is a rich source of growth factors and promoted significant changes in monocyte-mediated proinflammatory cytokine/chemokine release. LXA4 was increased in PRP, suggesting that PRP may suppress cytokine release, limit inflammation, and thereby promote tissue regeneration.

Weibrich et al. observed an advantageous effect with platelet concentrations of approximately 106/μL. Further, they state that higher concentrations might have a paradoxically inhibitory effect.

Following the initial inflammatory phase, which typically lasts for two to three days, fibroblasts enter the site and begin the proliferative phase. Low pH and low oxygen levels stimulate fibroblast proliferation in the injury site. Fibroblasts become the most abundant cell by the seventh day. The fibroblasts are then responsible for deposition of collagen and ground substance. This phase lasts from two to four weeks. As these are primarily the deficient cells with chronic injury (lack of normal collagen in extracellular matrix), this stage is mandatory for MSK repair.

The Proliferative Phase
During the proliferative phase—peaking anywhere from day 5 to 15 and which can last for weeks—fibroblasts differentiate into myofibroblasts and actin contracts to make the wound smaller. Low pH and hypoxemia also stimulates neovascularization. Neovessels begin to form at approximately day 5 to 7 and this process proceeds until the neovessels disappear near completion of the remodeling phase.

The Remodeling Phase
During the remodeling phase, collagen matures and strengthens. Tissue repair starts when the production and breakdown of collagen equalizes. This phase can last over one year. During this period, type III collagen is replaced by type I collagen, reorganization occurs, and the blood neovessels disappear.

Cell Proliferation Triangle
It has become apparent, then, that PRP grafts function via a triad of interactions, known as the cell proliferation triangle (see Figure 3). Each element of this triangle must be present for effective tissue repair and pain relief.

When preparing a graft for clinical use, the constituents of each of these three must be considered—i.e. is there an inherent matrix to place the graft in, or will the graft be washed away with motion, synovial fluid, or repeated graft compression or distraction? Does the patient have an adequate response for inflammation and is there an adequate quantity of platelets to concentrate for progenitor cell mitogenesis and proliferation?

Biotensegrity—A Construct for Regeneration of Tissue
Biotensegrity refers to a dynamic construct of compressive and tensional forces acting on, and through, multiple levels of organization to maintain or repair tissue homeostasis. Biotensegrity, then, is a repeated pattern of structural and functional architecture of all living tissue.

The probable link though all levels of biotensegrity is the vascular endothelial system with its regenerative and neuroendocrine functions as subsequently described.
Endothelial cells line the lumen of all blood vessels as a single squamous epithelial cell layer. They are derived from angioblasts and hemangioblasts. Weibel-Palade bodies are specialized secretory granules found in endothelial cells. These vesicles store preformed hormones, cytokines, and growth factors; as well as enzymes, receptors, and adhesion molecules; which can be released and/or expressed on the cell surface without de novo protein syntheses by regulated exocytosis in response to stimulation of cell activation.6

Thus, the authors believe there is sufficient evidence to suggest that the vascular endothelial system links all of the biotensegrity levels together as the various factors are at work up and down the scale.

Contraindications to the Use of PRP Matrix Grafts

Absolute Contraindications include:

- Platelet dysfunction syndrome
- Critical thrombocytopenia
- Hypofibrinogenemia
- Hemodynamic instability
- Septicemia
- Sensitivity to bovine thrombin (if using bovine thrombin with calcium to make platelet gel)

Relative Contraindications include:

- Consistent use (anti-inflammatory use) of NSAID’s within 48 hours of procedure
- Corticosteroid injection at treatment site or systemic use of corticosteroids within 2 weeks of graft procedure
- Recent fever or illness
- Rash at graft donor site or at receptor site
- Cancer — especially hematopoietic or of bone
- Active history or history of Pseudomonas, Enterococcus or Klebsiella infection, as PRP has been shown in one study to potentially stimulate these pathogens.22
- HGB < 10 g/dl
- Platelet count less than 105/µL

Risk Involved With the Use of PRP Matrix Grafts

While there have been no reports of worsened pain or function following tissue maturation that the authors could find, few randomized, placebo controlled trials exist regarding the utilization of these grafts. In the primary author’s experience of performing approximately 20 to 30 cases of percutaneous PRP Matrix Grafts per week for the last two years, no patients reported worsened pain or function. It is felt by the authors—and often expressed in the available literature—that this procedure technique is safe and effective.

Pain at the treatment site is common for a short period following injection. One of the primary author’s patients reported worsened pain for six months at a treated lateral epicondyle. This subsequently resolved and has been absent for over one year. This stresses the fact that remodeling of the tissue is necessary to see the effects of therapy. No tendon rupture or partial rupture was noted and the authors can find no reports of tendon or ligament rupture following PRP. In fact, Olena Virchenko and Per Aspenberg noted, in a rat achilles tendon transection model, that one postoperative injection resulted in increased strength after four weeks. This effect was obliterated with the use of botox at the site.33

Other risks that may occur at time of injection include injury from pain-induced syncope. Indeed, the main complaint received from patients is the injection pain of the PRP. There is also the risk of limb injury following the graft procedure since local or regional anesthesia is used at the time of procedure. The primary author had a patient who stepped from a ladder about four hours following an achilles and peroneal tendon injection, with subsequent inversion and fracture of the ankle—most likely due to proprioceptive and sensory loss from anesthesia.

As with any percutaneous needle technique, there is a slight risk of puncturing a hollow organ or infection, but this risk is not expected to be above or below that of other needle techniques employed in clinical medicine. The accepted risk of introduction of infection with percutaneous techniques has been reported as 1:50,000 injections. Since PRP is an autologous preparation, the risk of introducing foreign material and the risk of transmissible infection or allergic reaction is effectively eliminated—although the entire procedure must be carried out in sterile conditions. PRP—with its initial inflammatory phase—is also bacteriocidal, particularly against Staphloccoccus aureus and Escherichia coli as shown by Bielecki et al.22 The temporary formation of platelet and fibrin plugs at the wound site has also been noted to prevent the entry of microorganisms.22 However, PRP gel seems to induce the in vitro growth of Ps aeruginosa, suggesting that it may cause an exacerbation of infections with this organism. There was no activity against Klebsiella pneumoniae or Enterococcus faecalis.

Other considerations come into play if the procedure is not performed with completely autologous preparations. PRP gel techniques that rely upon the use of bovine thrombin, which may contain contaminants like bovine Factor Va as a platelet activation source, may result in antibodies to Factors V and VI, with potentially life threatening coagulopathies resulting. Other concerns with bovine thrombin include prion disease, although none are reported in the literature. The authors have neither seen nor heard of any infections occurring with the percutaneous use of PRP or biocellular therapeutic grafts.

Regarding the question of carcinogenesis, growth factors act on cell surface receptors only, do not enter the cell, and do not cause DNA mutation. There is no plausible mechanism by which growth factors would result in neoplastic development, and there have been no reports of this in the literature. Furthermore, Scott and Pawson showed that growth factors (PGF) activate normal, rather than abnormal, gene expression.23

Typical Treatment Regimen With PRP Consent

- Average series of injections is two to three at four- to six-week intervals
- Different sites or areas of treatment may expand or contract with further treatment
- You must functionally retrain the kinetic chain once the tissue has undergone some degree of healing

Risks

- 1:50,000 chance of introducing infection with injection procedure
- Allergy to local anesthetic(s)
- Syncope with pain/blood at the time of injection
- Injury occurrence with numbness or pain following procedure. i.e. falling, ankle sprain with inversion, etc.
- Though extremely rare, pain or function may worsen
- Puncture of tissue outside of intended graft site. i.e. vascular, neural, lung, or other tissue placements
Technique for Myotendinous or Tenosseous Sites
- Alcohol or Betadyne prep (we prefer Betadyne gel when using an ultrasound probe for 'live' injection guidance)
- +/- Ethyl Chloride spray
- Inject PRP with approximately 1cc PRP per cm² of tissue/interface
- Important to touch bone and ‘pepper’ the area of teno-osseous junction to stimulate the greatest number of fibroblast colonies
- For myotendinous sites use ultrasound to ensure layered treatment throughout the tendon
- Sterile band-aid applied post injection
- Kinesiotape to protect motion if needed

Technique For Intra-Osseous Sites
- Alcohol or Betadyne prep (we prefer Betadyne gel when using an ultrasound probe for 'live' injection guidance)
- +/- Ethyl Chloride spray
- Local anesthetic either mixed with the PRP graft or to sites of tenderness to ‘road test’ the area prior to using the graft. This ensures that the PRP matrix graft is placed in the proper areas.
- Aspirate degenerative joint fluid prior to PRP matrix graft placement
- Gel the PRP or utilize other stabilizing matrix for intra-articular sites. Ligaments, tendons, and inherent matrix sites do not require gel in the authors’ experience
- 8-10cc PRP matrix graft is the typical amount used for a knee or shoulder joint in our clinic
- “Treat regionally, not locally” (D. Crane, MD; e.g. treat all of the capsule that is tender along with tendinous and ligamentous sites of tenderness in addition to the intra-articular capsule)

It should be noted that Kevy and Jacobson have evaluated the mixture of common local anesthetics with PRP and find no significant platelet activation or diminution of graft growth factor functions.

Tendonosis and the Use of PRP
Anitua showed—from in vitro studies of collagen and tendon—that autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. Mishra performed an in vitro study which determined the effect of a platelet concentrate medium on the proliferation of human skin fibroblasts—the cells responsible for deposition of collagen. Buffered PRP was shown to augment human fibroblast proliferation when compared to control.

Schnabel evaluated gene expression patterns, DNA, and collagen content of equine flexor digitorum tendons cultured in a media consisting of PRP and other blood products. PRP at 100% concentration stimulated the greatest number of collagen type I, collagen type III, and cartilage oligomeric protein (COMP) molecule genes without increasing expression of the pro-inflammatory matrix metalloproteinases. ELISA detected higher levels of PDGF and TGF-B in the PRP group.

Hesham El-Sharkawy et al. measured platelet derived growth factor (PDGF)-AB, PDGF-BB, transforming growth factor-b1, insulin-like growth factor-I, fibroblast growth factor-basic (FGF-b), epidermal growth factor (EGF), vascular endothelial growth factor, interleukin-12 (p40/70) and, regulated on activation, normal T-cell expressed and secreted (RANTES) levels by enzyme-linked immunosorbent assay. Cytokine, chemokine, and LXA₄ levels, as well as monocyte chemotactic migration, were analyzed. PRP led to significantly increased levels of growth factors and significantly suppressed inflammation by promoting secretion of LXA₄.

These growth factors stimulated the proliferation of fibroblasts and periodontal ligament cells, as well as extracellular matrix formation, and promoted collagen and total protein synthesis while stimulating the synthesis of hyaluronate from gingival fibroblasts. IGF-I levels in PRP in this study were not significantly different from the cyclolignan picropodophyllin (PPP), suggesting that other cell types could be responsible for the release of this growth factor.

Tissue culture studies performed by du Toit et al. for use in dermal regeneration confirmed the potent mitogenic stimulation of human fibroblasts, keratinocytes, chondrocytes, neural tissue, and myoblasts.

In Vivo Human Studies: Reviews and Case Examples

Tendon and Ligament Use of PRP
Mishra evaluated 20 patients that failed non-operative treatment for chronic epicondylar pain. These 20 patients were randomized to a single PRP injection or injection with bupivacaine. Mishra comments that the IRB would not allow a blood draw from the control patients to blind the study. All PRP patients had lower pain and greater ROM than control (bupivacaine). Eight weeks after the treatment, the platelet-rich plasma patients noted 60% improvement in their visual analog pain scores versus 16% improvement in control patients... At 6 months, the patients treated with platelet-rich plasma noted 81% improvement in their visual analog pain scores...”
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bands of the most affected plantar fascia with promising results. Seven out of nine patients had complete resolution of their plantar fascial pain at one year and all the patients in the study had improvement that was noted on diagnostic ultrasound. One of the patients was considered a failure because of a subsequent steroid injection even though all pain had resolved.30

Scarpone reported on a prospective study carried out in 14 patients with shoulder pain. The patients all had rotator cuff tears with no significant AC joint thickness with impingement and no other significant symptomatic pathology such as labral tears, glenohumeral arthritis, or gross instability. All of the patients failed non-operative treatments such as NSAIDs, physical therapy, and corticosteroid injections and all were considering surgical options. Of the 14 patients, 12 had statistically significant improvements in their pain scale and their strength and endurance at eight weeks. Of the 12 patients, six had radiographic evidence of healing of their tendinopathy on MRI at eight weeks. Of the four patients who were considering surgery because of persistent pain, only two went on to have rotator cuff surgery. No significant complications were noted.31

Ventura et al. evaluated PRP in ACL repair. A total of 20 patients with anterior cruciate ligament (ACL) injuries were treated by quadrupled hamstring tendon graft (QHTG)—with or without PRP gel growth factor (GF) application. CT highlighted a significant difference (P<0.01) between ACL density of the two groups. CT densities of the ACL and posterior cruciate ligament (PCL) were similar in the GF-treated group. In the control group, however, the intensity of the signal was heterogeneous and the new ACL was not clearly identifiable with respect to the PCL. A different density of the ACL was also noted: in the GF-treated group this density was uniform and the new ACL was more structured, while in the control group the ligament was less structured and did not completely fill the femoral and tibial tunnels. In the PRP treated group, one patient had a synovitic reaction. On CT, the new ACL was increased and hypertrophic and surrounded by a soft-tissue reaction. MRI confirmed this finding.32

Sanchez reported on a case-control study of twelve athletes with complete achilles rupture. All twelve had open achilles repair; six had PRGF. The treatment group had no wound complications and experienced earlier functional restoration: ROM (7 vs. 11 wks), jogging (11 vs. 18 wks), and training (14 vs. 21 wks). The authors of this study measured IGF-1, TGF-B1, PDGF-AB, EDF, VEGF, and HGF and noted that the number of platelets held direct correlation to the level of growth factors.33

Case Example: Chronic Tendonopathy
A 63-year-old male ironman distance triathlete presented with a history of left achilles pain longer than three months. The patient had no relief with physical therapy or ultrasound (U/S) therapy for six weeks duration. The patient was diagnosed by MRI with stress fracture of the fibula with no discrete cortical line or fracture in addition to an achilles tendinopathy. Diagnostic U/S in our office showed an 8cm segment of tendon collagen change consistent with a tendinopathy with associated peritenon fibrosis (see Figure 4).
The patient undergoes three separate series of PRP at four-week intervals to the Achilles tendon and fibula along with the peroneal tendon sheath at the myotendinous junction. Subsequent ultrasounds show improved fibrosis and less scarring along with collagen pattern reorganization consistent with improved vascularity and tendon structure (see Figure 5).

The patient has greater than 90% pain reduction after the three PRP matrix grafts and returns to ironman distance racing after the three months of restricted training. Supportive compression sleeves are utilized for three months to allow for load distribution until strength in the peroneal muscles and achilles is 90% of the unaffected right side.

Muscle Strain and the Use of PRP
Sanchez reported a 20 patient prospective muscle injury pilot study with six-month follow-up. Ultrasound guided injection of PRP within the injured muscle enhanced healing (echo-graphic images) and functional capacities 50% faster than the control group.

Case Example: Quadriceps VMO Muscle Strain
A 56-year-old male presented with right thigh pain occurring for approximately one year. The pain is worse on the bike and, in fact, is more prevalent when seated and pushing large gears or uphill climbing. The patient has no significant pain with running. The patient is an ironman distance triathlete and remembers no injury of significance one year ago at onset. Ultrasound shows a vastus medialis injury/strain pattern with associated fiber tearing and fibrosis. This is near the VMO myotendinous junction at the right knee (see Figure 6). No evidence of knee pathology is noted on physical exam or on ultrasound. Palpable tenderness exists at the strain site on the medial thigh. Pain is also reproduced on eccentric loading of the VMO muscle group. No improvement had been obtained previously with three weeks of NSAID use, physical therapy, or myofascial therapy work.

The patient undergoes a single injection of PRP (4cc) along with 1cc of injectable collagen for matrix stabilization at two discrete sites in the VMO muscle with ultrasound guidance (see Figure 7).

The patient's pain after one month is more than 80% resolved and the patient has no pain on the bike or with activity as previously noted. Resumption of training occurred one week following injection with swimming, running, and protected cycling.

Articular Cartilage and the Intra-Articular Use of PRP
Everts, Devilee, et al. reported that autologous platelet gel and fibrin sealant enhance the efficacy of total knee arthroplasty by improved range of motion, decreased length of stay, and a reduced incidence of arthrofibrosis. Everts’ team also investigated whether the use of autologous derived platelet gel and fibrin sealant would reduce postoperative blood loss, decrease the impaired range of motion, and reduce the incidence of arthrofibrosis. Study group patients (n=85) were treated with the application of autologous platelet gel and fibrin sealant at the end of surgery. Eighty patients were operated without the use of platelet gel and fibrin sealant and served as the control group. During a five-month postoperative period, patients were followed to observe the incidence of arthrofibrosis. In patients in the treatment group, the hemoglobin concentration in blood decreased significantly less when compared to the control group. They also showed a superior postoperative range of motion when compared to those of the control group (P<0.001). The incidence of arthrofibrosis and subsequent forced manipulation was significantly less (P<0.001) in patients managed with platelet gel and fibrin sealant.

Case Example: Severe Hip Osteoarthritis With a History of Congenital Hip Dysplasia
A 56-year-old female presented with increasing left hip pain greater than one year duration. The patient has a history of bilateral hip dislocations at birth (birth country Poland — no x-rays available) with evidence of shallow acetabular deformity noted on x-ray (see Figure 8).

The patient is active in dance and is of normal weight and BMI. Some relief is obtained with NSAID therapy but pain is now affecting sleep and is interfering with activities of daily living and her dance regimen. The patient undergoes one PRP injection to the left hip using an anterior approach. 8cc PRP is placed with ultrasound guidance as noted (see Figure 9).

Figure 7. U/S pictures (2) post injection with PRP and gel matrix at the myotendinous junction and the insertion.

Figure 8. X-ray of pelvis and affected left hip pre-treatment with PRP matrix graft.

Figure 9. X-ray of left hip following PRP matrix graft series. X-ray shows subtle smoothing of the irregular femoral head surface.
After 3 months, the patient reports 75% pain improvement and some improvement in ROM is also reported. The night pain has resolved and the patient’s pain is controlled with acetaminophen. She is able to resume dance and activities for fitness and health.

**Bone and Periosteal Use of PRP**

Gandhi et al. observed normalized cellular proliferation and chondrogenesis with an improved mechanical strength when PRP was injected percutaneously in a diabetic experimental femur fracture model.36

Sanchez et al. utilized PRP after reattachment of a large (2 cm) loose chondral body in its crater in the medial femoral condyle. Autologous plasma (PRP) was injected into the area between the crater and the fixed fragment. They state that complete articular cartilage healing was considerably accelerated, and the functional outcome was excellent, allowing a rapid resumption of symptom-free athletic activity.37

PRP has been used successfully in maxillofacial surgery in several studies including a randomized trial of 88 patients with mandibular defects treated with cancellous cellular marrow grafts with, or without, PRP. Grafts with PRP showed twice the radiographic maturity at six months follow up.2

Another case report describes a fifty-year old woman with nonunion of humerus who had undergone two unsuccessful operations. Union was obtained by the use of autologous platelet-rich gel (PRG). At the 8th week, over 75% of the circumference of the bone at the defect site had resolved and, during later visits, remodeling of the union was observed on X-ray films and DEXA examinations. Maximum healing was reached at the 18th week. Twelve months after PRG injection, the intramedullary nail that had previously been placed was removed.38

**Case Example: Bilateral Pars Interarticularis Stress Fractures (Spondyloysis)**

A 14-year-old softball player presented with a history of developing back pain over a period of six weeks, made worse following a minor motor vehicle crash four weeks prior to visit. The patient had initial pain and localized tenderness on the right low back L4-5 area with a positive stork test. X-ray and MRI confirm spondyloysis (see Figure 10).

The patient undergoes extensive physical therapy for approximately 8 months with subsequent relief. The patient then returns to sport specific activity but develops pain. After appropriate discussion of the benefits and risks, a PRP matrix graft is placed on the right L5-S1 facet joint and the L5 pars with ultrasound guidance. On return to activity, the patient notes the absence of pain on the right pars or low back area. The patient is allowed to slowly return to activity. Two months following the initial PRP graft, the patient develops pain in the opposite, left lumbar area after repeated throwing drills. A repeat MRI shows a left sided spondylolysis. No listhesis is appreciated. Evidence of healing is noted on the right pars stress fracture to a small degree (see Figure 11).

A PRP matrix graft—with a total 8cc PRP at a six-fold concentration and mixed with 2 cc 50:50 lidocaine 1% with marcaine 0.5%—is then placed an additional X3 on the right and X3 on the left, with approximately 5cc placed at the lev-
els of the L5 pars as well as the accompanying facet joints. The patient is started on physical therapy at two weeks into the graft injection series with progression at 6 weeks to pilates therapy and then sport specific activity with heavy focus on the mechanics of core stabilization and kinetic chain reintegration. A repeat MRI is obtained two months following PRP (see Figure 12).

Figure 12 shows interval slight healing of the fracture sites. The patient has not developed any reoccurrence of pain and is back to softball activities with no bracing. No tenderness remains at the prior fracture sites on physical exam.

In Vivo Studies: Skin Healing, Range of Motion, and Pain With the Use of PRP

A prospective, single-blind pilot study comprising 80 full-thickness skin punch wounds (4mm diameter) was conducted on the thighs of eight healthy volunteers. With each subject serving as his or her own control (five punch sites per leg), PRP was applied topically on one thigh, while an antibiotic ointment and/or a semi-occlusive dressing was applied on the other thigh. On day 17, the percentage of closure was 81.1% for the PRP-treated sites and 57.2% for the control sites. Also, the PRP wound closure velocities were significantly faster than those of the controls (P=.001). When the platelet count in the gel was more than six times the baseline intravascular platelet count in some subjects, epithelialization and granulation formation appeared three days earlier in the PRP-treated group.49

Everts et al. noted improved wound healing when platelet leukocyte gel was applied during wound closure after total knee arthroplasty.9

In a study examining PRP gel for diabetic foot ulcers, Driver et al. noted that 13 of 19 patients in the study group (68.4%) had complete healing, compared with only 9 of 21 (42.1%) of the control group (saline gel). This study was a prospective, randomized, controlled trial with both groups receiving a blood draw for blinding purposes. The treating providers and patients were blinded to the gel applied. It should be noted that no treatment serious adverse events were reported and bovine thrombin used for PRP gel did not cause any Factor V inhibition.10

In another study from Everts et al., platelet leukocyte gel (PLG) was injected in the subacromial space during wound closure in patients who underwent an open subacromial decompression.41 In the PLG-treated patients, a decrease in the VAS pain score was observed (P<0.001) compared to the non-treated patients. Consequently, the use of pain medication was significantly less (P<0.001) in PLG-treated patients. Furthermore, treated patients demonstrated a significantly improved range of motion earlier after surgery with a high shoulder functional index. A significant reduction in pain was also observed after PRP use by Fanning et al. after applications in gynecologic surgery42; Gardner and co-workers following total knee replacement surgery43; and Crovetti and associates in patients with chronic wounds.44

Conclusion

PRP matrix grafts along with other biologic grafting techniques are becoming more prevalent in the treatment paradigms of musculoskeletal medicine. These PRP matrix grafts provide effective, safe, relatively low-cost treatment options to patients who have the time and wherewithal to allow collagen synthesis and maturation at the graft site. PRP matrix grafts appear to restore tissue homeostasis and biotensegrity of collagen. Other pain inhibiting effects are also present in PRP matrix grafts which allow earlier resumption of pain free activity. It is the authors' experiences that these grafts, along with other regenerative grafting options, are at times the only viable treatment option for a select group of patients with degenerative myofascial tissue injuries. The authors recommend appropriate first line therapies such as relative rest, appropriate bracing and kinesiotaping, evaluation of kinetic chain mechanics, and physical therapy—with or without eccentric loading protocols—prior to the utilization of these PRP matrix grafting protocols.

Reduction in pain after PRP applications has been observed by several authors. However, an explanation of this phenomenon has not always been given. The authors believe that serotonin released from activated platelets might be responsible for decreased pain, as described by Everts41 and Fanning.42 Except for the growth factors in the Alpha-granules, large amounts of serotonin45 are contained within the dense platelet granules. Since platelet counts of the PRP are generally almost six-fold higher when compared to whole blood levels, it stands to reason that serotonin levels are therefore also significantly increased at the wound site. This phenomena has been explained in detail by Sprott et al.46 who reported on pain reduction following acupuncture and measured a decrease in serotonin concentration in platelets from these patients and an increase in serotonin levels in plasma—suggesting normalization of plasma serotonin levels due to the mobilization of platelet serotonin.
Other grafting tools such as the use of autologous bone marrow aspirate stem cells (BMAC) with PRP matrices have not been explored in this article but may be explored in further detail by the authors. These stem cell/growth factor grafts are being utilized for severe degenerative states with associated tissue hypoxemia. Hence, PRP and other regenerative biocellular therapeutic matrices deserve further study to determine their effects in animal and human models.

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Peter A.M. Exerts, PhD heads the Department of Peri-Operative Blood Management at Catharina Hospital at Eindhoven, The Netherlands. He is currently the chairman of Foundation FERET which is the research leader in the PRP medical field. Dr. Exerts has done pioneering work involving autologous platelet rich plasma in both animal and human trials and wrote the 2007 thesis titled: Autologous Platelet-Leukocyte-Enriched Gel: Basics and Efficacy, which describes a novel method to support soft tissue and bone healing (ISBN 10:90-8590-016-6, ISBN 13:978-90-8590-016-0). Dr. Exerts may be contacted via email at exerts@elcive.nl.

References
10. Rivera J and Stephenson F. Personal communication with author. 03/06.
An Evidence-Based Model Comparing the Cost-effectiveness of Platelet-Rich Plasma Gel to Alternative Therapies for Patients with Nonhealing Diabetic Foot Ulcers

Edward J. Dougherty, MS

ABSTRACT

OBJECTIVE: A cost-effectiveness analysis compared the potential economic benefit of an autologous, platelet-rich plasma (PRP) gel to alternative therapies in treating nonhealing diabetic foot ulcers.

DESIGN: An economic model used peer-reviewed data to simulate clinical and cost outcomes and quality-adjusted life-years (QALYs) associated with PRP gel and other treatment modalities.

PATIENTS: The model varies rates of healing, recurrence, infection, amputation, death, and associated costs for a hypothetical group of 200,000 patients with full-thickness, nonhealing diabetic foot ulcers for 5 years or until death.

MAIN OUTCOME MEASURES: The model simulates the clinical, cost, and QALY outcomes associated with PRP gel versus other modalities in treating nonhealing diabetic foot ulcers over a 5-year period.

MAIN RESULTS: The average 5-year direct wound care cost per modality and QALYs were PRP gel, $15,159 (2.87); saline gel, $33,214 (2.70); standard of care, $40,073 (2.65); noncontact kilohertz ultrasound therapy, $32,659 (2.73); human fibroblast–derived dermal substitute, $40,569 (2.65); allogenic bilayered culture skin substitute, $24,374 (2.79); bilayered cellular matrix, $37,340 (2.71); negative pressure wound therapy, $20,964 (2.81); and recombinant human platelet-derived growth factor BB, $47,252 (2.69).

CONCLUSION: Use of PRP gel resulted in improved quality of life and lower cost of care over a 5-year period than other treatment modalities for nonhealing diabetic foot ulcers. Although actual treatment outcomes may differ from those modeled, PRP gel represents a potentially attractive treatment alternative for insurers and health care providers to address the cost burden and health effects of nonhealing diabetic foot ulcers.

INTRODUCTION

A cost-effectiveness analysis of a platelet-rich plasma (PRP) gel was developed using the published clinical data on the PRP gel and pricing information received from the manufacturer of PRP gel, as well as companies and distributors of alternative therapies, to evaluate the potential economic and quality-of-life impact of use. The economic model described in this article was developed through a review of the published literature available at the time the model was developed. Results are derived from a simulation of the probabilities of various health outcomes of cohorts of hypothetical subjects. These results are not intended to predict or guarantee actual treatment outcomes associated with any of the products discussed.

The prevalence of diabetes mellitus is growing in the United States and worldwide. An estimated 7.8% of Americans, or approximately 23.6 million persons, are afflicted with the disease, and with the longevity of the population increasing, the prevalence of diabetes-related complications will also continue to rise. Foot disorders are a major source of morbidity and a leading cause of hospitalization for persons with diabetes.

Diabetic foot ulcers are among the most common complications shown in people with diabetes. It is estimated that 15% of patients with diabetes will develop a lower extremity ulcer during the course of their disease. A landmark study published in 1990 documented the components of the causal pathway of lower extremity amputations in the diabetic patient. These include ulceration (84%), faulty wound healing (81%), initial minor trauma (81%), neuropathy (61%), infection (59%), gangrene (55%), and ischemia (46%). A nonhealing ulcer was the most frequent cause of amputation. Subsequent research has corroborated these findings. Mayfield et al documented that although veterans with diabetes comprise approximately 16% of the Veterans Administration service population, they...
account for more than half of all hospitalizations for lower extremity ulceration.

More than 60% of nontraumatic lower-limb amputations in the United States occur among people with diabetes. In 2004, about 71,000 nontraumatic lower-limb amputations were performed in people with diabetes. Costs for amputation have been estimated to be between $20,000 and $60,000 per case. An efficient process for healing diabetic foot ulcers would reduce their cost burden to society.

The PRP gel (AutoloGel; Cytomedix, Rockville, Maryland) used in this analysis is a system that uses patient blood for deriving autologous PRP and activating it to become a gel-like substance consisting of a fibrin matrix scaffold and the platelet releasate contents, which contain multiple growth factors. In 2006, the manufacturer of this PRP gel system published the results of a prospective, randomized, controlled, blinded, multicenter trial of the PRP gel compared with a saline gel control (Normlgel; Mölnlycke Health Care, Norcross, Georgia), which is a US Food and Drug Administration (FDA)–cleared wound dressing used in addition to standard of care for the treatment of nonhealing diabetic foot ulcers. The trial included nonhealing, full-thickness, diabetic foot ulcers that extended through the dermis without bone, muscle, ligament, or tendon exposure and which had been present for more than 4 weeks. The primary efficacy end point was the incidence of total wound closure at 12 weeks. Among 35 patients (16 treated with PRP gel and 19 treated with saline gel) who completed the study per protocol and who had a wound area 7 cm² or less and a wound volume 2 cm³ or less (the most common wound size for diabetic foot ulcers), the healing efficacy at 12 weeks was 81.3% in the PRP gel group and 42.1% in the saline gel group (this difference was significant at \( P = .036 \)).

A cost-effectiveness analysis of the PRP gel was conducted using the published clinical data on the PRP gel, as well as pricing information received from the manufacturer, to evaluate the potential economic and quality-of-life impact of its use. The research methodology, model structure, assumptions, and other inputs were developed from the peer-reviewed literature.

OBJECTIVE
The objective of this research was to perform a cost-effectiveness analysis of the PRP gel compared with alternative therapies to evaluate its potential economic benefit to health care insurers and providers who treat patients with nonhealing diabetic foot ulcers. Each of these products would be used in addition to the standard of care.

MATERIALS AND METHODS
Decision Model Structure and Process
The accepted standard of care for diabetic foot ulcers includes wound debridement, pressure relief in the wound area, appropriate wound management (eg, moist wound healing), infection management, ischemia management, medical management of comorbidities, and surgical management as needed. Changing wet-to-moist saline gauze dressings 3 to 4 times per day has been the traditionally accepted wound treatment modality.

Rather than using these wet-to-moist gauze dressings, which tend to dry easily, a normal saline gel dressing left on the wound for up to 4 days was selected as the control in this PRP gel trial. Healing rates associated with PRP and saline gels, as well as those found in the recent literature for other diabetic foot ulcer therapies, were used in the model. To ensure a follow-up period of 1 year, recurrence rates were determined based on published data of autologous platelet releasates (similar to PRP gel) and published standard of care in comprehensive wound care programs.

A computerized decision analysis model was developed (Figure 1), using TreeAge Pro 2006 to simulate the cost-effectiveness of the PRP gel compared with alternative therapies in the treatment of nonhealing diabetic foot ulcers. The model has 2 treatment arms: (1) the PRP gel and (2) the normal saline gel. The patient population and treatments in the model were based on the described PRP gel prospective, randomized, clinical trial as published. The model is also used to simulate the effects of other treatment modalities as published in the literature.

In the investigational and control arms of the model, patients received twice-weekly treatments with the PRP gel or the saline gel until the wounds had healed or for 12 weeks, whichever came first. If the ulcer had not healed at 12 weeks, the patient reverted to standard-of-care treatment for as long as the ulcer remained unhealed.

The model has 4 health states: unhealed ulcer, healed ulcer, amputation, and deceased. Hypothetical cohorts of 10,000 patients each enter each of the treatment arms of the model, 1 at a time, and are “followed” by the model on a weekly basis (a cycle) for 5 years as they transition between the health states. Each patient in each treatment arm begins in the unhealed ulcer health state of the decision tree. During each cycle (1 week) that the patient remains in the unhealed ulcer health state, the ulcer may either heal or remain unhealed. The probability of healing depends on the type of treatment and the time since treatment began.

In the unhealed ulcer health state, if the ulcer does not heal during a cycle (cases assigned to the “unhealed” branch of the
model), the patient is exposed to the risk of developing a severe infection consisting of cellulitis and/or osteomyelitis. Each patient is assumed to have no more than 1 severe infection during the time their ulcer remains unhealed, and each severe infection is assumed to have been treated for 6 weeks unless the patient undergoes amputation or dies first. All patients with unhealed ulcers are at risk of lower extremity amputation, which is classified according to 3 levels: toe, foot, or lower leg. If the patient undergoes an amputation, he or she transitions in the next cycle to the amputation health state of the model, where he or she remains for the rest of the simulation. As shown in Figure 1, patients with 1 amputation may require a second amputation.

In the unhealed ulcer health state, if the ulcer does heal during a cycle (heal), the patient transitions in the next cycle to the healed ulcer health state. Patients in the healed ulcer health state are at risk of ulcer recurrence (recur). If the ulcer recurs during a cycle, the patient transitions back to the unhealed ulcer health state during the next cycle. Otherwise, the patient remains in the healed ulcer health state until the end of the simulation or death, whichever comes first.

Figure 1.
COST-EFFECTIVENESS DECISION ANALYSIS MODEL
During each cycle, patients are exposed to the risk of death. If a patient dies during a cycle (unhealed ulcer, healed ulcer, or amputation health state), in the next cycle, they transition to the deceased state. Presently, the risk of death depends only on the age of the patient at the start of the simulation and the fact that the patient has diabetes. The model can be adjusted to allow for the added risk of death due to amputation, if there is evidence to support an association between amputation and increased risk of death independent of confounding comorbidity.

During the simulation, each patient accrues the costs of managing the ulcer, severe infection, and amputation, if it occurs. The weekly costs of managing an unhealed ulcer depend on whether the patient is assigned to the PRP gel or the saline gel arm of the model, and on whether the patient has a severe infection. Each arm of the model was run a total of 10 times for a total of 200,000 observations over a 5-year period.

**DATA INPUTS**

**Probabilities**

Probability data for the model were obtained from the PRP gel/saline gel randomized, controlled trial and the scientific literature (Table 1).

**Ulcer Healing**

The most important clinical (in contrast to cost or utility) variable in the model is the ulcer healing rate in each of the treatment arms. The baseline rates of ulcer healing in the PRP gel arm and the saline gel arm were obtained from the prospective, randomized, controlled trial comparing the PRP gel and the saline gel. In this trial, among 35 patients (16 treated with PRP gel and 19 treated with saline gel) who completed the study per protocol and who had a wound area 7 cm² or less and a wound volume 2 cm³ or less, the healing efficacy at 12 weeks was 81.3% in the PRP gel group as compared with 42.1% in the saline gel group (this difference is significant at P = .036). This size wound seems to be the most common size of diabetic foot ulcer (see Discussion and Tables 5 and 6). Subjects randomized to PRP gel in the total wound (n = 40) and intent-to-treat (n = 72) groups of the study also reported improved rates of healing at 12 weeks when compared with the saline gel control group. Healing rates for weeks 13 through 52 were derived through statistical approximation based on reported healing at 12 weeks and at 1 year.

**Ulcer Recurrence**

The longest documented follow-up measure to evaluate ulcer recurrence was derived from the scientific literature. The rate of ulcer recurrence was determined from 2 major categories of wound treatments for diabetic foot ulcers: (a) treatment with autologous platelet releasates containing multiple growth factors (a precursor to PRP gel) and (b) quality standard of care at specialty wound care programs. A weighted average was determined for each group based on the published data and determined for a 12-month follow-up period. If a published study did not document the recurrence rate, a

---

Table 1.

<table>
<thead>
<tr>
<th>MAIN PROBABILITY INPUTS</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input</td>
<td>PRP Gel</td>
</tr>
<tr>
<td>Ulcer healing</td>
<td>81.3% at 12 wk⁷</td>
</tr>
<tr>
<td>2.8% per wk between 12 and 20 wk⁸</td>
<td>0.9% per wk between 12 and 20 wk⁹</td>
</tr>
<tr>
<td>0.09% per wk between 20 and 52 wk²⁰</td>
<td>0.09% per wk between 20 and 52 wk²⁰</td>
</tr>
<tr>
<td>0% after 52 wk</td>
<td>0% after 52 wk</td>
</tr>
<tr>
<td>Ulcer recurrence</td>
<td>4% at 1 y¹⁰⁻¹²</td>
</tr>
<tr>
<td>Severe infections¹⁵</td>
<td>9% at 1 y</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>3.6% at 1 y</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>19% at 1 y</td>
</tr>
<tr>
<td>First amputation¹⁶</td>
<td>44%</td>
</tr>
<tr>
<td>Toe²</td>
<td>14%</td>
</tr>
<tr>
<td>Foot³</td>
<td>42%</td>
</tr>
<tr>
<td>Leg⁴</td>
<td>11% at 1 y after the first first</td>
</tr>
<tr>
<td>Second amputation¹⁷</td>
<td>10.2% per y</td>
</tr>
</tbody>
</table>

¹Percentages are among those with an unhealed ulcer.
²Percentages are among those undergoing amputation.
³Among type 1 diabetic patients aged 65 years.

Table 2.

<table>
<thead>
<tr>
<th>COST INPUTS (UPDATED TO 2006 DOLLARS)²³</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input</td>
<td>PRP Gel</td>
</tr>
<tr>
<td>Cost per month Uncomplicated ulcer</td>
<td>$4841²⁴</td>
</tr>
<tr>
<td>Severe infection</td>
<td></td>
</tr>
<tr>
<td>Cellulitis</td>
<td>$2492</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>$4619</td>
</tr>
<tr>
<td>Total Event Cost</td>
<td></td>
</tr>
<tr>
<td>First amputation</td>
<td></td>
</tr>
<tr>
<td>Toe</td>
<td>$27,607</td>
</tr>
<tr>
<td>Foot</td>
<td>$51,892</td>
</tr>
<tr>
<td>Leg</td>
<td>$62,359</td>
</tr>
<tr>
<td>Second amputation</td>
<td></td>
</tr>
<tr>
<td>Healed ulcer</td>
<td>0.8</td>
</tr>
<tr>
<td>Unhealed ulcer</td>
<td>0.6</td>
</tr>
<tr>
<td>Amputation</td>
<td>0.7</td>
</tr>
</tbody>
</table>

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A conservative approach was used; the PRP gel recurrence rate was used for those without data.

**Severe Infection**
Rates of severe infection (cellulitis and/or osteomyelitis) also were gathered from the literature. Weekly rates were calculated for the model such that the cumulative rates over 52 weeks were consistent with those annual rates reported in the literature.

**Amputation (Primary and Secondary)**
The rate of first amputation was obtained from an observational cohort study of 185 patients presenting with new ulcers to a dedicated diabetic foot ulcer clinic. Patients were followed up for an average of 34 months. The 5-year amputation rate was 19% for the entire cohort. In the model, the weekly rate of amputation resulting from an unhealed ulcer was calculated such that the cumulative rate over 1 year was consistent with the annual rate reported in the cohort study. The distribution of amputation levels (toe, foot, leg) was obtained from the Centers for Disease Control and Prevention, Diabetes Public Health Resource (http://www.cdc.gov/diabetes/statistics/lealevel/table8_age.htm). Significant numbers of diabetic foot ulcer patients undergoing amputation require a second amputation. The literature indicates that during 12 months after the initial amputation, 9% to 13% of amputees require a new (ipsilateral) or second leg (contralateral) amputation. A rate of 11% within 1 year was selected as the baseline rate for the model.

**Table 3. BASELINE RESULTS**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>PRP Gel</th>
<th>Baseline Treatment (Saline Gel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cost (baseline healing rates)</td>
<td>$15,159</td>
<td>$33,214</td>
</tr>
<tr>
<td>QALYs</td>
<td>2.87</td>
<td>2.70</td>
</tr>
</tbody>
</table>

**Table 4. COSTS AND OUTCOMES OF THE PRP GEL VERSUS ALTERNATIVE THERAPIES**

<table>
<thead>
<tr>
<th>Product</th>
<th>No. Wounds in Study</th>
<th>No. Wounds Mean Area &gt;0.5 to &lt;7 cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP gel</td>
<td>72</td>
<td>68</td>
</tr>
<tr>
<td>Noncontact kilohertz ultrasound therapy²⁷</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Human fibroblast dermal substitute²⁸</td>
<td>245</td>
<td>245</td>
</tr>
<tr>
<td>Allogenic bilayered culture skin substitute²⁹</td>
<td>208</td>
<td>208</td>
</tr>
<tr>
<td>Bilayered cellular matrix³⁰</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>rhPDGF-BB³¹</td>
<td>118</td>
<td>118</td>
</tr>
<tr>
<td>rhPDGF-BB³²</td>
<td>382</td>
<td>382</td>
</tr>
<tr>
<td>rhPDGF-BB³³</td>
<td>1000</td>
<td>882</td>
</tr>
<tr>
<td>rhPDGF-BB³⁴</td>
<td>992</td>
<td>992</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3112</td>
<td>2978</td>
</tr>
</tbody>
</table>

Percentage of wounds >0.5 to <7 cm²: 95.7%

**Table 5. NUMBER AND SIZE OF WOUNDS IN DIABETIC FOOT ULCER PUBLISHED RANDOMIZED CONTROLLED TRIALS**

<table>
<thead>
<tr>
<th>Product</th>
<th>No. Wounds in Study</th>
<th>No. Wounds Mean Area &gt;0.5 to &lt;7 cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP gel</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Noncontact kilohertz ultrasound therapy²⁷</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Human fibroblast dermal substitute²⁸</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Allogenic bilayered culture skin substitute²⁹</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Bilayered cellular matrix³⁰</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>rhPDGF-BB³¹</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>rhPDGF-BB³²</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>rhPDGF-BB³³</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>rhPDGF-BB³⁴</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>rhPDGF-BB³⁵</td>
<td>900</td>
<td>900</td>
</tr>
<tr>
<td>rhPDGF-BB³⁶</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3100</td>
<td>3100</td>
</tr>
</tbody>
</table>

Percentage of wounds >0.5 to <7 cm²: 95.7%

**Table 6. NUMBER AND SIZE OF WOUNDS REPORTED IN MARGOLIS DIABETIC FOOT ULCER STUDY³⁶**

<table>
<thead>
<tr>
<th>Wound Care Centers Database</th>
<th>No. in Study</th>
<th>No. with Mean Wound Area &gt;0.5 to &lt;7 cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic foot ulcers</td>
<td>26,599</td>
<td>15,959</td>
</tr>
<tr>
<td>Percentage of wounds &gt;0.5 to &lt;7 cm²</td>
<td>60%</td>
<td></td>
</tr>
</tbody>
</table>

Of 26,599 diabetic wounds (not all were on the plantar surface of the foot) in this large database, 60% had a mean wound area of >0.5 and <7 cm².
Mortality
In the literature, amputation is often linked to excess mortality. The 1995 edition of Diabetes in America documented that there was a mortality rate of 38% to 68% within 5 years after amputation. However, it is unclear whether amputation itself is a risk factor for death, or if patients requiring amputation are at higher underlying risk of death. Several studies have failed to show an association between amputation and death among diabetic foot ulcer patients, adjusting for patient covariates. Therefore, in the model, a conservative assumption was made that amputation does not increase the risk of death. Presently, we have assumed a baseline mortality rate consistent with patients aged 65 years with type 1 diabetes.

Costs
Costs were obtained from companies or distributors in 2006 and are shown in Table 2. For purposes of the economic model, the “per treatment” price of the PRP gel was assumed to be $450. In this analysis, it was assumed that treatments with the PRP gel are provided twice per week. In addition, a sensitivity analysis was conducted using a per-treatment price of $600.

The baseline costs of managing an uncomplicated ulcer (no infection or local infection) and of managing severe infection (consisting of cellulitis and/or osteomyelitis) were converted to weekly costs. The monthly cost of managing an uncomplicated ulcer, as reported in the literature, was assumed to reflect the standard of care. The monthly cost of wound care for an ulcer not complicated by infection is $942, as compared with the monthly cost of $2492 for an ulcer complicated by cellulitis and $4619 for an ulcer complicated by osteomyelitis (these costs have been updated to 2006 dollars using the medical care component of the Consumer Price Index). In general, hospitalizations represent the most significant contribution (70% to 80%) to the cost of care. Drug costs generally do not drive total costs.

The costs of amputation depend on the level of the amputation. The total costs of amputation, including initial inpatient admission and prostheses, are “loaded” into the week in which the amputation occurs. Conservatively, all second amputations were assumed to be of the foot, rather than a more costly below-the-knee amputation, and the cost of the second amputation was assumed to be identical to the first foot amputation. All costs were inflated to 2006 dollars using the medical care component of the Consumer Price Index, All Urban Consumers (http://www.bls.gov/cpi/).

Utilities
Each week, the model assigns a utility value to each patient depending on their present health state in the model (unhealed ulcer, healed ulcer, amputation, and deceased). Utilities range from 0 to 1 and represent the proportion of full health (utility = 1) the patient feels about his or her present health. Utilities for unhealed ulcer, healed ulcer, and amputation were obtained from the literature (Table 2).

Model Outputs
For each patient entering each of the treatment arms, the model calculates the total cost of managing the ulcer plus amputation (when it occurs) during 5 years or until death, whichever comes first.

As previously discussed, hypothetical cohorts of 10,000 ulcer patients enter each of the treatment arms of the model one at a time. The model generates a total cost and total quality-adjusted life-years (QALYs) for each patient. Ten analyses of 10,000 simulations per analysis were run for each arm of the model for a total of 200,000 observations. The mean cost and mean QALYs across the 10 analyses are reported.

RESULTS
Baseline Analyses
The results of the baseline analysis are presented in Table 3. As shown, with baseline probability and cost inputs included in the model, the mean total cost in the PRP gel arm of the model was $15,159 compared with $33,214 in the control (saline gel) treatment arm, a savings of $18,055 during the 5 years because of improved ulcer healing and lower ulcer recurrence in the PRP gel arm of the model.

Sensitivity Analyses
The results of the sensitivity analysis, in which the impact of varying the price of the PRP gel was explored, indicate that the expected 5-year cost per patient changed little when the cost per treatment was increased to $600 (the average 5-year cost per patient was $16,835). This seems to be the result of the high rate of healing in the PRP gel treatment arm of the model.

As shown in Table 4, sensitivity analyses in which the cost-effectiveness of the PRP gel relative to alternative treatments was examined show that the PRP gel is both less costly and more effective than all of the other options considered by the study when the rate of healing from the majority wound group is used. The average 5-year direct wound care cost per modality and QALY were as follows: PRP gel, $15,159 (2.87); saline gel, $33,214 (2.70); standard of care, $40,073 (2.65); noncontact kilohertz ultrasound therapy, $32,659 (2.73); human fibroblast-derived dermal substitute, $40,569 (2.65); allogenic bilayered culture skin substitute, $24,374 (2.79); bilayered cellular matrix, $37,340 (2.71); negative pressure wound therapy (NPWT), $20,964 (2.81); and recombinant human platelet-derived growth factor BB (rhPDGF), $47,252 (2.69).
healing from the total per-protocol group is used, the PRP gel remains less costly and more effective than other treatment modalities. In a cost-effectiveness analysis, when one technology is both less costly and more effective than another, it is said to dominate that technology.

**DISCUSSION**

A computerized decision-analysis model was constructed to assess the cost-effectiveness and potential quality-of-life benefit of the PRP gel compared with several other treatment alternatives when used to treat patients with diabetic foot ulcers. Based on the assumptions in the model, the findings suggest that the PRP gel would result in important cost savings and better quality of life compared with the other treatment alternatives evaluated.

Even when the per-treatment cost of the PRP gel ($450) was included, and assuming 2 treatments per week for up to 24 treatments per course of therapy (it should be noted that 81% of the patients who were part of the per-protocol majority wound group in the trial achieved full healing in an average of 6 weeks of twice-weekly treatments, ie, in 12 treatments), the expected 5-year cost per patient was $18,055 lower in the PRP gel arm of the model as compared with care in the saline gel treatment arm. In addition, QALYs were longer in the PRP gel arm of the model than the saline gel arm. In a cost-effectiveness analysis when one technology is both less costly and more effective than another, it is said to dominate that technology. In this instance, the PRP gel dominates standard of care as described in the published literature, and proves more effective than treatment with saline gel.

The data for PRP gel used in this economic model were taken from the results reported for the Per Protocol Majority Wound group of the PRP gel study. These were determined to be appropriate inputs for the model because protocol violations in the study’s intent-to-treat (ITT) group (n = 72) made treatment outcomes reported for this group a potentially less reliable measure of expected treatment outcomes using PRP gel. However, the cost data for this group were included in the sensitivity analysis (Table 4).

Also, during the FDA-supported size stratification analysis on the per-protocol group, it was found that most wounds (n = 35) fell into a specific size range, that is, wound area was 0.5 cm² or greater and 7 cm² or less. This wound size was compared with the size of wounds in other major prospective, randomized, controlled studies of diabetic foot ulcer treatments, as well as to data from the largest study documented on diabetic foot ulcers (in total, including nearly 30,000 patients). It was found that these Majority Wound Group wounds were the same size as most wounds in these published studies. Of 3112 diabetic foot wounds in 9 prospective trials, nearly 96% of the wounds had a mean wound area of 0.5 cm² or greater and 7 cm² or less (Table 5). Sixty percent of the wounds in the large database also fell into this size range (Table 6).

Because the wound area of the Per Protocol Majority Wound group was consistent with the average wound area as commonly reported in other randomized, controlled studies of diabetic foot ulcer treatments documented in this analysis, the healing rates reported in the PRP gel study for this group were included in the economic model. These comparisons highlight the potential clinical significance of the outcomes associated with the PRP gel in populations with these common sizes of diabetic foot ulcers. Even when the economic data for the ITT and Per Protocol Wound groups were analyzed, use of PRP gel in these groups also demonstrated increased cost-effectiveness and quality of life when compared with other treatment modalities. Despite the study’s limitation, the results of the cost-effectiveness model suggest that improved healing rates expected with the PRP gel could result in substantial cost savings to diabetic foot ulcer patients because of fewer infections, osteomyelitis, and amputations, in addition to improved patient quality of life, relative to alternative therapies.

Finally, PRP gel was compared with other advanced therapies. Assuming healing rates for the PRP gel are consistent with rates observed in the Majority Wound Group of the clinical trial (n = 35), and with the overall per-protocol group (n = 40), the PRP gel is both less costly and more effective than all other therapies considered in the model.

The present model has limitations that could affect the findings. Although amputation is often associated with increased risk of death, we elected not to link amputation to mortality in the model. It is not completely clear from the literature whether amputation results in excess mortality or whether underlying risk factors for amputation also are risk factors for mortality. We believe that our decision is conservative in that it may result in underestimating the impact of the PRP gel on patient outcomes.

Although the author’s conclusion that the Majority Wound Group represents the most-common-size wounds reported in the peer-reviewed literature (with wound area measuring ≥ 0.5 and ≤ 7 cm²), we recognize that the rate of healing for PRP gel is based on a relatively small number of observations (35 in total). Even with the small number of wounds, the author believes that the statistically significant difference between treatment arms makes this a valid cohort to model.

Finally, several of the published studies did not report recurrence rates so the same recurrence rate included in the PRP gel analysis was used. If these other modalities had higher rates of ulcer recurrence, their cost data would increase, causing
an even greater impact on the cost-effectiveness of the PRP gel. This PRP gel contains a physiological normal concentration of platelets (1.2× baseline). The literature documents other PRP gel systems in which the PRP is manipulated causing a platelet concentrate (6-10× baseline). There are no published, peer-reviewed, prospective, randomized, controlled trials demonstrating that this higher concentration is efficacious for the treatment of diabetic foot ulcers. Thus, although this physiological platelet level PRP gel has demonstrated efficacy and cost savings in the most common diabetic foot ulcers, that efficacy and resulting cost savings have not been demonstrated in the platelet concentrate systems as of this time.

**CONCLUSION**

An economic model developed to evaluate the clinical and cost outcomes associated with using an autologous, physiological PRP gel compared with other modalities currently used to treat nonhealing diabetic foot ulcers demonstrated that use of this PRP gel resulted in better treatment outcomes, improved quality of life, and lower cost of care.

**REFERENCES**


23. Cost inputs are inflated to 2006 dollars using the Consumer Price Index—all urban consumers. The index uses the average annual CPI-U for a given year. For the current year, the latest monthly index value is used (in this case, November 2006). http://www.bls.gov/cpi/. Last accessed October 8, 2008.
Prospective randomised study on the effect of autologous platelets injection in lateral epicondyritis compared with corticosteroid injection.

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Lateral epicondyritis is not an inflammatory condition, but rather an angiofibroblastic degeneration caused by failure of normal tendon repair mechanisms, including vascular responses. Corticosteroids have shown to relieve symptoms, but the positive effect lasts for approximately 6 weeks and tendon healing is not stimulated.

We studied the experimental treatment with injection of autologous platelets, since platelets contain growth factors, which are necessary for tissue regeneration.

We obtained platelet rich plasma (PRP) by centrifugation of autologous blood. The PRP is reported to have a 5-8 times higher concentration than does whole blood.

The study was designed as a prospective double-blind RCT, level 1. 100 patients were included. Follow-up occurred after 4-8-12-24 weeks after the injection using VAS- and DASH-scores.

We saw a significant decrease in the scores in both groups compared to the initial scores. However after 24 weeks the corticosteroid group did not have a significant improvement anymore, but the PRP group remained at low VAS and DASH scores.

We conclude that injection of PRP has a positive effect on the course of lateral epicondyritis, the effect actually exceeds the effect of corticosteroids, known as the golden standard. We await the 1- and 2-year results.
Use of Autologous Platelet-rich Plasma to Treat Muscle Strain Injuries

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Background: Standard nonoperative therapy for acute muscle strains usually involves short-term rest, ice, and nonsteroidal anti-inflammatory medications, but there is no clear consensus on how to accelerate recovery.

Hypothesis: Local delivery of platelet-rich plasma to injured muscles hastens recovery of function.

Study Design: Controlled laboratory study.

Methods: In vivo, the tibialis anterior muscles of anesthetized Sprague-Dawley rats were injured by a single (large strain) lengthening contraction or multiple (small strain) lengthening contractions, both of which resulted in a significant injury. The tibialis anterior either was injected with platelet-rich plasma, was injected with platelet-poor plasma as a sham treatment, or received no treatment.

Results: Both injury protocols yielded a similar loss of force. The platelet-rich plasma only had a beneficial effect at 1 time point after the single contraction injury protocol. However, platelet-rich plasma had a beneficial effect at 2 time points after the multiple contraction injury protocol and resulted in a faster recovery time to full contractile function. The sham injections had no effect compared with no treatment.

Conclusion: Local delivery of platelet-rich plasma can shorten recovery time after a muscle strain injury in a small-animal model. Recovery of muscle from the high-repetition protocol has already been shown to require myogenesis, whereas recovery from a single strain does not. This difference in mechanism of recovery may explain why platelet-rich plasma was more effective in the high-repetition protocol, because platelet-rich plasma is rich in growth factors that can stimulate myogenesis.

Clinical Relevance: Because autologous blood products are safe, platelet-rich plasma may be a useful product in clinical treatment of muscle injuries.

Keywords: skeletal muscle; injury; strain; muscle regeneration

Muscle injury occurs from either acute or repetitive trauma and results in a decreased ability to produce force that does not recover after a short period of rest. When an activated muscle lengthens because the external load exceeds the tension generated by the muscle contraction, this is termed a lengthening (or eccentric) contraction. Submaximal lengthening contractions are used in everyday life, but it is well known that high-force lengthening contractions are associated with muscle damage and pain.13,18,30 The force generated during a maximal lengthening contraction is approximately 2-fold the force developed during a maximal isometric contraction.9,18

The generation of high force by muscles is a goal of strength training; this is evident in training protocols that use lengthening contractions, or “negatives,” to increase strength. Although lengthening contractions are common and often occur without causing damage, high-force lengthening contractions are more likely to produce damage than either isometric or concentric contractions, resulting in pain and damage.16,18,23,30 In clinical lexicon, the injury resulting from a high-force lengthening contraction is termed a muscle strain; such strains are the most common cause of muscle injuries.15,37
To conduct a well-controlled study, we developed an animal model of muscle injury and a reproducible mechanism for generating muscle strain. We recently reported that recovery of contractile function after injury by a single, large-strain lengthening contraction involves repair of damaged sarcolemma with minimal myogenesis, whereas recovery from multiple, small-strain lengthening contractions requires myogenesis, with minimal sarcolemmal repair.\(^\text{25}\) Here we used both protocols in our established injury model to test the effects of autologous platelet-rich plasma (PRP) on recovery in the tibialis anterior muscle of rats. Platelet-rich plasma is isolated by a technique involving centrifugation of whole blood, allowing extraction of the specific part of the plasma containing a high concentration of platelets. These platelets are rich in growth factors that can stimulate myogenesis\(^\text{7,27,32,36}\) and mitigate inflammation.\(^\text{8,26}\) We hypothesized that the local delivery of PRP to injured skeletal muscles accelerates recovery, and we present data from experiments that support this hypothesis.

**MATERIALS AND METHODS**

**Injury Model**

All protocols were approved by the university Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (n = 72) weighing 341 ± 21 g (approximately 4 months of age) were anesthetized with isoflurane (2% with oxygen flow rate of 0.5 L/min). The Sprague-Dawley rats were inbred, allowing us to consider them syngeneic. The injury model results in a significant and reproducible injury and has been described previously.\(^\text{11,23-25,33}\) In brief, anesthesia was confirmed by lack of response to a normally painful stimulus (pinching the foot); the left hindlimb was stabilized and the foot was secured onto a footplate. The axis of the footplate was attached to a stepper motor (T8904 NMB Technologies, Chatsworth, California), a potentiometer to record angular position, and a torque sensor (QWFK-8M, Sensotec, Columbus, Ohio) to measure torque. The fibular nerve was stimulated via subcutaneous needle electrodes (Harvard Apparatus 723742, Cambridge, Massachusetts), and proper electrode position was determined by a series of isometric twitches. In addition to visual confirmation of isolated dorsiflexion, an increase in twitch torque in response to increasing voltage indicated that opposing plantar flexor muscles were not being simultaneously stimulated.\(^\text{3}\) A custom program was used (Labview version 8.5, National Instruments, Austin, Texas) to synchronize contractile activation and onset of ankle rotation. Impulses generated by an S48 square pulse stimulator (Grass Instruments, West Warwick, Rhode Island) were 1 millisecond in duration and passed through a PSIU6 stimulator isolation unit (Grass Instruments).

To induce injury in the tibialis anterior muscle (TA), we superimposed a lengthening contraction onto a maximal isometric contraction (Figure 1), using either a single repetition (large strain) or multiple repetitions (small strain). Specifically, a maximal isometric contraction was obtained in the dorsiflexors and after 200 milliseconds they were lengthened through an arc of motion at 90 deg/s. The majority of torque produced by the dorsiflexors is from the TA,\(^\text{15}\) and we have shown previously that this model results in injury to this muscle.\(^\text{11,23-25}\) The TA remained stimulated throughout lengthening and was injured using 1 of 2 protocols: a single lengthening contraction through a 90° arc or 45 lengthening contractions through a 60° arc. For multiple repetitions, the lengthening contractions were spaced 2 minutes apart.

**Outcome Measures**

For both protocols, a maximal isometric contraction (200-millisecond duration) of the dorsiflexors was used to measure maximal torque before injury. For each animal, maximal isometric torque was also measured 4 minutes after injury (to measure force lost because of injury). Animals were returned to their cages after recovery from anesthesia, and maximal isometric torque was retested after injury (ISO-POST).

**Figure 1.** Representative trace recordings of torque from the lengthening contractions. For both single and multiple repetitions, muscles were stimulated for 200 milliseconds to induce a peak isometric contraction before lengthening by the footplate. Maximal isometric torque (without lengthening) was measured before injury (not shown, but equal to the plateau of the isometric portion of the trace recordings, indicated by filled arrows). A, superimposed recordings from the multiple-repetition protocol (a 60° arc of motion) showing the first (ECC 1), middle (ECC 15), and last (ECC 45) eccentric contractions. Note that even with injury, the eccentric torque is still relatively high compared with isometric torque (the peak isometric torque measured after injury is not shown but is similar to the isometric portion of the last trace recording, indicated by the open arrow). B, superimposed recordings of the single-repetition protocol (ECC 1, a 90° arc of motion) and the isometric contraction to measure torque loss after injury (ISO-POST).

Animals were returned to their cages after recovery from anesthesia, and maximal isometric torque was also measured 4 minutes after injury (to measure force lost because of injury). Animals were returned to their cages after recovery from anesthesia, and maximal isometric torque was retested after injury (ISO-POST).

After functional data were collected, tibialis anterior muscles were harvested from the anesthetized rat, snap frozen in liquid nitrogen, and stored at –80°C. The animal was then sacrificed by carbon dioxide inhalation or with pentobarbital sodium (200 mg/kg) administered intraperitoneally.
TABLE 1
Timeline for an Individual Animal

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<th>Day</th>
<th>Measure maximal isometric torque</th>
<th>Induce injury</th>
<th>Wait 5 minutes and retest torque</th>
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*a*PRP, platelet-rich plasma; PPP, platelet-poor plasma. Each mouse was anesthetized, and maximal isometric torque was measured in vivo. An injury was induced, and the loss of force was determined by another measure of maximal isometric torque. Subsequent measures of torque and injections of PRP/PPP were as shown.

**Platelet-rich Plasma**

Twenty milliliters of whole blood was collected from 5 adult male Sprague-Dawley rats (withdrawn from the femoral vein, renal vein, or cardiac puncture). The syngeneic nature of the Sprague-Dawley inbred rat allowed us to consider the blood of one animal autologous to blood from another animal. Autologous PRP was then separated from the blood using the Symphony II Platelet Concentration System (Depuy, a Johnson & Johnson Company, Warsaw, Indiana). The centrifugation results in the formation of 2 layers within the plasma: a platelet-poor plasma (PPP) component and a PRP component. The PPP supernatant was carefully removed and used as a control vehicle. The remaining PRP was conditioned using 10 seconds of high-frequency ultrasound to lyse platelets and release growth factors, thereby enriching the PRP before injection. One hundred microliters of PRP was injected into the TA of the injured hindlimb in each rat of the treated group at days 0 (day of injury), 3, 5, and 7 (Table 1). Both the conditioned PRP and the PPP were refrigerated and used within a few days.

Enzyme-linked immunosorbent assays (ELISAs) (R&D Systems, Minneapolis, Minnesota) were performed following the manufacturer's instructions, to determine whether PRP is enriched in myogenic growth factors. Both PRP and PPP were separated and subjected to ELISAs to detect and quantify the presence of platelet-derived growth factor (PDGF) and insulin growth factor-1 (IGF-1). We also assayed conditioned plasma, as described previously.

**Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)**

Two micrograms of total RNA was isolated from frozen rat tibialis anterior muscle with TRIzol (Invitrogen, Carlsbad, California) and was reversed-transcribed with Superscript II First strand Synthesis System (Invitrogen), per the manufacturer's instructions. The resulting complementary DNA was used as a template for PCR amplification. The primers for rat MyoD were (forward) 5'-CACTCCTCCAATTGTC-3' and (reverse) 5'-TTTATTCCACCGTAGAG-3'. The primers for rat myogenin were 5'-ACCTTCCCGAGTGAGACC-3' and 5'-AAGAATGCCCCAGAGACC-3'. The primers for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5'-ACGACCCCTTCATTGACC-3' and 5'-ATCACGCCCACTTCCC-3'. The NCBI reference numbers are M24393, M017008, and M84176 for MyoD, myogenin, and GAPDH, respectively. The PCR products were run on 1% agarose gels, stained with ethidium bromide, and scanned. The intensity of bands was quantified using NIH Image J software, and relative expression was quantified based on the total GAPDH expressed in a particular muscle sample.

**Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblotting**

Western blot analysis was used to assess semiquantitative changes in the levels of MyoD and myogenin proteins. Extracts of unfixed TA muscles were snap frozen in liquid nitrogen, pulverized, and homogenized with a PowerGen 125 homogenizer (Fisher Scientific, Waltham, Massachusetts) at a wt/vol ratio of 0.05 in homogenate buffer (10 mmol/L NaPO₄, 2 mmol/L EDTA, 140 mmol/L NaCl, 1% NP40, pH 7.4) with protease inhibitors (Complete Protease Inhibitor Tablets, Roche Diagnostics, Indianapolis, Indiana). Samples were boiled and centrifuged, and the protein concentration of the supernatant was determined using a Bradford assay. Samples were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 4% to 12% gradient gel and transferred onto nitrocellulose electrophoretically. The nitrocellulose was blocked in 3% milk-PTA, washed, and then incubated with anti-myOD (sc 760) or anti-myogenin (sc 576) polyclonal (rabbit) antibodies (Santa Cruz Biotechnology, Santa Cruz, California) for 6 hours. Excess antibodies were washed off and the nitrocellulose was incubated with donkey anti-rabbit secondary antibodies conjugated to alkaline phosphatase (Jackson Laboratories, Bar Harbor, Maine). The excess secondary antibodies were then washed off and the bands were visualized by a chemiluminescent assay method (Tropix, Bedford, Massachusetts).

**Hematoxylin and Eosin Staining**

Tissue was frozen in isopentane-cooled liquid nitrogen, and transverse sections were cut on a cryostat (10-µm thickness). Sections were collected onto glass slides (Superfrost Plus; VWR, West Chester, Pennsylvania) and stained with hematoxylin and eosin for counting centrally nucleated fibers. Sections were randomized and viewed at ×100 magnification in a Zeiss Axioskop light microscope, and pictures were taken with a digital camera (AxioCam HR using AxioVision 3.0 AXIOVISION 3.0, Carl Zeiss Inc, Germany).
Each optical field contained an average of $38 \pm 7$ fibers, and more than 45 fields were counted per muscle.

**Statistical Analysis**

Contractile data from each experiment were analyzed using a single-factor analysis of variance (ANOVA, Sigma-Stat, San Rafael, California). When a significant ratio was found, a Tukey post hoc analysis was performed to determine where significant differences had occurred ($P < .05$). Each ELISA contained 3 replicates, and the results were analyzed with a 1-way ANOVA. For statistical analysis of RT-PCR, densitometry was performed for messenger RNA (mRNA) of myoD or myogenin and GAPDH for each blot. The mRNA/GAPDH ratios were calculated and analyzed using the Holm-Sidak pairwise multiple comparison test.

**RESULTS**

**Assessment of Growth Factors**

We quantified growth factors such as PDGF and IGF-1, both known to stimulate myogenesis, in the PRP and PPP groups. We examined conditioned (sonicated) and unconditioned PRP and PPP using ELISA kits. Figure 2 shows that the concentrations of PDGF and IGF-1 ($20,745 \pm 520$ pg/mL and $65,550 \pm 500$ pg/mL, respectively) in PRP were significantly higher than in PPP ($P < .001$), and concentrations were further increased (a 5-fold increase in PDGF and a 27% increase in IGF-1) by mechanical perturbation of the platelets (see Methods). Because of the significant increase of growth factors with conditioning, we tested the effects of conditioned PRP on muscle injury, as stated in the methods.

**Functional Recovery**

We induced injury and studied recovery of function in the whole-ankle dorsiflexor group. We harvested and then examined TA muscles, which account for most of the torque generated by this muscle group. The peak isometric torque generated by each animal was measured before injury and was considered 100% for that animal (the mean $\pm$ SD torque for all animals was $44 \pm 5$ N-m). For each animal, peak isometric torque was also measured after injury. The torque measurements immediately after injury and during recovery were expressed as the percentage of maximal isometric torque (out of 100%) for a given animal. The mean percentage of recovery at each time point is presented in Figure 3. Both injury protocols resulted in a significant loss of muscle function ($P < .001$) followed by gradual recovery. The multiple-repetition protocol results in a larger force deficit and takes longer to recover. The PRP treatment had little effect on the single-repetition protocol but did significantly ameliorate the force loss ($P = .003$) at 1 time point (day 3, Figure 3A), even though the animals had received only 1 injection by this time point (injections were immediately after injury and on days 3, 5, and 7 after injury). In the multiple-repetition protocol, PRP treatment significantly improved contractile function at 2 time points (days 7 and 14, $P < .001$), effectively shortening the time to full recovery from 21 days to 14 days (Figure 3B).

**Muscle Regeneration**

We harvested TA muscles from at least 2 animals at each time point for each protocol. We used 2 different markers to assay muscle regeneration. The first was to assay levels of myoD and myogenin. These are major muscle regulatory factors (MRFs) and are only expressed when satellite cells are activated to proliferate. The GAPDH was used as an internal control. The RT-PCR data (Figure 4A) show that mRNA transcripts for both of these muscle-specific transcription factors were present 7 days after injury but clearly elevated in the muscles treated with PRP compared with sham-treated muscles (PPP) or controls (not shown). Muscle tissue from other time points was also analyzed (data not shown), but the most obvious differences in mRNA levels occurred on day 7 ($P < .001$). The GAPDH transcript was tested and did not show altered transcription after muscle injury. Densitometry was performed and...
normalized to GAPDH to compare relative expression of myoD and myogenin (Figure 4B). To confirm that changes were not just in the expression of mRNA, we performed Western blotting and probed for myoD and myogenin. The results confirm that these 2 markers of muscle regeneration were increased in muscle samples injected with PRP (Figure 4C).

The second assay we used to detect myogenesis was quantification of centrally nucleated fibers (CNFs). Muscle fibers are multinucleated with the nuclei located at the periphery of the fibers, but within weeks after injury, nuclei are observed in the cytoplasm (Figure 5A) and these CNFs are widely accepted as a marker of muscle regeneration.6,12,16

Figure 3. Maximal torque was measured in each animal before injury (CTL) and immediately after injury (D0), as well as at selected time points after injury (days 3, 5, 7, 14, and 21). One hundred percent represents peak torque before injury. The percentage of recovery for each animal was calculated and the mean is expressed as the percentage of maximal isometric torque (out of 100%) at each time point. A, after a single repetition through a 90° arc of motion, there is a significant decrease in torque and gradual recovery to full contractile function by day 7. The PRP had a significant effect only on day 3 ($P = .003; n = 8$ animals each group). B, after multiple repetitions through a 60° arc of motion, there is a significant loss of torque followed by a gradual recovery by day 21. The PRP had a significant effect on days 7 and 14 ($P < .001$), by which time the injured muscle had returned to the pre-injury level of strength ($n = 8$ animals each group). No Rx, injury only (no injections). *$P < .05$.

Figure 4. Myogenesis after the multiple-repetition injury. A, 2 µg of total RNA was isolated from frozen rat tibialis anterior muscle, and reverse transcriptase polymerase chain reaction (RT-PCR) was performed at various time points after injury using primers for 2 different genes involved in muscle regeneration (myoD and myogenin) as well as a gene used as an internal control (glyceraldehyde 3-phosphate dehydrogenase, or GAPDH). The gel shows representative PCR products from muscles injected with PPP or PRP 7 days after injury. B, densitometry of the bands was performed and the results quantified relative to expression of the total GAPDH expressed in a particular muscle sample, as described in the methods. Thus, the histogram shows the mRNA transcript levels of myoD and myogenin from muscles injected with PPP or PRP ($n = 3$). C, muscle samples from the same time point (day 3) were homogenized and proteins separated by electrophoresis. Immunoblots confirmed the increase protein expression of myoD (38 kD) and myogenin (36 kD). *$P < .05$. 
Muscle strains are among the most common complaints treated by physicians\(^{10,17}\) and account for the majority of all sport-related injuries.\(^5,37\) Except for complete ruptures of muscles, displaced avulsions, and recalcitrant symptoms from myositis ossificans, almost all muscle injuries are uniformly treated with nonoperative therapy. Standard nonoperative therapy for acute muscle injuries usually involves rest, ice, compression, and elevation (RICE). Beyond the principle of short-term rest and ice, there is no clear consensus on treatment of muscle injuries.\(^5\)

In the laboratory setting, investigators have used toxins, lacerations, freeze damage, and contusions to study muscle damage, but by far the majority of muscle injuries during sports are attributable to excessive strain of an activated muscle (ie, forceful lengthening, or eccentric, contractions).\(^5,10,17\) Because muscle strains are so common, we used an animal model of contraction-induced injury. Some models that remove part or all of the muscle to perform load to failure or other such tests have certainly yielded useful information, but this is less representative of normal physiology. We used an in vivo model where the neurovascular supply and anatomical attachments are undisturbed, inducing a muscle strain injury under conditions that are as similar to the clinical scenario as possible, while preserving control over the biomechanical parameters to yield a consistent injury. One of the reasons muscle injury is difficult to study is that there are so many parameters to yield a consistent injury. Even with the in vivo model, the response to injury can vary widely based on the timing of activation, the amount of strain, the number of repetitions, and level of activation.

We compared the effects of conditioned PRP on 2 in vivo protocols of contraction-induced injury. The 90° arc of plantar flexion uses a large part of the available range of motion, but this magnitude of stretch is required to induce a detectable and reliable injury after just a single contraction, which we used to mimic an acute strain.\(^24\) The 60° arc of plantar flexion yields a significant and reliable injury, but only with multiple repetitions. Despite the different protocols, they yield a similar force loss,\(^25\) presumably because the amount of active strain (arc of motion in this study) is a key determinant of muscle injury.\(^20\)

Previously, we found that recovery of muscle contractile function after injury by a single, large-strain lengthening contraction involves sarcolemmal repair, whereas recovery from multiple, small-strain lengthening contractions requires muscle regeneration, with minimal sarcolemmal repair.\(^28\) Here we used both protocols to test the effects of autologous PRP on recovery. In the single-repetition protocol, use of PRP did somewhat improve the ability of the muscle to generate force, but only at the day 3 time point. Otherwise, the overall recovery—and time to full return of function—was not altered. Alternatively, in the multiple-repetition protocol, use of PRP resulted in significant improvement at several time points as well as a quicker return to full function. This is most likely attributable to the enhancement of myogenesis, a process required to recover from this protocol.

Myogenesis is not restricted to prenatal development but also occurs in regenerating muscle after some injuries. A large body of evidence suggests that individual growth factors play a role during muscle regeneration/myogenesis. Insulin growth factor-1, fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), and transforming growth factor-β1 (TGF-β1) are thought to be key regulators for myogenesis. For example, IGF-1 is able to stimulate the proliferation and differentiation of myoblasts (precursors of muscle cells) and improves muscle regeneration in mouse skeletal muscles.\(^27\) In vivo, FGF-2 enhances the
diameter and number of regenerating fibers.\(^1^9\) In vitro, HGF is able to activate quiescent satellite cells,\(^3\) the stem cells of skeletal muscle committed to a myogenic lineage. Transforming growth factor-\(\beta1\) supports other growth factors, specifically PDGF, which stimulates satellite cell activation.\(^1^4,2^8,3^1\) Satellite cells are dormant in healthy skeletal muscle but can be stimulated by injury to proliferate or differentiate into mononucleated myoblasts, which then fuse to form multinucleated myotubes. These myotubes can then form new skeletal muscle, replacing damaged or lost tissue.\(^4,1^6,3^4\)

Platelet-rich plasma contains up to 8 times the concentration of platelets found in whole blood,\(^7\) and these platelets contain \(\alpha\)-granules, which can release a multitude of growth factors, such as PDGF, IGF-1, TGF-\(\beta\), FGF, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and HGF.\(^7,8,3^2\) The fact that PRP contains several different growth factors, present in physiological proportions, is an appealing benefit compared with using isolated growth factors. Other advantages are that it is relatively simple and easy to obtain PRP from a human blood sample, and there is little risk of developing an immune response from autologous PRP.

Given the common nature of muscle strain injuries, a treatment that can improve recovery time could have a tremendous effect in athletics. Platelet-rich plasma can be isolated from a centrifuge about the size of a small microwave oven; this can increase the convenience and feasibility of using this treatment method in an athletic training room. After an injury, whole blood could be drawn from an athlete and centrifuged, and autologous PRP could be retrieved and injected at the site of injury. Conditioning of the PRP could be performed via sonication but necessarily under sterile conditions to avoid infection. This would be a convenient and cost-effective way to administer concentrated growth factors locally. Injection of isolated growth factors, even if effective, could be prohibitively expensive. A potential limitation to the use of PRP in the clinical setting, at least with elite athletes, is the concern expressed by the World Anti-Doping Association (WADA) regarding the use of growth factors in sports.\(^5\) One possible solution for professional athletes is to obtain an exemption from a WADA-approved anti-doping organization for therapeutic use.

The mechanisms for the improved muscle recovery resulting from the use of PRP after injury need to be elucidated. We have shown via RT-PCR analysis and counts of CNFs that myogenesis is enhanced with the use of PRP; however, this does not preclude other possibilities. Platelet-rich plasma may alter cytokine release, limit inflammation, or have other effects not yet examined. Although we did not attempt to quantify inflammation, the inflammatory process is likely altered in the presence of PRP. This may explain the improvement seen only at day 3 of recovery after the single-repetition protocol, as this is when inflammation peaks after a muscle strain.\(^2^9\) Another possibility is that muscle fiber membrane damage or repair is altered or that damage or repair of contractile and/or structural proteins is altered by the use of PRP. These and other hypotheses have not been rigorously tested.

We operationally defined muscle injury as a loss in the ability of the muscle to produce force. Torque of a muscle is represented by the equation \(T = F \cdot d\), where \(T\) is torque, \(F\) is muscle force, and \(d\) is the moment arm of the muscle. Because we use a maximal tetanic contraction and we measured torque at a fixed ankle position, our measure of torque ultimately reflects muscle force.\(^2^,2^1,2^2,2^3\) After the initial injury, there is sometimes another decrease in force, as seen after our single-repetition protocol (Figure 3A). This secondary injury is thought to be attributable to the increase in inflammation that occurs several days after the muscle strain and is also the presumed cause of delayed-onset muscle soreness.\(^3\) We did not see a secondary injury with the multiple-repetition protocol. Although it is not clear why, it could be that we missed a further decline in force production because of our selected time points. For example, force in the multiple-repetition protocol may have decreased even further than the initial injury, but within 24 or even 48 hours, well before our day 3 measurement.

An animal model provides several obvious advantages, such as control over force of contraction, type of contraction, lengthening velocity, diet, activity level, and access to tissues for analysis. Animal models also provide control over many threats to internal validity of a study, such as history, selection bias, and maturation. Yet, this study has several limitations. The first and most obvious is that findings from animal studies are not always applicable to humans. Second, the high level of control over experimental parameters (timing of contraction, arc of motion, etc) is less representative of the wide array of injuries that occur in humans. For example, we use maximal stimulation to recruit all motor units within the muscle (to obtain a consistent injury), but this is not representative of the graded recruitment of motor units that occurs in humans. A third possible limitation is that we did not identify which components of PRP are responsible for the improvements we found. However, because PRP is easily obtainable through several commercial centrifuge devices and no negative side effects have been reported, it may not be necessary to isolate the specific growth factors within PRP that account for the enhanced recovery from injury.

Like any experiment, this work raises new questions, which we hope to address in future work. We did not examine the effects of conditioned PPP. The ELISAs indicate no discernible difference with conditioning (Figure 2), but the ELISA results are based on only 2 representative growth factors. It is possible that a small fraction of platelets persisted in the PPP, containing other growth factors that could affect recovery from injury. Because of the significant increase in growth factors with high-frequency ultrasound (conditioning), our PRP treatment consisted of conditioned PRP. This allowed us to minimize the number of animals needed for the study and improved our chances of finding an effect. It is likely that the platelets from the unconditioned PRP would rupture during injection and still release their contents, either immediately or soon after injection, but we did not test this.

Another question is what type of strain injury PRP is appropriate for. Our results do not determine whether PRP...
would be beneficial for an acute strain versus an overuse injury in humans. It is clear that injuries that require myogenesis are better candidates for PRP therapy, but muscle injury is highly variable between subjects. Finally, we do not know what dose and delivery methods are optimal. We did not test various permutations of frequency, amount, and duration of injections, and it is likely that other delivery methods may work as well as or better than repeated injections.

To our knowledge, this is the first study to use PRP in a model of muscle strain injury. We demonstrated that PRP extract can hasten recovery from a muscle strain injury and that enhanced myogenesis is the probable mechanism underlying this effect. Delivery of growth factors at the site of injury is a potential therapy to treat muscle injuries, and because autologous blood products are safe, PRP may be a useful product in treatment of muscle injuries.

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CHEMOTACTIC AND MITOGENIC Stimulation of HUMAN MESENCHYMAL STEM CELLS BY PLATELET RICH PLASMA SUGGESTS A MECHANISM FOR ENHANCEMENT OF BONE REPAIR

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INTRODUCTION

Platelets are known to perform multiple functions during injury and tissue repair. While their role in hemostasis is well understood, their mechanism of action in promoting wound healing requires further characterization. As a repository of multiple growth factors such as PDGF, EGF, VEGF, and TGF-β, degranulation of platelets at wound sites serves to initiate or enhance the healing cascade. Armed with this knowledge, clinicians have used platelet concentrates in conjunction with bone graft materials to enhance osseous repair. In addition, experimental evidence has shown that when PDGF or platelet concentrates are combined with demineralized bone or certain other materials, augmentation of bone formation ensues.

The purpose of this study was to begin elucidating the cellular mechanisms that underlie these observations. Since mesenchymal stem cells (MSCs) are known to be an essential component of the tissue repair process, we sought to characterize elements of their response to platelet concentrates in the controlled in vitro environment.

PLATELET CONCENTRATION

Platelet rich plasma (PRP) was isolated from approximately 55 ml of fresh human blood (IRB-approved protocol) using the Symphony™ Platelet Concentration System (DePuy AcroMed, Raynham, MA), designed to be used at the point-of-care for obtaining a platelet concentrate from a small amount of blood. Samples of the starting material and platelet concentrates were analyzed to determine the absolute concentrations and yields of platelets. PRP, platelet poor plasma (PPP) and whole blood were clotted with thrombin (1000 U/ml in 10% CaCl₂) by adding 1 part thrombin stock solution to nine parts PRP, PPP or blood to yield a final thrombin concentration of 100 U/ml. The soluble platelet releasates from the clotted preparations were isolated by centrifugation and cleared by ultrafiltration.

PRP and PPP releasates were diluted in serum-free DMEM to generate appropriate final dilutions of platelet releasate. Similar to previously published results, we obtained very high efficiency of platelet concentration. Also, the efficiency of the platelet concentration was reproducible across the various samples as can be seen by the low standard deviation. The specific levels of various growth factors were not measured in this study. However, it has been previously reported that in platelet concentrates processed using the current system, the concentration of growth factors increase linearly with the platelet concentration.
CELL PREPARATION

In order to evaluate the mitogenic activity of PRP, human MSCs (hMSCs) were isolated and culture-expanded from bone marrow (IRB-approved protocol) using published techniques. The growth media (GM) for the selection and culture-expansion of hMSCs consisted of DMEM supplemented with 10% fetal bovine serum (FBS). The specific lot of FBS was chosen for its ability to optimize MSC selection and growth.

Control media consisted of serum free medium (SF), or DMEM supplemented to 10% (v/v) with the following preparations: PPP releasate alone, or serum from clotted peripheral blood (PB). Test media consisted of DMEM supplemented with undiluted PRP releasate or PRP releasate diluted with PPP, such that the final concentration of PRP releasate ranged from 0.625- to 10-fold of that in media supplemented with peripheral blood. To achieve the 5-fold concentration and the 10-fold platelet concentration, the PRP releasate was added to the media at 10% and 20% (v/v), respectively. To achieve the lower platelet concentrations, PRP diluted with an appropriate amount of PPP was added to the media at 10% (v/v).

CHEMOTACTIC MIGRATION

The ability of concentrated platelet releasate to stimulate the chemotactic migration of hMSCs was measured using a Neuroprobe AC48 Boyden Chamber with 5 µm pore size polycarbonate filters. 7,500 hMSCs in 50 µl serum-free medium were added to the upper chambers of each well. Lower chambers contained test media. Cells were allowed to migrate for 4 hours at 37°C, at which time non-migratory cells were scraped from the filter. Migratory cells on the underside were stained with crystal violet dye and counted. PRP releasate and VEGF each stimulate chemotactic migration of hMSCs in a dose-dependent manner. Since VEGF is a component of PRP releasate, it is at least partially responsible for the chemotactic activity of PRP releasate on hMSCs.

Photomicrographs of hMSCs after chemotaxis due to PRP releasates and proper controls (original magnification 200x).

Chemotactic Migration of hMSCs in Response to Various Media Additives

Chemotactic Migration of hMSCs in Response to PRP Releasate

PRP-Releasate Stimulates Dose-Dependent Chemotactic Migration of hMSCs

VEGF Stimulates Dose-Dependent Chemotactic Migration of hMSCs
However, extrapolating from the reported levels of VEGF in the PRP, it is clear that VEGF by itself is unlikely to account for the majority of the chemotactic effects of PRP. Other known chemotactic molecules, such as TGF-β probably contribute to the chemotactic response of PRP.

**MITOGENIC STIMULATION**

In order to evaluate the mitogenic activity of PRP, second passage hMSCs were replated at a density of 3x10^3/cm^2 in serum-free DMEM. Cells were allowed to attach and incubate for 48 hours, at which time culture medium was replaced with the various media. hMSCs were allowed to incubate in test and control media for 7 days with complete media changes taking place on day 4. At the end of the 7 day time course, cells were released with trypsin and counted with a hemocytometer.

PRP releasate stimulates proliferation of hMSCs in a dose-dependent manner. While these experiments demonstrate that serum from a fresh human blood clot, and even PPP, can stimulate hMSC proliferation, approximately 90% of the mitogenic activity in PRP is derived from the platelet releasate.

**OSTEOGENIC DIFFERENTIATION**

The ability of PRP to support mitotic expansion of hMSCs without loss of their osteogenic potential was demonstrated by first expanding hMSCs in DMEM-LG supplemented to 10% with 5x PRP releasate, PPP releasate, or GM. After 5-7 days of mitotic expansion in the various test media, hMSCs were harvested and reformatted at 3x10^3/cm^2 and allowed to attach overnight in serum-free DMEM-LG. The next day, culture media were switched to standard GM plus Osteogenic Supplements (OS) consisting of 10^-7M dexamethasone, 10m M beta-glycerophosphate and 50 μM ascorbic acid-2-phosphate. On days 4, 8, 12 and 16 cultures were analyzed for alkaline phosphatase expression and calcium deposition into the cell layer.

As expected, PRP releasate by itself does not cause osteogenic differentiation of hMSCs. In hMSC samples grown in the presence of PRP releasate without OS, the dominant effect was proliferation with no evidence of differentiation. This trend continued in samples grown with PRP (or PPP) releasates plus OS, as cell proliferation was nearly double that observed in GM plus OS samples. The net effect of this potent mitogenic activity was to keep cells cycling, thus preventing their entry into the osteogenic differentiation pathway.

In samples that were exposed to osteogenic differentiation signals, after rapid expansion in the PRP-supplemented media, the levels of various osteogenic markers were similar or greater than those observed in the controls when normalized to a per cell level. Thus, mitogenic stimulation of hMSCs by PRP releasate occurs without alteration of the cell's phenotype or the loss of its osteogenic development potential. Furthermore, the proliferation rate continued to be higher in the samples initially expanded in PRP, thus leading to an overall increase in osteogenic matrix output in these samples as compared to the controls. This effect was similar to data generated by Slater, et al. using human fetal osteoblastic cells.
CONCLUSIONS & DISCUSSION

- PRP releasate and Vascular Endothelial Cell-Derived Growth Factor (VEGF) each stimulate chemotactic migration of hMSCs in a dose-dependent manner.
- PRP releasate stimulates proliferation of hMSCs in a dose-dependent manner. Approximately 90% of the mitogenic activity in PRP is derived from the platelet releasate.
- Mitogenic stimulation of hMSCs by PRP releasate occurs without alteration of the cell's phenotype or loss of its osteogenic developmental potential.

These observations are consistent with in vivo wound healing models in which degranulated platelets initiate or enhance the healing cascade through the transient chemotactic attraction and mitotic stimulation of reparative cells, which is then followed by morphogenic signals from other sources that induce cell differentiation. These studies represent the first published data showing a direct effect of PRP releasate on purified human MSCs, which play a pivotal role in the process of musculoskeletal tissue repair. The observation that this easily prepared, autologous source of concentrated growth factors possesses chemotactic and mitogenic activity lends further credence to its therapeutic role in clinical orthopaedics. In view of the data presented, we suggest that local application of PRP causes migration of hMSCs to the wound site, followed by their massive replication to form a repair blastema. As the bioactive factors diffuse away from the fibrin scaffold, now densely populated by hMSCs, the cells cease dividing and are primed to respond to the endogenous inductive cues that stimulate differentiation. The local and transient activity of PRP in this model of tissue repair is responsible for initiating and accelerating the natural healing cascade.

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Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions

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Abstract Platelet-rich plasma (PRP) is a natural concentrate of autologous blood growth factors experimented in different fields of medicine in order to test its potential to enhance tissue regeneration. The aim of our study is to explore this novel approach to treat degenerative lesions of articular cartilage of the knee. One hundred consecutive patients, affected by chronic degenerative condition of the knee, were treated with PRP intra-articular injections (115 knees treated). The procedure consisted of 150-ml of venous blood collected and twice centrifuged: 3 PRP units of 5 ml each were used for the injections. Patients were clinically prospectively evaluated before and at the end of the treatment, and at 6 and 12 months follow-up. IKDC, objective and subjective, and EQ VAS were used for clinical evaluation. Statistical analysis was performed to evaluate the significance of sex, age, grade of OA and BMI. A statistically significant improvement of all clinical scores was obtained from the basal evaluation to the end of the therapy and at 6–12 months follow-up ($P < 0.0005$). The results remained stable from the end of the therapy to 6 months follow up, whereas they became significantly worse at 12 months follow up ($P = 0.02$), even if still significantly higher respect to the basal level ($P < 0.0005$). The preliminary results indicate that the treatment with PRP injections is safe and has the potential to reduce pain and improve knee function and quality of live in younger patients with low degree of articular degeneration.

Introduction

The incidence of articular cartilage pathology has grown due to the marked increase in sports participation and greater emphasis on physical activity in all age groups [5]. Unfortunately, articular cartilage lesions, with their inherent limited healing potential [1, 3, 16, 26], are hard to treat and remain a challenging problem for orthopedic surgeons.

A variety of agents, such as nonsteroidal anti-inflammatory drugs, glucosamine, chondroitin-sulphate, hyaluronic acid, and glucocorticoids have been proposed as non-invasive solutions for pain treatment, improvement in function, and disability, and ultimately modification [10] of severe
chondral degeneration and osteoarthritis with varying success rates. The initial pharmacologic management typically begins with analgesia and anti-inflammatory agents, through acetaminophen and NSAIDS [12]: the potential cardiovascular and gastrointestinal toxicity, the large apparent variation in the individual response to each drug and the absence of clear clinical data regarding the therapeutic potency could represent limits for a correct administration of symptoms [31]. Topical agents have only been proven useful for short-term use of mild-to moderate pain in osteoarthritis [14]. Intra-articular injections of corticosteroids, indicated by some studies, are of short-term benefit [22]. Moreover, some evidence suggests that they are not able to alter the natural history of the disease and may have deleterious consequences on knee structures [18]. Glucosamine, chondroitin-sulphate, and intra-articular hyaluronic acid have not been clearly demonstrated to be effective either, and due to the continuing controversies and lack of common accepted beneficial evidence should not be considered ideal procedures for the treatment of chronic severe chondropathies or osteoarthritis [4, 20].

Current research is investigating new methods of stimulating repair or replacing damaged cartilage, such as matrix metalloproteinase inhibitors, gene therapy, cytokine inhibitors, artificial cartilage substitutes, and growth factors [29]. In particular, the most recent knowledge regarding tissue biology highlights a complex regulation of growth factor for the normal tissue structure and the reaction to tissue damage. The influence of the growth factors in cartilage repair is now being widely investigated in vitro and in vivo [6, 9, 11, 25, 28]. Platelet-rich plasma (PRP) is a natural concentrate of autologous growth factors from the blood. The method is simple, low cost, and minimally invasive. Currently, a wide range of experiments is taking place in different fields of medicine in order to test the potential of enhancing tissue regeneration [2, 22].

The aim of our study is to explore this novel approach in treating degenerative lesions of articular cartilage. The objective of this pilot study is first to evaluate the safety of our protocol, by gathering and assessing the number, timing, severity, duration, and resolution of related adverse events. The second aim of the study is to analyze the short-term results obtained, to determine feasibility, indication criteria, and application modalities for further wider studies.

Our hypothesis is that the utilization of PRP could bring a stimulation of the chondral anabolism and a reduction of the catabolic processes. PRP may also influence the overall joint homeostasis, reducing synovial membrane hyperplasia.

Materials and methods

One hundred consecutive patients were enrolled and treated with PRP intra-articular knee injections. The following criteria for patient selection were used: history of chronic (at least 4 months) pain or swelling of the knee and imaging findings (radiograph or MRI) of degenerative changes in the joint. Exclusion criteria were systemic disorders, such as diabetes, rheumatoid arthritis, major axial deviation (varus > 5°, valgus > 5°), hematological diseases (coagulopathies), severe cardiovascular diseases, infections, immunodepression, patients in therapy with anticoagulants–antiaggregants, use of NSAIDs in the 5 days before blood donation, patients with Hb values of <11 and platelets values of <150,000/mmc. Ninety-one patients were prospectively evaluated at a 2, 6, and 12 months follow-up, 5 were lost at follow up, whereas 4 were excluded from the study because they did not complete the treatment: 3 patients stopped the treatment after the first injection for personal reasons and, in the other case, we decided to stop treatment because of a marked swelling and pain response after the injection. The patients analyzed were 57 men and 34 women, with a median age value of 47 years (range 24–82). Sixty-seven patients were affected by a monolateral lesion, whereas 24 patients presented a bilateral lesion, for a total of 115 knees treated. The mean BMI was 25 ± 3 (ranging from 18 to 32) and 27 patients had previously undergone knee surgery. All the patients presented a chronic degenerative condition; 58 knees presented a degenerative chondral lesion (Kellgren 0), 33 an early osteoarthritis (Kellgren I–III), while 24 knees had advanced osteoarthritis (Kellgren IV).

Platelet-rich plasma preparation

The procedure consisted of a 150-ml venous blood sample (collected in a bag containing 21 ml of sodium citrate) taken for every lesion treated. A complete peripheral blood count was also collected at the time of the initial blood draw. Then two centrifugations (the first at 1,800 rpm for 15 min to separate erythrocytes, and a second at 3,500 rpm for 10 min to concentrate platelets) produced a unit of 20 ml of PRP. All the procedures were performed in the same office setting. The unit of PRP was divided into 4 small units of 5 ml each. All the open procedures were performed in an A-class sterile hood. One unit was sent to the laboratory for analysis of platelet concentration and quality tested (platelet count and bacteriological test), one unit was used for the first injection within 2 h, and the other 2 units were stored at −30°C. The total number of platelets per millilitre in the PRP represented a mean increase of 600% compared with whole blood values, and an average of 6.8 million platelets were given to the lesion site at every injection. Injections were administered every 21 days; for the second and third treatments, the samples were thawed in a dry-thermostat at 37°C for 30 min just before application. Before the injection, 10% of
Ca-chloride (\(\text{Ca}^{2+} = 0.22 \text{ mEq} \times \text{dose}\)) was added to the PRP unit to activate platelets.

Treatment procedure and follow-up

The skin was sterilely dressed and the injection was performed through a classic lateral approach using a 22-g needle. At the end of the procedure, the patient was invited to bend and extend the knee a few times, to allow the PRP to distribute itself throughout the joint before becoming gel (Fig. 1). After the injection, the patients were sent home with instructions to limit the use of the leg for at least 24 h and to use cold therapy/ice on the affected area for pain. During this period, the use of non-steroidal medication was forbidden. During the treatment period, rest or mild activities (such as an exercise bike, mild exercises in pool) were indicated, and subsequently the gradual resumption of normal sport or recreational activities was allowed as tolerated. All complications and adverse events were recorded. Patients were prospectively clinically evaluated before the treatment, at the end of the treatment (2 months after the first injection) and at the 6 and 12 month follow-ups. All results are presented as the number of knees (not the number of individuals). IKDC, objective and subjective, and EQ VAS were used in clinical evaluation. The patient’s satisfaction was also recorded.

Statistical analysis

All statistical analyses were carried out using the SPSS (Statistical Package of Social Sciences, Chicago, IL, USA) for Windows software program version 13.0. A \(P\) value of less than 0.05 was considered statistically significant. The results were expressed as mean ± SD. The Wilcoxon test, the Mann–Whitney test, the Paired \(T\) test, the Kruskal–Wallis test and the One Way ANOVA test were used to test for significant differences between baseline band various follow-up measurements. The Spearman’s and the Pearson’s statistical correlations were used to determine the parameters that statistically influenced the clinical outcome.

This clinical experimentation was approved by the Hospital Ethics Committee and the informed consent of all patients was obtained before the treatment.

Results

No major adverse events related to the injections were observed during the treatment and follow-up period. In only one case, a patient presented a marked pain response with swelling after the injection, which spontaneously resolved itself after 2 weeks. In some cases, slight pain was present during first 2 or 3 days. A statistically significant improvement of all clinical scores was obtained from the basal evaluation, to the end of the therapy and then at both the 6 and 12 month follow-ups. Eighty per cent (73/91) of patients were satisfied with their treatment results. The IKDC objective score passed from 46.1% of normal and nearly normal knees before the treatment (9A, 44B, 42C, and 20D) to 78.3% of normal and nearly normal knees (37A, 53B, 18C, and 7D) at the end of the therapy, then to 73.0% (36A, 48B, 20C, and 11D) and 66.9% (32A, 45B, 23C, and 15D) at 6 and 12 month follow-ups, respectively, showing a statistically significant improvement \((P < 0.0005)\) at each of the follow-up times with respect to the basal level. The improvement was maintained from the end of the therapy to the 6 month follow-up, with an only slight tendency of worsening \((P = 0.08)\), whereas a statistically significant decrease in the results was observed in the period between the 6 and 12 month follow-ups \((P = 0.018)\). Similarly, the IKDC subjective score improved markedly from the basal evaluation to the end of therapy and the follow ups at 6 and 12 months \((P < 0.0005)\), passing from 40.5 ± 10.4 before the treatment to 62.5 ± 15.9 at 2 months and 62.6 ± 18.6 and 60.6 ± 18.9 at the 6 and 12 month follow-ups, respectively. The results remained stable from the end of the therapy to the 6 month follow-up, whereas they became significantly worse at the 12 month follow-up \((P = 0.02)\),
even if still significantly higher with respect to the basal level \((P < 0.0005)\) (Fig. 2). The same trend was confirmed by the EQ VAS evaluation, which improved from 50.3 ± 16.4 to 71.2 ± 15.2 at 2 months, 70.6 ± 17.5 at 6 months and 69.5 ± 17.4 at the final evaluation, with statistically significant higher scores at all the follow-up times with respect to the basal level \((P < 0.0005)\), and a tendency (even if not statistically significant in this case: \(P = 0.2\)) of worsening over time (Fig. 3).

In order to establish the indications for this type of treatment, we tried to determine the parameters that influenced the clinical outcome. We found overall lower objective scores in older patients, before the treatment \((P = 0.043)\) and at the different follow up times \((P = 0.005\) at 2 months, \(P = 0.001\) at 6 months and \(P = 0.013\) at 12 months), and most importantly, a scarce response to the treatment, with a lower improvement at the 6 months follow-up \((P = 0.049)\) with respect to younger patients. A lower effect of the platelet concentrate in older patients was also confirmed analyzing the IKDC subjective evaluation \((\text{rho} = -0.331, P < 0.0005)\) and EQ VAS scores \((\text{rho} = -0.389, P < 0.0005)\) (Fig. 4). However, older patients also presented more severe changes of the joint \((P < 0.0005)\), and the degree of articular degeneration was also significantly correlated to the clinical outcome (Table 1; Fig. 5). While analyzing older patients (>65 years) affected by advanced osteoarthritis separately, we found a significant improvement in the IKDC subjective evaluation in only 30% of the cases (3/10). Clinical results were not influenced by previous surgery. Finally, further analysis showed worse results were shown in women \((P < 0.0005)\) in the subjective evaluation, and a significantly lower improvement at the 2 months follow-up in patients with higher BMI \((\text{rho} = -0.187, P = 0.045)\), with a similar tendency at the 6 months follow-up \((\text{rho} = -0.1637, P = 0.08)\).

Discussion

The most important finding of the present study was to investigate this novel biological approach for the treatment of knee degenerative pathology. In recent years, there has been an increasing prevalence of the use of autologous blood products that might provide cellular and humoral mediators to favor tissue healing in a variety of applications. The rationale is based on the activity of blood growth factors. The growth factors are a diverse group of polypeptides that have important roles in the regulation of growth and tissue development, determining the behavior of all cells, including chondrocytes. The understanding of their effects on chondrocytes is progressing rapidly and many growth factors have been identified as aiding in the regulation of articular cartilage. Most of the studies include the transforming growth factor-beta super-family (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF) [17].

In particular, TGF-β is one of the most important factors involved in the process of cartilage regeneration; its functions include the increase of chondrocyte phenotype expression [19, 27], the chondrogenic differentiation of mesenchymal stem cells [15, 29], matrix deposition [6] and counteract with most of the suppressive effects of inflammatory mediators IL1 on cartilage-specific macromolecules synthesis [19]. PDGF also plays an important role in the maintenance of hyaline-like chondrogenic phenotype, increases chondrocyte proliferation, upregulates proteoglycan synthesis, and is a potent chemotactic factor for all cells of mesenchymal origin [25]. IGF is
another important cartilage anabolic factor [13] and it may have a role in augmenting the effects of other growth factors found in cartilage [17, 29]. Many other growth factors are involved in cartilage regeneration and metabolism, like FGF and HGF, and they may have chondro-inductive actions, independently or even more so with additive effects and synergistic interaction [17]. PRP is a blood product that allows in a simple, low cost, and minimally invasive way to obtain a concentration of many of these growth factors [6, 9, 11, 25, 28]. Platelets contain storage pools of growth factors including PDGF, TGF β, IGF-1, FGF and many others. Cytokines, chemokines, and newly synthesised metabolites are also released [22]. PRP derived from centrifugation of autologous whole blood contain a platelet concentration four to five times higher than that of normal blood. The platelet concentrate is activated by the addition of calcium chloride, and this results in the formation of platelet gel and the release of a cascade of growth factors. The administration in the form of platelet gel provides an adhesive support that can confine secretion to a chosen site [2].

Blood derived growth factors have already been studied for their potential in helping cartilage repair and documented in the literature. Frisbie [7] administrated autologous conditioned serum (ACS) in horses with experimentally induced osteoarthritis, obtaining significant clinical improvement in lameness, decreased synovial membrane hyperplasia, less gross cartilage fibrillation, and synovial membrane hemorrhage and an increased synovial fluid concentration of interleukin-1 receptor antagonist. Gaissmaier [8] investigated the effect of human platelet supernatant (hPS) on chondrocyte proliferation and differentiation and concluded that addition of hPS may accelerate chondrocyte expansion, even though it can also lead to their dedifferentiation. Saito [21] documented preventive effects against OA progression with the administration of gelatin hydrogel microspheres containing PRP in a rabbit model. PRP has also been used as injectable scaffold for tissue engineering. Wu [30] investigated the feasibility of PRP to support chondrogenesis: the gelled PRP provided a 3-dimensional environment for seeded chondrocytes and was successfully used to deliver chondrocytes in cartilage defects in a rabbit model. Sánchez [24] described a case report where plasma rich in growth factors was used to treat an articular cartilage avulsion in a soccer player, obtaining an accelerated and complete articular cartilage healing. Finally, he reported [23] preliminary results about the effectiveness of intra-articular injections of an autologous preparation rich in growth factors for knee OA treatment in

Fig. 4 Correlation between age and clinical outcome: older patients obtained worse IKDC subjective results at all the follow up times (2, 6, and 12 months) evaluated
an observational retrospective cohort study on 30 patient, suggesting the safety and usefulness of this treatment approach.

These studies and others suggest an important role for these potent biological regulators of chondrocytes in cartilage repair. However, for the time being, the evidence base for PRP clinical use is still in its infancy, and there are only a few papers that specifically address treatment applications in the orthopedic field.

The primary objective of this pilot study was to evaluate the safety of our protocol, by gathering and assessing the number, timing, severity, duration, and resolution of related adverse events. No complications such as infection, marked muscle atrophy, deep vein thrombosis, fever, hematoma, tissue hypertrophy, adhesion formation, or other major adverse events occurred among study subjects. Only minor adverse events were detected, such as a mild pain reaction and effusion after the injections, which persisted for not more than 2 days, except in one case where marked pain and swelling were successfully treated in 2 weeks (in this case, we preferred to stop the treatment).

The secondary aim of the study was to evaluate the preliminary results obtained, in order to determine the feasibility and potential of this new therapeutic approach, and to analyze indication criteria and application modalities for further studies. For this purpose, a group of 100 consecutive patients affected by degenerative pathology of articular knee cartilage was enrolled for the treatment with autologous PRP via multiple injections. We analyzed 91 patients (115 knees) who completed the treatment and were available for the 2, 6, and 12 months follow-up, and obtained a statistically significant improvement in all the parameters evaluated. The good results achieved at the end of the therapy were maintained at the 6 months follow-up, whereas a tendency of worsening was observed at 1-year evaluation. However, even if a significant improvement was demonstrated, the mean clinical outcome achieved allowed most of the patients only a normal daily activity life. In any case, due to the high average age, the low score at the end of the therapy was often explained by the low patient activity level, rather than from any persistent knee pain or functional limitation. Further analysis was performed in order to evaluate the influence of the different variables. Better results were achieved in younger patients, and a similar correlation was observed in the most degenerated joints. This could be expected and easily explained by the low percentage of living and vital cells and therefore the low response potential to the growth factors. In addition, extensive joint damage in severe osteoarthritis is hardly reversible. A correlation was also found with sex, with worse results in women. Results were also correlated with the patients BMI: at 2 months, worse results were found in patients with a higher BMI; the low number of overweight patients in our study, however, did not allow us to better analyze the influence of weight at the

Table 1  Correlation between the degree of joint degeneration and the clinical outcome at different follow-up times

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>2 months</th>
<th>6 months</th>
<th>12 months</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degenerative chondropathy</td>
<td>50.9 ± 15.6</td>
<td>75.6 ± 12.6</td>
<td>78.0 ± 13.7</td>
<td>78.9 ± 13.3</td>
<td>NS</td>
</tr>
<tr>
<td>Early osteoarthritis</td>
<td>51.8 ± 17.2</td>
<td>71.6 ± 14.9</td>
<td>67.5 ± 18.9</td>
<td>69.2 ± 17.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Advanced osteoarthritis</td>
<td>50.3 ± 16.4</td>
<td>60.0 ± 16.4</td>
<td>57.1 ± 15.0</td>
<td>56.9 ± 15.9</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

Fig. 5  Patients with degenerative chondropathy achieved better results with respect to patients affected by early osteoarthritis, who presented a higher improvement compared to patients with advanced osteoarthritis.
different follow up times. Finally, other factors, such as previous surgery, did not influence the clinical outcome. Unexpectedly good results, however, were also obtained in three cases in older patients with severe osteoarthritis. We suppose that this could be explained by the fact that injected platelets may act at different levels and are not stimulating the chondral anabolism or slowing the catabolic processes. PRP may also influence the overall joint homeostasis, reducing synovial membrane hyperplasia and modulating the cytokine level, thus leading to an improvement in the clinical outcome, even if only temporarily and without affecting the cartilage tissue structure and joint degenerative progression [7]. Further studies are needed to confirm the results obtained and to understand the mechanism of the action, evaluating if there is only a temporary symptom improvement or if PRP may also play a more important role through disease modifying properties. The limitations of this study are the lack of a control group and the fact that the evaluation of the results only took place at a short term follow up. We analyzed our patients at a maximum follow up period of 12 months because, as for other injective therapies, the treatment can be repeated after a certain time interval. In any case, we believe that the main benefit of this type of therapy is expected in the short term. Promising results were obtained regarding safety, feasibility and the short-term effectiveness of this treatment option. This report documents our experience on autologous platelet-derived growth factor injections as a treatment of knee cartilage degeneration, which may represent a low-invasive and safe alternative in patients with chondropathy or early osteoarthritis. We suppose that PRP injections have a clinical relevance reducing inflammatory and degenerative articular processes and improving knee function and quality of life.

Conclusion

The preliminary short-term results of our pilot study are encouraging and indicate that treatment with autologous PRP intra-articular injections is safe, and may be useful for the treatment of early degenerative articular pathology of the knee, aiming to reduce pain and improve knee function and quality of life. However, randomized controlled studies will be needed to confirm the real potential and to evaluate the durability of this procedure, to better identify indication criteria and to improve application modalities. Further studies evaluating this new technique for treating cartilage degenerative pathology are in progress.


References

Treatment of Tendon and Muscle Using Platelet-Rich Plasma

Allan Mishra, MDa,*, James Woodall, Jr., MDb, Amy Vieira, PA-Ca

**KEYWORDS**
- Platelet-rich plasma • PRP • Growth factors • Tendon
- Muscle • Tendonitis

Musculoskeletal injuries and impairments result in over 100 million office visits in the United States per year. Tendons and muscle-related issues account for a significant percentage of these visits. As our population ages and remains active, the number of orthopedic-related problems will rise dramatically.1 Younger and older patients expect faster recovery from their injuries with less invasive procedures. Within this landscape, PRP has become a potential standalone or adjunctive treatment.

The concept of using the growth factors within PRP to help heal wounds dates back to the early 1980s.2 Its use in orthopedic surgery, however, began during this decade and initially focused on the augmentation of bone grafting. The efficacy of PRP to accelerate bone healing continues to be debated in the literature.3–8 Employing PRP to augment tendon healing, however, has been advocated only recently.9,10

**PLATELET-RICH PLASMA BIOCHEMISTRY**

PRP is a bioactive component of whole blood. The specific elements of PRP have not been uniformly defined in the literature. PRP, in general, has a higher concentration of platelets compared with baseline blood. Clinically valuable PRP, however, typically contains 1 million platelets or more per microliter.11 Some authors define PRP as only platelets whereas others note that PRP may also have increased concentrations of white blood cells. The white blood cells within some forms of PRP contain important cytokines and enzymes. For example, Horsburgh and colleagues12 found that platelet-derived mediators may be responsible for increased monocyte adherence in vitro. This adherence may be important for long-term tissue regeneration that is macrophage-based.

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mediated. Importantly, in vitro studies have also found that PRP significantly inhibits the growth of *Staphylococcus aureus* and *Escherichia coli*. In one of these studies, PRP was found to have no activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, or *Enterococcus faecalis*.13,14

PRP activation and the pH of PRP are other parameters that are being debated in the literature. Thrombin and calcium have historically been used to activate platelets. This combination results in the formation of a gel that may be used in open surgery but cannot be injected even through a large-gauge needle. Thrombin and calcium activation results in rapid release of contents of the granules within platelets. This requires immediate use of the PRP. Platelets, however, can be slowly activated by exposure to tendon-derived collagen.15 This can produce in vivo activation and allows for administration of PRP through a small-gauge needle. Variations in partial activation with calcium are also being explored.16 Liu and colleagues17 have also found the release of growth factors from PRP to be pH dependent.

When platelets are activated either ex vivo or in vivo, they release the growth factors and proteins that reside within their alpha and dense granules. The alpha granules contain cytokines including platelet-derived growth factor, transforming growth factor-β, and vascular endothelial growth factor, among many others (Table 1).18 Concentrations of these growth factors rise linearly with increasing platelet concentration.11,19 After release, the cytokines are free to bind to transmembrane receptors on the surface of local or circulating cells. They then initiate intracellular signaling, which results in the expression of proteins responsible for cellular chemotaxis, matrix synthesis, and proliferation. Tissue regeneration through angiogenesis, extracellular matrix production, and collagen synthesis is orchestrated by the autocrine and paracrine effects of the growth factors. Everts and colleagues20 have elegantly outlined the electron micrographic properties of how PRP releases these proteins. Properly prepared PRP in an unactivated form clearly reveals an abundance of platelets in a photomicrograph at high power (Fig. 1).21

Much emphasis has been placed on alpha granules but dense granules also play a role in tissue modulation and regeneration. The dense granules contain adenosine, serotonin, histamine, and calcium.

Adenosine is a nucleoside that plays an important role in many biochemical processes, including transfer of energy. Adenosine is a primary cytoprotective agent that prevents tissue damage. Adenosine receptor activation has been shown to have an anti-inflammatory effect during the inflammatory process associated with diabetic nephropathy.23 In laboratory studies, adenosine A2A receptor agonists applied topically to diabetic foot wounds have been effective in tissue repair and reconstruction and their effect on difficult wounds in humans is currently under investigation.24 Adenosine also has the ability to increase IL-10 production by macrophages in some cases.25 This increase in IL-10 could indicate a change in the character of the

### Table 1

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Actions</th>
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<tbody>
<tr>
<td>Platelet-derived growth factor</td>
<td>Chemoattractive for mesenchymal stem cells and monocytes</td>
</tr>
<tr>
<td>Transforming growth factor- β</td>
<td>Mitogen for fibroblasts and enhances extracellular matrix production</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
<td>Stimulates angiogenesis</td>
</tr>
</tbody>
</table>

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macrophage to an anti-inflammatory state. In other instances, it appears that adenosine can function to activate macrophages to produce pro-inflammatory cytokines, IL-1 and IL-18.26

Serotonin is a monoamine neurotransmitter. This hormone can be exponentially more effective at increasing capillary permeability than even histamine.27 Serotonin also acts as a chemoattractant for fibroblasts and increases their proliferation. Interestingly, macrophage cells have receptors that are sensitive to serotonin. Serotonin injected locally into tissue induces an influx of macrophages into that tissue.28 Serotonin has also been shown to effect macrophages by suppression of IFN-gamma-induced 1α expression at sites of inflammation.29 These data suggest strong interactions between serotonin and macrophages. This relationship and the particular effect serotonin has on cellular interactions should be considered when evaluating the effects of PRP on inflammation and healing.

Histamine is a biogenic amine involved in local immune responses. Locally, it also acts as a vasodilator. Histamine enhances permeability of the microvascular system of capillaries and venules. This increased permeability is due to the contraction of endothelial cells and removal of fenestrated diaphragms blocking gaps in the endothelial lining.30 At the time of injury, histamine is released, acting as a vasodilator that actively increases endothelial membrane permeability. This increase in membrane permeability allows inflammatory and immune cells greater access to marginate and enter the local area. Histamine is also a strong activator of macrophages.

Calcium is the final component of the dense granules. Involvement of calcium in wound healing is mainly in keratinocyte proliferation and differentiation. Skin fibroblasts require calcium but are far less sensitive than keratinocytes to its effects. Calcium may also be required in epidermal cell migration and regeneration in the remodeling phase. Although calcium dressings are meant to serve in the hemostatic phase of healing, it is unclear whether their effect carries over into the later remodeling phase. The effect of calcium is essential in wound management, and the calcium content within the dense granules of platelets may play a vital role in its delivery to the site of injury.31

The unique combination and concentration of bioactive molecules that exist within PRP have profound effects on the inflammatory, proliferative, and remodeling phases of wound healing. Researchers worldwide are evaluating how PRP produces these effects. Not all cytokines within PRP have been characterized. These cytokines also exist in hyperphysiologic concentrations in PRP when compared with whole blood.
Since healing of tendon and muscle is similar to wound healing in some respects, PRP has great potential to improve soft tissue healing. The concept of using PRP to restore tendons and muscle after injuries is explored in this context.

TENDON INJURIES AND HEALING

Tendon injuries and disorders come in many forms (Table 2). The generic term, tendinopathy, is best used to describe these many forms. The spectrum of problems ranges from acute tendonitis to chronic tendinosis to full-thickness tearing. Extrinsic factors, for example, a hooked acromion in the shoulder, combined with intrinsic factors, such as age-related degeneration, can contribute to tendinopathy. Repetitive microtrauma or exposure to fluoroquinolone antibiotics has also been implicated. Genetic factors, matrix metalloproteases (MMPs), and apoptosis may further contribute to tendon degeneration.

Tendon healing occurs through 3 phases: inflammation, proliferation, and remodeling. These overlapping phases are controlled by a variety of growth factors. They are also linked through complex cellular signaling cascades. For example, the temporal expression of growth factors has been reported to be important in supraspinatus tendon healing. Since PRP contains many of these cytokines and cells in hyperphysiologic doses, it may be a reasonable choice to help initiate or accelerate tendon healing. The use of PRP for tendon disorders is presently being investigated for significant tendon disorders, such as chronic severe tendinosis, or in combination with surgery for complete tendon tears.

**Use of PRP in Tendinopathy**

In vitro studies have found that PRP can enhance human stromal and mesenchymal stem cell proliferation. Conversely, Woodall and colleagues found that PRP suppresses macrophage proliferation and IL-1 production within the first 72 hours after exposure. This differential induction of cells has important implications for tendon and muscle healing. It may be possible for PRP to initially inhibit excess inflammation while stimulating proliferation and maturation. This may be especially important in preventing the fibrous scar tissue healing that occurs with macrophage-mediated tendon-to-bone healing. Future studies should evaluate the possibility that PRP may also stimulate tendon stem cells that have recently been identified.

Equine and human cell culture studies support the use of PRP for the treatment of tendon injuries and disorders. Schnabel and colleagues reported enhanced type I

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Types of tendon problems</th>
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</thead>
<tbody>
<tr>
<td><strong>Type of Tendon Problem</strong></td>
<td><strong>Findings</strong></td>
</tr>
<tr>
<td>True tendonitis</td>
<td>Associated with acute increase in activity, eg, patellar tendonitis with hill running</td>
</tr>
<tr>
<td>Tendinosis</td>
<td>Common, misdiagnosed as “tendonitis.” A chronic degeneration of a tendon, eg, “tennis elbow”</td>
</tr>
<tr>
<td>Torn tendon</td>
<td>Common, can occur with trauma or spontaneously through chronic tendinosis, eg, Achilles tear or rotator cuff tear</td>
</tr>
<tr>
<td>Tendinopathy</td>
<td>Generic term for tendon disorder</td>
</tr>
<tr>
<td>Tendon-related pain</td>
<td>What the patient complains of and what the clinician needs to treat</td>
</tr>
</tbody>
</table>
collagen gene expression in PRP-cultured tendon cells, with no concomitant increase in catabolic molecules, such as matrix metalloproteinase 3 (MMP-3). Other authors, however, have found that PRP not only stimulates human tenocyte proliferation and total collagen production but also slightly increases MMP-3 expression. Anitua and colleagues reported that the balance between TGF-β and other secreted cytokines may control angiogenesis and fibrosis.

Aspenberg and Virchenko reported greater maturation in tendon callus when PRP was used to augment rat Achilles tendon tears. They also reported increased force to failure and ultimate stress in PRP-treated animals. In a landmark study, Kajikawa and colleagues found that PRP enhances the mobilization of circulation-derived cells to an area of injection. They also found that PRP induced type I collagen production and increased the proliferation of macrophages at 3 and 7 days. This article did not, however, measure macrophage proliferation in the first 48 hours, so it is not possible to directly compare it to the work of Woodall et al, which showed initial macrophage suppression during that period.

Mishra and Pavelko were the first to report the use of PRP for patients considering surgery for chronic severe elbow tendinosis. All of the patients had failed a standardized nonoperative treatment protocol. In this prospective, controlled pilot study using unactivated and buffered PRP, the authors found 60% improvement in pain scores for PRP-treated patients versus a 16% improvement in control patients 8 weeks after treatment. This was a small, nonrandomized study. At final follow-up (mean, 25.6 months; range, 12–38 months), however, the PRP patients reported over 90% reduction in pain compared with pre-treatment scores. Ninety-three percent of patients were also fully satisfied with the treatment. A double-blind prospective randomized trial of 230 patients using this protocol in the United States has been initiated. No significant complications or worsening of symptoms has been reported using this technique.

Anitua and colleagues found faster recovery in athletes undergoing PRP-enhanced Achilles tendon repair. In their study, athletes treated with surgery and PRP were compared with a retrospective control group of athletes treated with surgery alone. The PRP patients recovered range of motion earlier, had no wound complications, and returned to training activities in less time than control patients. The cross-sectional area of the PRP-treated tendons was also smaller than that in nontreated tendons when measured by ultrasound. Randelli and colleagues recently

Fig. 2. Injection of PRP for chronic elbow tendinosis.
reported a case series of patients treated with PRP-augmented arthroscopic rotator cuff repairs. They found the technique to be safe without any reported complications and all patients recovered full passive range of motion within 1 month post-treatment. Gamradt and colleagues\textsuperscript{50} reported on another technique for potentially enhancing rotator cuff repair with a different form of PRP. This method is presently being evaluated in a prospective, randomized trial.

Several other trials are underway in the United States and Europe to clarify the value of PRP for tendon injuries. Gosen and colleagues\textsuperscript{51} are using unactivated PRP in a prospective double-blind randomized controlled trial of 100 patients to test PRP against cortisone in patients with chronic lateral epicondylar tendinosis. Preliminary data from their study find PRP patients demonstrating more reduction in pain and higher DASH scores at 24 weeks. Aspenberg and colleagues are presently conducting a prospective, randomized trial of PRP-augmented Achilles tendon repairs in humans. They will also be able to report biomechanical data because they are implanting tantalum balls above and below the repair site. This will allow measurement of tendon elongation postoperatively. Similar findings in the United States and Europe also support the use of PRP in the treatment of chronic Achilles tendinopathy (Figs. 3 and 4). (Mishra, personal communication, June 2008).

The Role of PRP in Muscle Injuries

Muscle injuries may be caused by a contusion by way of a direct blow, a strain, or occasionally a laceration. Rapid eccentric contraction is responsible for many of these injuries and the musculotendinous junction is the most common location of injury. Contact, sprinting, and jumping sports yield the most muscle injuries.\textsuperscript{52} Although imaging studies may be included in the workup, diagnosis is based largely on patient history and physical examination. While there is no universal classification system for muscle injuries, the most common one has been adapted from Ryan’s system (Table 3).\textsuperscript{53}

Muscle healing, like tendon healing, occurs in a series of overlapping phases, including inflammation, proliferation, and remodeling. These events are also
coordinated by growth factors and cell-to-cell interactions. Healing is dependent on local vascularity and regeneration of intramuscular nerve branches, both of which may be enhanced by PRP.\textsuperscript{54,55} The speed of progression through these phases of healing depends on the severity of the injury and the efficiency of the patient’s own biology in combination with any prescribed therapy and rehabilitation.

Despite the significance of this type of injury there are few clinical studies evaluating treatment options. Standard treatment plans attempt to decrease the bleeding and swelling associated with the injury. Recommendations include rest from activity, immediate application of ice, compressive dressings, and elevation of the affected limb. Administration of anti-inflammatory medications may alleviate pain; however, there is some evidence that this may interfere with the ability of the muscle tissue to heal. Nonsteroidal anti-inflammatory drugs may inhibit fusion of myogenic precursor

Fig. 4. Achilles MRI before and after PRP treatment. (A) MRI before PRP injection, partial Achilles tendon tear. (B) MRI 4 mo after PRP, healing of partial tear.
cells, thus impairing muscle healing. Rehabilitation often involves a gradual return of the injured muscle to resistance exercise after the inflammatory phase has subsided. The ideal treatment for muscle injuries would accelerate the process of muscle healing while enhancing the quality of repaired tissue. The role of several growth factors in the natural repair of injured muscle is evident based on increased levels of these cytokines found in healing muscle tissue. PRP is known to contain many of these bioactive proteins.

The Role of PRP in Muscle Healing

Several growth factors within PRP have been evaluated in muscle healing. In vitro results investigating individual growth factors on skeletal muscle are variable, but certain growth factors are capable of enhancement of muscle regeneration and improved muscle force after injury. Growth factors along with macrophages and the products of the COX-2 pathway regulate the inflammatory phase of skeletal muscle healing. Transforming growth factor-β1 and PGE2 may also function synergistically to balance the level of fibrosis during skeletal muscle healing. In a mouse model of muscle laceration, insulin-like growth factor 1 and fibroblast growth factor-β improved muscle healing and increased fast-twitch and tetanus strength compared with controls at 1 month. Autologous platelet concentrate used to treat muscle injury in a rat gastrocnemius contusion model resulted in increased satellite cell activation and myofibril width. Acceleration of functional restoration was found in a human trial of elite athletes injected with ultrasound-guided PRP following muscle injury. These high-level athletes returned to sport at full strength in as early as half the expected recovery time without any evidence of excess fibrosis. There are, however, no randomized controlled human studies supporting the use of PRP for muscle injuries. This is clearly an area that needs further in vitro and in vivo investigation. A prospective randomized trial using ultrasound-guided PRP for Grade 3 or Grade 4 injuries in elite athletes with return to play as an end point would provide helpful information.

DISCUSSION AND FUTURE CONSIDERATIONS

Athletes of all types are presently dissatisfied with their treatment options for tendon and muscle injuries. They are requesting better and less invasive methods to enhance or accelerate healing. Biologic options include the use of stem cells, gene therapy, and autologous or bioengineered cytokines. However, all of these possibilities are

<table>
<thead>
<tr>
<th>Grade</th>
<th>Tissue Damage</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Few muscle fibers involved</td>
<td>Not apparent until conclusion of activity; very little swelling and pain only with activity</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Moderate number of fibers involved with intact fascia</td>
<td>Immediately painful and moderately sore to palpation</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Many fibers involved with incomplete fascial injury</td>
<td>Immediately painful and sore to palpation; patient may limp to avoid pain, severe pain with flexion vs. resistance and/or full extension</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Complete dissociation of fibers and fascia; complete rupture</td>
<td>Immediate severe pain; ecchymosis below area; palpable defect</td>
</tr>
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</table>

Table 3
Muscle injury grades

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currently experimental and are not available for clinical use. Growth factors, in the form of PRP, meet many of the criteria for the ideal biologic treatment. PRP is made from the patient’s own blood, which makes rejection or an adverse reaction unlikely. It can also be prepared immediately at the point of care, which makes it simple and less expensive than stem cell therapies, which often require a period of sorting and culturing before clinical use.

The exact mechanisms by which PRP initiates cellular and tissue changes are presently being investigated. It is clear that PRP induces proliferation of a variety of cell types. PRP has also been found to recruit reparative cells. This helps explain why a single PRP application can have a lasting effect on the healing process. Through interaction with macrophages, PRP may control the inflammatory reaction and thus improve tissue healing and regeneration. It is clear from in vitro studies that PRP initially inhibits IL-1 production from macrophages and reduces their proliferation. By day 4, however, this inhibition turns to stimulation of IL-1 and macrophage division. This initial suppression of macrophage activity may prevent the excessive early inflammation that can lead to dense scar tissue formation. It may further be possible for PRP to regenerate tissue phenotypically closer to normal tendon and muscle by stimulating quiescent stem cells. This has yet to be evaluated but should be investigated. Finally, investigating specific gene expression patterns in vitro and in vivo will contribute to a more detailed understanding of the mechanism of action of PRP.

The foregoing hypothesis supports the following one of how PRP may regenerate tendon or muscle function. PRP is applied in an unactivated form that becomes activated by the collagen within connective tissue. The PRP then releases its growth factors and cytokines. These bioactive proteins in turn stimulate local stem cells and enhance extracellular matrix gene expression. Recruitment of reparative cells from the local circulation or bone marrow then occurs. Simultaneously, PRP inhibits excess inflammation, apoptosis, and metalloproteinase activity. These interactive pathways may result in the restoration of tendon or muscle tissue, which can withstand loading with work or sports activity, thereby diminishing pain. PRP may also modulate the microvascular environment or alter efferent or afferent neural receptors. Much more investigation is required to verify the mechanism(s) of action of PRP.

Clinical investigation of PRP for tendon and muscle injuries and disorders is just beginning. There are only a few small, nonrandomized trials supporting the use of PRP for tendinosis or acute tendon tears. Virtually no published evidence supports the use of PRP for muscle injuries in human clinical trials. Basic science data, however, point to a theoretical value. Fortunately, several prospective, double-blind randomized trials have been initiated for both tendon and muscle injuries. The results of these trials will guide future treatment recommendations.

As we look forward to these trials, it will be important to evaluate the inclusion and exclusion criteria rigorously. Defining the best types of tendon and muscle problems to treat with PRP will be a difficult but important task. The anatomic location of the injury may also be salient. For example, tendons have 3 distinct zones: the myotendinous junction, the midsubstance, and the osseotendinous junction. PRP most likely affects these zones differently. This has yet to be studied. The dosage and type of PRP employed, clearly, will also be critical elements for further study. Presently, there are proprietary PRP formulations and equipment to produce it. Standardized dosing and composition will be required to compare results. In addition, the value of ultrasound or other guidance mechanisms for injection need to be investigated. Finally, post-procedure protocols and rehabilitation methods must be coordinated to produce the best overall outcomes. For example, it may be better to gently load the tendon in the first few weeks to enhance healing.
The tendon injuries that may be improved using PRP include, but are not limited to, repairs of Achilles, patellar, quadriceps, or rotator cuff tendon tears. Chronic tendinosis of any tendon may also benefit. Specifically, it could be possible to treat an acute Achilles tendon tear nonoperatively using a PRP injection. Careful evaluation will be required to determine if the rerupture rate and the tendon strength are equivalent to operative repair, without the increased risk of infection and wound complications that accompany surgical repair. Acute muscle injuries treated with hematoma aspiration and PRP injection may be another potential indication. A study of this type of injury in elite athletes has been initiated at the authors’ institution.

SUMMARY

In summary, PRP has emerged as a promising, but not proven, treatment option for tendon and muscle injuries and disorders. Basic science and animal investigation have begun to help in understanding the mechanism by which PRP affects tissue restoration. Because PRP is autologous and is prepared at the point of care, it also has an excellent safety profile. It may have the ability to transform the care of muscle and tendon injuries in both elite and recreational athletes. Well-designed prospective randomized trials will be required to best understand how, when, and where to use PRP most effectively.

REFERENCES

51. Gosen T, Sluimer J. Prospective randomized study on the effect of autologous platelets injection in lateral epicondylitis compared with corticosteroid injection.
Positive Effect of an Autologous Platelet Concentrate in Lateral Epicondylitis in a Double-Blind Randomized Controlled Trial: Platelet-Rich Plasma Versus Corticosteroid Injection With a 1-Year Follow-up

Joost C. Peerbooms, Jordi Sluimer, Daniël J. Bruijn and Taco Gosens

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Positive Effect of an Autologous Platelet Concentrate in Lateral Epicondylitis in a Double-Blind Randomized Controlled Trial

Platelet-Rich Plasma Versus Corticosteroid Injection With a 1-Year Follow-up

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Background: Platelet-rich plasma (PRP) has shown to be a general stimulation for repair.

Purpose: To determine the effectiveness of PRP compared with corticosteroid injections in patients with chronic lateral epicondylitis.

Study Design: Randomized controlled trial; Level of evidence, 1.

Patients: The trial was conducted in 2 teaching hospitals in the Netherlands. One hundred patients with chronic lateral epicondylitis were randomly assigned in the PRP group (n = 51) or the corticosteroid group (n = 49). A central computer system carried out randomization and allocation to the trial group. Patients were randomized to receive either a corticosteroid injection or an autologous platelet concentrate injection through a peppering technique. The primary analysis included visual analog scores and DASH Outcome Measure scores (DASH: Disabilities of the Arm, Shoulder, and Hand).

Results: Successful treatment was defined as more than a 25% reduction in visual analog score or DASH score without a reinvention after 1 year. The results showed that, according to the visual analog scores, 24 of the 49 patients (49%) in the corticosteroid group and 37 of the 51 patients (73%) in the PRP group were successful, which was significantly different (P < .001). Furthermore, according to the DASH scores, 25 of the 49 patients (51%) in the corticosteroid group and 37 of the 51 patients (73%) in the PRP group were successful, which was also significantly different (P = .005). The corticosteroid group was better initially and then declined, whereas the PRP group progressively improved.

Conclusion: Treatment of patients with chronic lateral epicondylitis with PRP reduces pain and significantly increases function, exceeding the effect of corticosteroid injection. Future decisions for application of the PRP for lateral epicondylitis should be confirmed by further follow-up from this trial and should take into account possible costs and harms as well as benefits.

Keywords: lateral epicondylitis; platelet rich plasma; corticosteroids; pain; function

Lateral epicondylitis is the most commonly diagnosed condition of the elbow, affecting approximately 1% to 3% of the population. The condition mostly occurs in patients whose activities require strong gripping or repetitive wrist movements. Individuals between the ages of 35 and 50 years are at high risk. The dominant arm is most frequently affected.

The cause of lateral epicondylitis is unknown. It is thought that lesions occur in the common origin of the wrist and finger extensors on the lateral epicondyle owing to a combination of mechanical overloading and abnormal microvascular responses.

Numerous methods have been advocated for treating elbow tendinosis, including rest, nonsteroidal anti-inflammatory medication, bracing, physical therapy, extracorporeal shock wave therapy, and botulism toxin injection. Injection of corticosteroids (once considered the gold standard but now controversial), whole blood injections, and various types of surgical procedures have also been recommended.
In an animal model, the addition of growth factors to the ruptured tendon has been shown to increase the healing of the tendon. In humans, it has been shown that the injection of whole blood into the tendon decreases the pain.

Platelet-rich plasma (PRP) is promoted as an ideal autologous biological blood-derived product that can be exogenously applied to various tissues, where it releases high concentrations of platelet-derived growth factors that enhance wound healing, bone healing, and tendon healing. In addition, PRP possesses antimicrobial properties that may contribute to the prevention of infections. When platelets become activated, growth factors are released and initiate the body’s natural healing response.

In a double-blind randomized trial, we investigated whether injection of concentrated autologous platelets improves the outcome of patients with lateral epicondylitis more so than corticosteroid injection. The primary outcome parameters were pain and daily use of the elbow.

METHODS

This double-blinded randomized trial included 100 consecutive patients with lateral epicondylitis scheduled for injection therapy in 2 Dutch training hospitals between May 2006 and January 2008.

All procedures used the same injection procedure, performed by an orthopaedic consultant or a supervised orthopaedic resident. Criteria for participation included lateral epicondylitis for longer than 6 months and pain of at least 50 on a visual analog score (VAS) for pain (0, no pain; 100, maximum pain possible). Lateral epicondylitis was defined as pain over the lateral epicondyle on direct palpation and pain in that area during resisted wrist extension. All affected elbows were screened with radiography and all proved to be normal, except for some calcifications of the common extensor origin. Sonography and magnetic resonance imaging were not standardly used. Patients had a clinical diagnosis of lateral epicondylitis, or lateral elbow pain increased by pressure on the lateral epicondyle and during resisted extension of the wrist. All patients suffered for more than 6 months. Before 6 months of the trial, they were treated with cast immobilization, injections with corticosteroids, or physiotherapy.

Exclusion criteria were as follows: age less than 18 years, pregnancy, history of carpal tunnel syndrome or cervical radiculopathy, and systemic disorders such as diabetes, rheumatoid arthritis, and hepatitis. Also, patients were excluded if they had been treated for lateral epicondylitis with surgical intervention or with a corticosteroid injection in the past 6 months.

The primary endpoint was a 25% reduction in the VAS score or DASH Outcome Measure score (DASH: Disabilities of the Arm, Shoulder, and Hand) without a reintervention after 1 year. In the current study, we tested the hypothesis that the injection of concentrated autologous platelets increases the healing of patients with tendinitis compared with those treated with a steroid injection.

Statistical data were collected to determine the power of both groups. Successful treatment in the PRP group was determined by using the results of Mishra and Pavelko. In this study, 93% of the patients with chronic lateral epicondylitis that received PRP were considered successful—that is, with more than a 25% decrease in pain. Successful treatment in the control group was determined by using the results of Hay and colleagues, who studied the effect of corticosteroid injections for chronic lateral epicondylitis. Full recovery or decrease in complaints without complications was seen in 65% of the patients in the corticosteroid group.

With a bilateral alpha of .05 and a power of 90% (p1 = .93 and p2 = .65), 42 patients per group are necessary to measure the difference with the chi-square test. To correct for the patients who where lost to follow-up, we included a minimum of 50 patients in each group. The Medical-Ethical Committee and the National and Institutional Review Board approved the study.

Randomization

Randomization was performed after patients were deemed eligible and had provided informed consent. Patients were randomly allocated to the concentrated autologous platelet group (PRP group) or the corticosteroid group (control group). A computer using block randomization of 10 patients was used to create a randomization schedule. Treatment assignments (placed in sequentially numbered opaque envelopes) were assigned by the trial managers, who also arranged the facilities needed for the procedure.

PRP Preparation

In the group randomized to receive PRP, the patient’s own platelets were collected with the Recover System (Biomet Biologics, Warsaw, Indiana). This device uses a desktop-size centrifuge with disposable cylinders to isolate the platelet-rich fraction from a small volume of the patient’s anticoagulated blood, drawn at the time of the procedure.

As part of the double-blind procedure, blood was also collected from the patients in the control group. In sum, 27 mL of whole blood was collected from the uninvolved arm into a 30-mL syringe that contained 3 mL sodium citrate. The platelet-rich fraction was prepared according to the instructions of the Recover System. Approximately 3 mL PRP was obtained for each patient. The PRP was then buffered to physiologic pH using 8.4% sodium bicarbonate, and bupivacaine hydrochloride 0.5% with epinephrine (1:200000) was added. No activating agent was used. After masking the tubes with opaque tape, the investigator returned and injected 3 mL of this PRP into the patient. The total time from blood draw to injection in the patients was about 30 minutes. No specialized equipment was required, other than the centrifuge to process the Recover disposable cylinders. All the procedures were performed in the same office setting by an independent person certified for blood management, without the investigator or the patient present.

Injection Technique. Approximately 1 mL of PRP or corticosteroids (kenacort 40 mg/mL triamcinolon acetonide) with bupivacaine hydrochloride 0.5% with epinephrine...
(1:200000) was injected directly into the area of maximum tenderness. Then, using a 22-gauge needle and a peppering technique, the investigator injected the remaining PRP or corticosteroids with bupivacaine hydrochloride 0.5% with epinephrine (1:200000, 6 mL) into the common extensor tendon. This technique involved a single skin portal and 5 penetrations of the tendon.

Postprocedure Protocol. Immediately after the injection, the patient was kept in a supine position without moving the arm for 15 minutes. Patients were sent home with instructions to rest the arm for approximately 24 hours. If necessary, patients were allowed to use acetaminophen, but the use of nonsteroidal anti-inflammatory medication was prohibited. After 24 hours, patients were given a standardized stretching protocol to follow for 2 weeks under the supervision of a physiotherapist. A formal eccentric muscle- and tendon-strengthening program was initiated after this stretching. At 4 weeks after the procedure, patients were allowed to proceed with normal sporting or recreational activities as tolerated. The VAS and DASH scores are compared with a validated upper limb functional score.22

Statistical Analysis

All data analysis was carried out according to a preestablished analysis plan, on a last-observation-carried-forward basis. The categorical values are compared with the Pearson chi-square test. The preoperative continuous variables are compared with the t test. The VAS and DASH scores are compared with an analysis of variance with repeated measurements test. The significance level was set at $P = .05$ for all tests, and SPSS 16.0 was used.

RESULTS

From May 2006 to January 2008, a total of 100 eligible patients with lateral epicondylitis were randomized into groups. Eight patients were lost to follow-up or had incomplete data sets; however, they needed no reintervention (Figure 1). Their data are included in the analysis until their last visit. Analysis of the demographics (sex, side, and center) between the protocol-compliant patients and those lost to follow-up showed no significant differences (Table 1).

The mean patient age was 47 years. There were 48 men and 52 women. The study included 63 patients with lateral epicondylitis on the right elbow and 37 patients with symptoms on the left elbow. The ratio between dominant and nondominant side was according to the literature: 65%. In most cases, the dominant side was involved.23 The ratio was equally distributed. The activity level of the patients, preintervention and postintervention, has been noted in the DASH score.

Eighteen patients needed a reintervention. The patients who needed a reintervention were all scored as nonsuccessful. Between the 2 hospitals, there were no significant differences between the protocol-compliant patients and the reintervention patients ($P = .168$). The primary analysis was conducted on a carried-forward principle and involved 100 patients.
In total, 18 reinterventions or operations were needed after an average of 5 months (range, 2-6 months). In the PRP group, 3 patients obtained an operation and 2 patients a reinjection with corticosteroids. In the corticosteroid group, 6 patients required an operation, 1 a reinjection with corticosteroid, and 6 a reinjection with PRP after 6 months of follow-up (Table 2). The percentages of reinter-
vention did not depend on age, gender, side, treatment, or preoperative VAS or DASH score.

Six months after the initial treatment, the patients who were operated had VAS and DASH scores (respectively, 54.3 ± 26 and 112.2 ± 75.2) that were significantly worse than those of the nonoperated patients (P = .04). The patients needing a second injection had comparable VAS and DASH scores (60.6 ± 29 and 94.6 ± 62.2; P = .0196) as the patients who did not have a second injection.

Initially, the PRP-treated patients had a mean VAS score of 70.1 ± 15.1 and a mean DASH score of 161.3 ± 62.3. The control patients had a mean VAS score of 65.8 ± 13.8 and a mean DASH score of 131.2 ± 58.2. Four weeks after the procedure, PRP-treated patients reported a mean improvement of 21% in their VAS scores (70.1 to 55.4) compared with the initial values, whereas the corticosteroid-treated patients reported a 32.8% improvement (65.8 to 44.2; P = .077) (Figure 2). Also, after 4 weeks, DASH scores had improved 15.7% (161.3 to 135.9) in PRP patients versus a 25.8% improvement (131.3 to 97.4) in corticosteroid-treated patients (P = .001) (Figure 3).

Eight weeks after the procedure, PRP-treated patients reported a mean improvement of 33.1% (70.1 to 46.9) in their VAS scores compared with the initial values, whereas the corticosteroid-treated patients reported a 34.8% improvement (65.8 to 42.9; P = .818) (Figure 2). After 8 weeks, DASH scores improved 29.7% (161.3 to 113.4) in PRP patients versus a 35.5% improvement (131.3 to 84.7) in corticosteroid-treated patients (P = .999) (Figure 3).

Twelve weeks after the procedure, PRP-treated patients reported a mean improvement of 44.8% (70.1 to 38.7) in their VAS scores compared with the initial values, whereas the corticosteroid-treated patients reported a 32.8% improvement (65.8 to 44.2; P = .206) (Figure 2). Also, after 12 weeks, DASH scores had improved 43.0% (161.3 to 92.0) in PRP patients versus a 29.8% improvement (131.3 to 92.2) in corticosteroid-treated patients (P = .060) (Figure 3).

Six months after the procedure, PRP-treated patients reported a mean improvement of 53.5% (70.1 to 32.6) in their VAS scores compared with the initial values, whereas the corticosteroid-treated patients reported a 14.0% improvement (65.8 to 56.6; P < .001) (Figure 2). Also, after 6 months, DASH scores had improved 50.7% (161.3 to 79.5) in PRP patients versus a 10.7% improvement (131.3 to 117.3) in corticosteroid-treated patients (P = .003) (Figure 3).

One year after the procedure, PRP-treated patients reported a mean improvement of 63.9% (70.1 to 25.3) in their VAS scores compared with the initial values, whereas the corticosteroid-treated patients reported a 24.0% improvement (65.8 to 50.1; P < .001) (Figure 2). Also, after 1 year, DASH scores improved 66% (161.3 to 54.7) in PRP patients versus a 17.4% improvement (131.3 to 108.4) in corticosteroid-treated patients (P = .001) (Figure 3).

Regarding the patients who failed their treatment, those who crossed over to the PRP group and those who received surgery did finally benefit. The patients who received a reinjection with corticosteroids did not see a resolution of pain and disability, according to the mentioned criteria.

Successful treatment was defined as more than a 25% reduction in VAS or DASH score without a reintervention after 1 year. The results showed that 24 of the 49 patients (49%) in the corticosteroid group and 37 of the 51 patients (73%) in the PRP group were successful with the VAS score, which was significant (P < .001). Twenty-five of the 49 patients (51%) in the corticosteroid group and 37 of the 51 patients (73%) in the PRP group were successful with the DASH score, which was also significant (P = .005).

No fevers or rashes were reported. Apart from the local inflammation causing increased pain 3 to 4 weeks after the injection, no systemic or other local reactions were seen. The effect can be characterized as a local mechanism, without systemic side effects.

If we set the criteria for success at 50% or 75% improvement of both scores (instead of 25% improvement), the results still show significant differences between both groups, as shown in Tables 3 and 4.

Regarding the cost, PRP is not cost-effective when compared with corticosteroid on a short-term basis. A PRP treatment costs around €200 (current US$300, as of November 2009). The DBC price for injection treatment

### TABLE 1
**Patient Demographics**

| Age, y | 47.3 ± 7.6 | 46.9 ± 8.4 | .797
| Sex: male | 25 | 26 | .840
| Side: right | 32 | 19 | .957
| Lost to follow-up | 1 | 3 | .700
| Reinterventions | 13 | 5 | .970

### TABLE 2
**Flow Chart of Patients**

<table>
<thead>
<tr>
<th>Corticosteroid Group</th>
<th>Platelet-Rich Plasma Group</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>Free of complications</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>Temporarily lost to follow-up</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Operation</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Corticosteroid injection</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Platelet concentrate injection</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>51</td>
</tr>
</tbody>
</table>
is €360 (US$540; DBC stands for Diagnose Behandeling Combinatie, or Diagnosis Treatment Combination). A DBC is an administrative code that combines diagnosis, treatment, and all the related costs; a DBC therefore includes all treatments per diagnosis, from the first visit to the last checkup. So the overall cost for a PRP injection will be around €560 (US$840) compared with the corticosteroid injection of around €200 (US$300). But this does not include all socioeconomic costs.

DISCUSSION

This randomized study was designed to test the use of concentrated autologous platelets in patients with lateral epicondylitis; its application proved to be both safe and easy. The corticosteroid group was actually better initially and then declined, whereas the PRP group progressively improved. There was a significant difference in decrease of pain and disability of function following the platelet application after 26 weeks and 1 year.

Lateral epicondylitis is a common problem with many available treatment methods. The most commonly recommended treatment is physiotherapy and bracing. Approximately 87% of the patients benefit from this combination of treatment methods.²⁰

Now controversial, corticosteroid injection was once considered the gold standard in the treatment of lateral epicondylitis. However, studies show that it is merely the best treatment option in the short-term, when compared with physiotherapy and wait-and-see policy. Poor results are often seen after the 12-week follow-up.¹⁸ Treatment with...
corticosteroids has a high frequency of relapse and recurrence, probably because intratendinous injection may lead to permanent adverse changes within the structure of the tendon and because patients tend to overuse the arm after injection as a result of direct pain relief.\(^{18}\)

In a meta-analysis, Smidt and colleagues\(^ {17}\) showed that the effects of steroid injections—as compared with placebo injection, injection with local anesthetics, injection with another steroid, or another conservative treatment—are not significantly different in the intermediate and long-term. However, the patients who were examined all had short-term lateral epicondylitis.

There are various types of surgical procedures for patients with chronic lateral epicondylitis. Verhaar and colleagues\(^ {21,23}\) noted an improvement in 60% to 70% of the patients after surgical treatment, although higher success rates (80% to 90%) have more recently been reported.\(^ {21,23}\) Patients remain, however, interested in an alternative to surgical intervention.

Platelet-rich plasma is promoted as an ideal autologous biological blood-derived product that can be exogenously applied to various tissues where, after being activated, it releases high concentrations of platelet-derived growth factors that enhance tissue healing.\(^ {5,26}\) With the Recover System, the patient’s own platelets can be collected into a highly concentrated formula. No activation agent was used during our procedure. The activation of the platelets will occur through the exposure of platelets to the thrombine, which is released from the tendon tissue during the peppering technique.

During the first 2 days of tendon healing, an inflammatory process is initiated by migration of neutrophils and, subsequently, macrophages to the degenerative tissue site. In turn, activated macrophages release multiple growth factors, including platelet-derived growth factor, transforming growth factors alpha and beta, interleukin-1, and fibroblast growth factor.\(^ {4}\) Angiogenesis and fibroplasia start shortly after day 3, followed by collagen synthesis on days 3 to 5. This process leads to an early increase in tendon breaking strength, which is the most important tendon healing parameter, followed by epithelization and, ultimately, the remodeling process. This was confirmed in an animal study.\(^ {1}\)

The treatment of tendinosis with an injection of concentrated autologous platelets may be a nonoperative alternative. Injection of autologous platelets has been shown to improve repair in tendinosis in several animal and in vitro models.\(^ {9,15}\) A possible explanation for the long-lasting

### Table 3

#### Pain Resolution for the Corticosteroid (CS) and Platelet-Rich Plasma (PRP) Groups

<table>
<thead>
<tr>
<th>Time, wks</th>
<th>Group</th>
<th>n</th>
<th>Average ± SE</th>
<th>t</th>
<th>&gt; 75%</th>
<th>50%-75%</th>
<th>25%-50%</th>
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<td>4</td>
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<td>–21.6 ± 3.5</td>
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<td>10.4</td>
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</tr>
<tr>
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<td>–22.9 ± 4.0</td>
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<td>31.3</td>
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<td>8.2</td>
<td>10.2</td>
<td>24.5</td>
<td>&lt; .001</td>
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</tbody>
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### Table 4

#### Disability Resolution for the Corticosteroid (CS) and Platelet-Rich Plasma (PRP) Groups

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<tr>
<th>Time, wks</th>
<th>Group</th>
<th>n</th>
<th>Average ± SE</th>
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<td>–22.4 ± 8.6</td>
<td></td>
<td>24.0</td>
<td>12.0</td>
<td>14.0</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRP</td>
<td>49</td>
<td>–106.6 ± 8.7</td>
<td>&lt; .001</td>
<td>57.1</td>
<td>4.1</td>
<td>14.3</td>
<td>24.5</td>
<td>.01</td>
</tr>
</tbody>
</table>
effect of platelets could be that platelets improve the early neotendon properties so that the cells are able to perceive and respond to mechanical loading at an early time point.1

The results of the present study confirm the suggested positive effect in vivo as described by Mishra and Pavelko.10 They reported a significant improvement of symptoms after 8 weeks in 60% of the patients treated with PRP versus 16% of the patients treated with a local anesthetic. After 6 months the improvement in patients treated with PRP was 81%. They compared PRP with a local anesthetic, which is not an accepted treatment for lateral epicondylitis in the Netherlands. Furthermore, they injected only 15 patients with PRP and compared them with 5 patients treated with a local anesthetic. The study was underpowered and the patients were not randomized.

Our results confirm the results of Edwards and Calandruccio.3 They injected whole blood into patients with lateral epicondylitis. Treatment success was seen in 79% of patients; however, multiple injections were necessary in 32% of patients. The limitation of this study is that all patients had failed previous nonsurgical treatments, including prior steroid injections. Furthermore, some patients had a beneficial effect after receiving more than 1 injection. In our study, a single percutaneous injection of PRP or corticosteroid was used with a peppering technique. Repeated injections might be beneficial in patients who had suboptimal results after the initial injection, although no evidence for a beneficial effect of more than one injection exists.

Twenty-six weeks (6 months) was chosen as the cutoff point to consider whether the therapy was successful or not; however, we achieved significant results after only 26 weeks. We know that the natural history of lateral epicondylitis predominantly results in healed patients (80%) within 1 year, but all patients in the present study had complaints for at least 6 months, thereby putting their improvement past the 1-year mark. In both the corticosteroid group and the PRP group, each patient has a natural history; as such and because the population was randomized, we can expect natural history to have the same influence on both groups.

In conclusion, this report describes the first comparison of an autologous platelet concentrate with the gold standard, corticosteroid injection, as a treatment for lateral epicondylitis in patients who have failed nonoperative treatment. It demonstrates that a single injection of concentrated autologous platelets improves pain and function more so than corticosteroid injection. These improvements were sustained over time with no reported complications. Perhaps for athletes it is less optimal, but all depends on the demands of the patient. We had no elite athletes in our population.

ACKNOWLEDGMENT

The study was sponsored by Biomet, Dordrecht, The Netherlands. The funding source had no involvement in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the work for publication. J.A.P. Hagenaars did the statistical analysis. He was an independent biostatistician from the Tilburg University, The Netherlands. He did not receive any funding for the statistical analysis. All authors declare that they participated in the writing of the article and that they saw and approved the final version. Taco Gosens declares that he had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. This trial was registered with ClinicalTrials.gov (identifier: 2007-004947-31; http://www.clinicaltrials.gov).

REFERENCES


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How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation

Olena Virchenko and Per Aspenberg

Background Mechanical stimulation improves the repair of ruptured tendons. Injection of a platelet concentrate (platelet-rich plasma, PRP) can also improve repair in several animal models. In a rat Achilles tendon transection model, 1 postoperative injection resulted in increased strength after 4 weeks. Considering the short half-lives of factors released by platelets, this very late effect calls for an explanation.

Methods We studied the effects of platelets on Achilles tendon regenerates in rats 3, 5 and 14 days after transection. The tendons were either unloaded by Botulinum toxin A (Botox) injections into the calf muscles, or mechanically stimulated in activity cages. No Botox injections and ordinary cages, respectively, served as controls. Repair was evaluated by tensile testing.

Results At 14 days, unloading (with Botox) abolished any effect of the platelets and reduced the mechanical properties of the repair tissue to less than half of normal. Thus, some mechanical stimulation is a prerequisite for the effect of platelets at 14 days. Without Botox, both activity and platelets increased repair independently of each other. However, at 3 and 5 days, platelets improved the mechanical properties in Botox-treated rats.

Interpretation Platelets influence only the early phases of regeneration, but this allows mechanical stimulation to start driving neo-tendon development at an earlier time point, which kept it constantly ahead of the controls.

Subcutaneous tendon repair starts with an organizing hematoma and ends with a remodeled tendon-like structure. Mechanical loading stimulates this development. Yet, mechanical stimulation is seldom used clinically in non-synovial tendon repair, obviously because of the fear of overloading and distracting the tendon. Other methods of stimulating repair are therefore called for. One possibility is the injection of growth factors or of a platelet concentrate into the hematoma. (Kurtz et al. 1999, Aspenberg and Forslund 2000, Forslund 2003, Zhang et al. 2003, Aspenberg and Virchenko 2004, Virchenko et al. 2005.) In rat and rabbit models, this increases tendon regenerate strength by one or two thirds, at a stage when the tendon is about half-healed. Remarkably, a single postoperative platelet injection in a loaded rat model causes improved material properties and histological maturity of the tendon regenerate as late as 3 weeks after the transection, well into the remodeling stage of repair (Aspenberg and Virchenko 2004). This is a remarkable effect, and it appears counter-intuitive that short-lived growth factors, released from the platelets into the hematoma, should influence the properties of the remodeling tendon regenerate after such a long time. In order to understand this phenomenon, we varied the mechanical conditions during repair, with the idea that some kind of interplay between the effects of mechanics and platelets might explain it.
Because the enigmatic effect was seen after a matter of weeks, we started by analyzing the 2-week time point. Our first goal was to see whether the effects of the platelet injection would disappear if mechanical stimulation were eliminated. We then went on to study the effects of increased loading. The results of these experiments then led us to hypothesize that mechanical stimulation starts first when healing has reached a threshold level, and that a platelet injection makes the early callus reach this level at an earlier time point. This would explain how a short-lived effect of the platelets could have long-term consequences. If this hypothesis is correct, there would be an early effect of platelets in unloaded tendons also, although it would not last. We finally tested whether this was the case.

**Animals and methods**

**Overview of experiments**

We used 130 Sprague Dawley rats. Platelet concentrate was prepared from the blood of 10 rats that were killed. Normally there were 10 rats in each treatment group. All experiments included tendon transection with spontaneous healing and were evaluated mechanically. All experiments except the final one were evaluated after 14 days. Firstly, 20 rats with unloading by Botox-induced paralysis of the calf muscles were randomized to receiving either the platelet or control injection. Then 20 rats were randomized to increased loading in activity cages or the control treatment (normal cages). We then repeated this experiment with addition of groups that received Botox and were randomized to activity cages or control. Finally, rats were randomized to platelet treatment or control (no treatment), and evaluated after either 3 or 5 days.

The study was approved by the regional animal ethics committee.

**Botulinum toxin A injections**

The rats were anesthetized with 5% Isoflurane gas (Forene; Abbot Scandinavia, Solna, Sweden) in an anesthetic induction chamber and then with 3.5% Isoflurane in a mask. The skin on the right hind limb was shaved. The *Botulinum* toxin A (Botox, Allergan) was reconstituted in sterile saline to a final concentration of 50 U/mL before each use.

**Activity cages**

The activity cages were manufactured by the workshop of our hospital. The cages consist of two floors connected by a narrow passage. The rats had nest boxes on the ground floor, from which they could climb up to the first floor, which was constructed as a maze. In two corners of the maze, food pellets were placed. Sawdust was spread both in the maze and on the ground floor and water was provided ad libitum on the ground floor (Figure 1). In a study on the behavior of untreated rats in this environment, the rats spent 75% of their time in the maze, and used less time for sleeping and grooming, which suggests increased physical activity (Carlsson 2005). The standard cages were 40 × 25 cm plastic containers with a metal grating above.

**Platelet concentrate and platelet gel preparation**

Whole blood was collected from 10 female Sprague Dawley rats (200 g; M&B, Ry, Denmark). The rats were anesthetized with Isoflurane, and 4–6 mL of whole blood was collected by cardiac puncture using a 10 mL syringe containing 1.5 mL antico-
agulant citrate phosphonate dextrose (CPD) buffer (0.15 mg CPD/mL), and a 1.2 mm needle. After blood collection, the animals were killed with CO₂ gas. The blood was then centrifuged at 220 × g for 20 minutes. The supernatant, containing platelet-rich plasma, was used for a second centrifugation at 480 × g for 20 min to form a pellet of rat platelets. The platelets were then resuspended in plasma and the cell density was adjusted to 8.3 × 10⁹ platelets/L. This preparation was used for platelet injections.

For platelet gel preparation, the same procedure was used and then the platelet concentrate was dispersed in 20 microwells, 50 µL per well, and activated by adding 0.25 U thrombin from bovine plasma (Sigma, St. Louis, MO) and 10% calcium chloride (Braun Melsungen; 1000 IE/mL CaCl₂–2H₂O) at 37°C 4 mM. Both the platelet concentrate and the platelet gel were stored at 4ºC for a maximum time of 24 h and were then applied to the transected Achilles tendons. The two different platelet preparations were used in this work because of a planning error. However, they appear to be equally efficacious in their effect on the mechanical properties of tendon at 14 days (data not shown; submitted for publication).

**Operating procedure and treatment**

The rats were anesthetized with 5% Isoflurane gas (Forene, Abbot Scandinavia, Solna, Sweden) in an anesthetic induction chamber and then with 3.5% Isoflurane in a mask. The skin on the right hind limb was shaved. 5-mg intramuscular injections of tetracycline (Engemycin; Intervet, Boxmeer, Holland), and analgesics in the form of 0.015 mg buprenorphine (Temgesic; Schering-Plough, Brussels, Belgium) were given preoperatively. The animal was placed prone on a warm pad (38.2°C) and the right hind leg was stretched backwards and washed with chlorhexidine ethanol. A 3-mm transverse incision was made in the skin lateral to the right Achilles tendon and the Achilles tendon complex was exposed. Approximately 7 mm of the plantaris tendon was then removed to simplify mechanical measurements at the end of the experiment. Subsequently, the Achilles tendon was cut transversely 1.5 and 4.5 mm proximal to the calcaneal insertion. Thus, a 3-mm segment was removed (Figure 2). The tendon was left unsutured with a gap between the tendon stumps and the skin sutured. There was no postoperative immobilization. After the operation, the animals were placed in clean cages under a heating lamp for a constant temperature of 30°C until they completely woke up.

The rats were divided into experimental and control groups by a lottery. For platelet treatment, this was done by an assistant during the operation. The rats either received 50 µL (1 piece) of platelet gel in the defect at the operation or a local injection of 50 µL of platelet concentrate 6h postoperatively, or control solution (saline).

**Evaluation**

After 3, 5 or 14 days, the rats were killed with CO₂ gas. The tendon with the attached calcaneal
bone was dissected free from other tissues and removed. Sagittal and transverse diameters were measured with a digital calliper. For clamping, the muscle was scraped off the tendon substance by blunt dissection to produce a fan of tendon fibers, which was then sandwiched between fine sandpaper in metal clamps. The calcaneus was fixated in a custom-made clamp in 30° dorsiflexion, relative to the direction of traction. Finally, the clamps were attached to a materials-testing machine (100R; DDL Inc., Eden Prairie, MN) and the tendon was pulled at a constant speed of 1 mm/s until failure. Mechanical parameters measured were force at failure, stiffness, and energy uptake at 10% droop of the curve. Transverse area and stress at failure were calculated.

**Statistics**

Statistical analysis was performed with StatView for Windows version 5.0.1 (SAS Institute, Cary, NC). Experiments with two groups were evaluated with Student’s t-test. When 4 groups were compared, this was done by 2-way analysis of variance.

### Results

5 rats were lost from mechanical follow-up because of various technical errors.

**No effect of platelets without mechanical loading at 14 days**

20 rats received Botox injections and underwent tendon transection 7 days later. At that time, the paralysis appeared complete. 6 h after transection, the animals were randomized to receive an injection of platelet concentrate into the hematoma (10 rats) or to receive buffer (10 rats). With unloading by Botox, all stimulatory effects of platelets were lost (Table 1), and the tendon regenerates were less than a third as strong as with normal loading. Stiffness and energy uptake were only a quarter of normal, transverse area half of normal, and stress at failure two thirds of normal (for normal data, see Tables 2 and 3). Thus, loading is a prerequisite for stimulation by platelets.

**Increased cage activity improves repair but not the response to platelets**

20 rats were randomized to normal cages or activity cages. After 1 week for acclimatization, they underwent tendon transection. Evaluation was done 14 days later. 2 tendons were excluded because of a fracture in the calcaneus at the mechanical test. Rats from the activity cages had increased force, energy uptake and transverse area (Table 2). We next repeated this experiment with addition of platelet gel. Thus, the rats were randomized to 4 groups: normal cage with control rats without any treatment; normal cage with platelet gel; activ-

### Table 1. No effect of platelets (PC) after 14 days in rats with calf muscles paralyzed with Botox

<table>
<thead>
<tr>
<th></th>
<th>PC Mean SD</th>
<th>Control Mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force (N)</td>
<td>16 6.3</td>
<td>15 2.5</td>
</tr>
<tr>
<td>Stiffness (N/mm)</td>
<td>3.7 1.2</td>
<td>3.0 0.6</td>
</tr>
<tr>
<td>Energy (N/mm)</td>
<td>22 11</td>
<td>25 12</td>
</tr>
<tr>
<td>Area (mm²)</td>
<td>4.3 0.8</td>
<td>4.0 0.8</td>
</tr>
<tr>
<td>Stress (MPa)</td>
<td>3.9 1.8</td>
<td>4.1 1.3</td>
</tr>
</tbody>
</table>

### Table 2. Effect of activity with or without platelets*

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Cage and treatment</th>
<th>n</th>
<th>Force (N) Mean SD</th>
<th>Stiffness (N/mm) Mean SD</th>
<th>Energy (Nmm) Mean SD</th>
<th>Area (mm²) Mean SD</th>
<th>Stress (MPa) Mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Activity</td>
<td>8</td>
<td>55 5.8</td>
<td>17 1.7</td>
<td>123 25</td>
<td>9.1 2.5</td>
<td>6.5 2.1</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>10</td>
<td>45 10</td>
<td>13 3.9</td>
<td>96 25</td>
<td>8.5 3.0</td>
<td>5.7 1.6</td>
</tr>
<tr>
<td>2</td>
<td>Activity control</td>
<td>9</td>
<td>48 6.5</td>
<td>12 2.2</td>
<td>136 13</td>
<td>11 1.8</td>
<td>4.6 0.9</td>
</tr>
<tr>
<td>2</td>
<td>Activity platelets</td>
<td>9</td>
<td>50 8.2</td>
<td>14 3.8</td>
<td>119 29</td>
<td>9.0 1.5</td>
<td>5.6 1.3</td>
</tr>
<tr>
<td>2</td>
<td>Normal control</td>
<td>10</td>
<td>47 9.1</td>
<td>13 1.8</td>
<td>126 37</td>
<td>7.7 2.5</td>
<td>6.4 1.5</td>
</tr>
<tr>
<td>2</td>
<td>Normal platelets</td>
<td>9</td>
<td>55 8.4</td>
<td>15 3.1</td>
<td>155 27</td>
<td>6.8 1.1</td>
<td>8.2 1.3</td>
</tr>
</tbody>
</table>

*Significant p-values as follows. Experiment 1: force 0.02; stiffness 0.03; energy 0.04. Experiment 2: Effect of activity: area < 0.001; stress < 0.001. Effect of platelets: stiffness 0.02; area 0.05; stress 0.005.
ity cage without any treatment; and activity cage with platelet gel. There was no significant effect on force. Platelets increased stiffness, reduced area and increased stress at failure. Activity increased area and reduced stress. No variable showed that cage activity influenced the response to platelets (Table 2).

Because of the methodological similarity, the first and the second activity cage experiments (platelet injections excluded) were combined to increase statistical power. We then found that activity increased force, energy uptake and area (Table 3). The 2 experiments differed regarding stiffness and energy. This is unexplained.

In conclusion, activity increased size (and thereby force and energy) and platelets improved material properties, so that the regenerates appeared to have come further ahead in the maturation process. No synergism between activity and platelets was found.

**Platelets have an early effect also without loading**

40 rats received Botox injections and underwent tendon transection 7 days later. They were randomized to platelet or control treatment. After 3 and 5 days, the platelets increased force, stiffness and area (Table 4).

**Discussion**

The basis of this study was the finding of a stimulatory effect of a single platelet injection on tendon repair, which can be traced with mechanical testing as late as 4 weeks afterwards (Aspenberg and Virchenko 2004). This late effect was surprising. We next noted that this effect (at 14 days) disappeared when the tendon was unloaded using Botox, which suggested a connection between the platelet effect and mechanical stimulation.
This was the motivation for the experiments that ensued.

Platelets had an early stimulatory effect in tendons unloaded with Botox, but loading was a prerequisite for this effect to remain at 14 days. Activity increased regenerate size, whereas loaded and platelet-treated specimens had improved material properties at 14 days.

The development of the tendon callus starts with an organizing blood clot. Mechanical stimulation can occur first when a sufficient number of fibroblasts is present and a matrix capable of transferring some load has been produced. A possible explanation for the long-lasting effect of platelets on the maturity of a regenerate (Aspenberg and Virchenko 2004) could be that platelets improve the very early callus properties so that the cells can perceive and respond to mechanical loading at an earlier time point. Once the non-platelet controls reach a sufficient stage of healing (at a later time point), they too will be responsive to loading, but the platelet-treated specimens will be further ahead in their development. This hypothesis occurred to us when we saw the 14-day data. This would explain the absent effect of platelets in the Botox-treated group: without mechanical stimulation, early responsiveness to loading is of no advantage. The hypothesis would also explain the lack of synergism between increased loading and platelets, because the factors act in sequence rather than at the same time (platelets during the first days, and loading thereafter). If the hypothesis is correct, one should be able to measure an early effect of platelets in the absence of loading, i.e. different callus properties at the time that loading would start exerting its stimulatory effects. Indeed, this is what we finally found. At the early time points, platelets stimulated callus formation.

This study could only give indirect proof of the principle. In order to show more directly at what time point loading starts to stimulate repair, one would need a model in which loading could be turned off and then on at defined time points, which is something which we do not have.

The clinical implication of this study is that mechanical stimulation is of utmost importance. This has been pointed out before in several studies (Enwemeka 1992, Maffulli and King 1992, Almekinders et al. 1995, Kannus et al. 1997, Iwuagwu and McGrouther 1998, Zeichen et al. 2000). However, the use of Botox for unloading of the healing tendon appears to be a new and practical model. As long as the muscle is relaxed and pliable, traction forces in the tendon callus should be low. Earlier models have used immobilization (Murrell et al. 1994, Ishida et al. 1996, Iwuagwu and McGrouther 1998, Yamamoto et al. 1999, Palms et al. 2002, Matsumoto et al. 2003). In animals, it is unclear to what extent immobilization really leads to unloading. Most animals will probably exert considerable traction forces by muscle contraction in spite of a brace or internal fixation. It is unknown how patients load their injured Achilles tendons while in plaster, but the situation in humans probably corresponds better to complete absence of muscle contraction with Botox than to animal cage activity with a brace. Thus, the dramatic inhibition of repair by unloading in the Botox model suggests that placing a patient in plaster with complete unloading could impair the repair process. Furthermore, platelet treatment for tendon ruptures may not be efficacious, unless it is combined with early physiotherapy. This must be early enough to benefit from the increased callus growth induced by the platelets.

Contributions of authors
Both authors participated in all parts of the study, but OL did most of the experimental work and PA most of the writing.

We thank Ali Sodeifi for assistance with rat surgery. This study was supported by the Swedish National Center for Sports Research, the Strategic Research Program "Materials in Medicine" (Östergötlands läns landsting, Linköping university), and by the Swedish Research Council (project no. 2031).


Treatment of Chronic Elbow Tendinosis With Buffered Platelet-Rich Plasma

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**Background:** Elbow epicondylar tendinosis is a common problem that usually resolves with nonoperative treatments. When these measures fail, however, patients are interested in an alternative to surgical intervention.

**Hypothesis:** Treatment of chronic severe elbow tendinosis with buffered platelet-rich plasma will reduce pain and increase function in patients considering surgery for their problem.

**Study Design:** Cohort study; Level of evidence, 2.

**Methods:** One hundred forty patients with elbow epicondylar pain were evaluated in this study. All these patients were initially given a standardized physical therapy protocol and a variety of other nonoperative treatments. Twenty of these patients had significant persistent pain for a mean of 15 months (mean, 82 of 100; range, 60-100 of 100 on a visual analog pain scale), despite these interventions. All patients were considering surgery. This cohort of patients who had failed nonoperative treatment was then given either a single percutaneous injection of platelet-rich plasma (active group, n = 15) or bupivacaine (control group, n = 5).

**Results:** Eight weeks after the treatment, the platelet-rich plasma patients noted 60% improvement in their visual analog pain scores versus 16% improvement in control patients ($P = .001$). Sixty percent (3 of 5) of the control subjects withdrew or sought other treatments after the 8-week period, preventing further direct analysis. Therefore, only the patients treated with platelet-rich plasma were available for continued evaluation. At 6 months, the patients treated with platelet-rich plasma reported 81% improvement in their visual analog pain scores ($P = .0001$). At final follow-up (mean, 25.6 months; range, 12-38 months), the platelet-rich plasma patients reported 93% reduction in pain compared with before the treatment ($P < .0001$).

**Conclusion:** Treatment of patients with chronic elbow tendinosis with buffered platelet-rich plasma reduced pain significantly in this pilot investigation. Further evaluation of this novel treatment is warranted. Finally, platelet-rich plasma should be considered before surgical intervention.

**Keywords:** platelet-rich plasma (PRP); tennis elbow; lateral epicondylitis; tendonitis; tendinosis

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Elbow epicondylar tendinosis is a common problem for patients whose activities require strong gripping or repetitive wrist movements. Histologic specimens from chronic cases confirm that tendinosis is not an acute inflammatory condition but rather a failure of the normal tendon repair mechanism associated with angiofibroblastic degeneration.8 The cause of elbow tendinosis is most likely a combination of mechanical overloading21 and abnormal microvascular responses.19 Further research into the precise cause of tendinosis is, however, still needed.

Numerous methods have been advocated for treating elbow tendinosis, including rest, nonsteroidal anti-inflammatory medication, bracing, physical therapy, iontophoresis,15 extracorporeal shock wave therapy, and botulism toxin.9 Injections of corticosteroids or whole blood,3 and various types of surgical procedures have also been recommended. The utility of several of these treatments has recently come into question. For example, one recent report noted that there is no difference between using corticosteroid or local anesthetic when treating elbow tendinosis with an injection.1

In this study, we evaluated the use of platelet-rich plasma (PRP) as a treatment for chronic severe epicondylar tendinosis. Platelet-rich plasma contains a more concentrated amount of platelets than does whole blood. Within platelets are powerful growth factors, including platelet-derived growth factor, transforming growth factor beta, and epidermal growth factor. Slater et al17 reported that the addition of platelets to a culture medium stimulated the proliferation of...
of human osteoblast-like cells. They further proposed that exogenous platelet growth factors may play a significant role in fracture repair.17

A recent review of common growth factors suggested PRP may be useful for tendon and ligament healing in vivo.11 The specific goal of this investigation was to measure the efficacy of buffered PRP as a potential new treatment for chronic severe elbow tendinosis.

MATERIALS AND METHODS

The study design and protocol were evaluated and approved by the authors’ investigational review board. This board refused to allow the drawing and discarding of 55 mL blood that would be required to blind the patients to their treatment. All patients signed a detailed informed consent form. Before starting the formal study, 2 healthy volunteers were treated with the PRP formulation and the protocol. No adverse effects were noted in these patients at 1 month, 3 months, 6 months, or 1 year after the procedure.

Patient Data

One hundred forty patients were evaluated in this study. Criteria for participation included elbow epicondylar pain for longer than 3 months of at least 60 of 100 on a visual analog score (0, no pain; 100, maximum pain possible), in spite of completing a standardized stretching and strengthening protocol. All patients had the same physical therapist instruct them in the same set of exercises. This specific protocol was posted on a Web site (www.emedx.com) for patients to follow. Patients also had failure with some combination of nonsteroidal medication, bracing, or corticosteroid injections. Elbow epicondylar tendinosis was defined as pain over the lateral or medial epicondyle, with direct palpation and pain at the elbow with resisted wrist extension (for lateral tendinosis) and resisted wrist flexion (for medial tendinosis). Exclusion criteria included pregnancy, history of carpal tunnel syndrome, cervical radiculopathy, and systemic disorders such as diabetes, rheumatoid arthritis, and hepatitis. Twenty (15%) of the 140 patients evaluated met these strict inclusion-exclusion criteria and were enrolled in the study.

There was a total of 15 PRP-treated and 5 control patients included in this study. The PRP-treated patients (15 patients: 14 lateral tendinosis, 1 medial tendinosis) received an injection of PRP that had been buffered to physiologic pH. The duration of symptoms in this group was 15.3 months, and the mean patient age was 48.1 years. The control patients (5 patients: 5 lateral tendinosis) received an injection of bupivacaine with epinephrine. The mean duration of symptoms in this group was 11.8 months, and the mean age was 42.2 years. Ten of the patients included in this report were part of a consecutive series, and 10 were part of a randomized trial. The randomization of the second group was done with an envelope randomization protocol. Both groups of patients were told that needling of the tendon by itself may produce improvement in symptoms. All patients were evaluated and treated during the same time frame (2002-2004).

Platelet-Rich Plasma Preparation

Fifty-five milliliters whole blood was collected from the uninvolved arm into a 60-mL syringe that contained 5 mL sodium citrate. A peripheral complete blood count was also collected at the time of the initial blood draw. The blood was then prepared according to the GPS System instructions (Cell Factor Technologies, Warsaw, Ind.). This device is a desktop-size centrifuge with disposable cylinders for the blood. All the procedures were performed in the same office setting. Approximately 5 mL PRP was obtained for each patient. The PRP was then buffered to physiologic pH using 8.4% sodium bicarbonate. No activating agent was used. Two milliliters of this PRP was then sent to the laboratory for analysis of platelet concentration, whereas the remaining 2 to 3 mL was used to inject into the patient. On average, 3.31 million platelets were given to each PRP-treated patient via this injection. The total number of platelets per milliliter in the PRP represented a mean increase of 539% compared with whole blood values in the active patient group. The total time from blood draw to injection in the patients was about 30 minutes. No specialized equipment, other than the GPS machine, was required. The cost of the machine and the kit with all the materials to perform the procedure has not been determined because they are not yet commercially available.

Injection Technique

Initially, bupivacaine with epinephrine was infiltrated into the skin and subcutaneous tissue of both groups as a local field block. Approximately 0.5 mL was also injected directly into the area of maximum tenderness. Then, either 2 to 3 mL PRP or 2 to 3 mL bupivacaine with epinephrine was injected using a 22-g needle into the common extensor or flexor tendon using a peppering technique. This technique involved a single skin portal and then 5 penetrations of the tendon (Figure 1).

Postprocedure Protocol

Immediately after the injection, the patient was kept in a supine position without moving the arm for 15 minutes. Patients were sent home with instructions to limit their use of the arm for approximately 24 hours and use hydrocodone or acetaminophen for pain. The use of nonsteroidal medication was prohibited. After 24 hours, patients were given a standardized stretching protocol to follow for 2 weeks. A formal strengthening program was initiated after this stretching. At 4 weeks after the procedure, patients were allowed to proceed with normal sporting or recreational activities as tolerated.

A 100-mm visual analog pain score (0, no pain; 100, worst pain possible) and a modified Mayo elbow score (best score, 100) were used as outcome measures. The patients were examined at 4 weeks, 8 weeks, and 6 months after the index procedure. A final follow-up overall evaluation was also done. Paired and unpaired t tests were used to statistically compare the 2 groups of patients. Initially, the visual analog pain score (0, no pain; 100, maximum pain possible), in spite of completing a standardized stretching and strengthening protocol.

Injection Technique

Initially, bupivacaine with epinephrine was infiltrated into the skin and subcutaneous tissue of both groups as a local field block. Approximately 0.5 mL was also injected directly into the area of maximum tenderness. Then, either 2 to 3 mL PRP or 2 to 3 mL bupivacaine with epinephrine was injected using a 22-g needle into the common extensor or flexor tendon using a peppering technique. This technique involved a single skin portal and then 5 penetrations of the tendon (Figure 1).

Postprocedure Protocol

Immediately after the injection, the patient was kept in a supine position without moving the arm for 15 minutes. Patients were sent home with instructions to limit their use of the arm for approximately 24 hours and use hydrocodone or acetaminophen for pain. The use of nonsteroidal medication was prohibited. After 24 hours, patients were given a standardized stretching protocol to follow for 2 weeks. A formal strengthening program was initiated after this stretching. At 4 weeks after the procedure, patients were allowed to proceed with normal sporting or recreational activities as tolerated.

A 100-mm visual analog pain score (0, no pain; 100, worst pain possible) and a modified Mayo elbow score (best score, 100) were used as outcome measures. The patients were examined at 4 weeks, 8 weeks, and 6 months after the index procedure. A final follow-up overall evaluation was also done. Paired and unpaired t tests were used to statistically compare the 2 groups of patients. Initially, the visual analog pain score (0, no pain; 100, maximum pain possible)

Injection Technique

Initially, bupivacaine with epinephrine was infiltrated into the skin and subcutaneous tissue of both groups as a local field block. Approximately 0.5 mL was also injected directly into the area of maximum tenderness. Then, either 2 to 3 mL PRP or 2 to 3 mL bupivacaine with epinephrine was injected using a 22-g needle into the common extensor or flexor tendon using a peppering technique. This technique involved a single skin portal and then 5 penetrations of the tendon (Figure 1).

Postprocedure Protocol

Immediately after the injection, the patient was kept in a supine position without moving the arm for 15 minutes. Patients were sent home with instructions to limit their use of the arm for approximately 24 hours and use hydrocodone or acetaminophen for pain. The use of nonsteroidal medication was prohibited. After 24 hours, patients were given a standardized stretching protocol to follow for 2 weeks. A formal strengthening program was initiated after this stretching. At 4 weeks after the procedure, patients were allowed to proceed with normal sporting or recreational activities as tolerated.

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Postprocedure Protocol

Immediately after the injection, the patient was kept in a supine position without moving the arm for 15 minutes. Patients were sent home with instructions to limit their use of the arm for approximately 24 hours and use hydrocodone or acetaminophen for pain. The use of nonsteroidal medication was prohibited. After 24 hours, patients were given a standardized stretching protocol to follow for 2 weeks. A formal strengthening program was initiated after this stretching. At 4 weeks after the procedure, patients were allowed to proceed with normal sporting or recreational activities as tolerated.

A 100-mm visual analog pain score (0, no pain; 100, worst pain possible) and a modified Mayo elbow score (best score, 100) were used as outcome measures. The patients were examined at 4 weeks, 8 weeks, and 6 months after the index procedure. A final follow-up overall evaluation was also done. Paired and unpaired t tests were used to statistically compare the 2 groups of patients. Initially, the visual analog pain score (0, no pain; 100, maximum pain possible)
scores and modified Mayo elbow scores were not statistically different between the 2 groups. All patients were either fair or poor as measured by the Mayo elbow score (Table 1).

RESULTS

During follow-up examinations, all patients reported complete compliance with the recommended postprocedure exercise program. All patients had the same physical therapist instruct them in this standardized protocol. No complications were noted in either group at any time. Specifically, there were no infections, neurovascular changes, or worsening of the patients’ epicondylar pain. Initially, the PRP-treated patients had a mean visual analog pain score of 80.3 (range, 60-100) and a mean Mayo elbow score of 50.3 (range, 38-68). The control patients had a mean visual analog pain score of 86 (range, 80-100) and a mean Mayo elbow score of 50 (range, 43-53).

Four weeks after the procedure, PRP-treated patients reported a mean of 46% improvement (80.3 to 43.4) in their visual analog pain scores versus 17% improvement (86.0 to 71.0) in the control patients \((P = .028)\) (Figure 2). Also, after 4 weeks, Mayo elbow scores had improved 42% (50.3 to 71.3) in PRP-treated patients versus a 20% improvement (49.5 to 59.5) in control patients \((P = .120)\) (Figure 3).

Eight weeks after the treatment, PRP-treated patients reported a mean of 60% improvement (80.3 to 32.0) in their visual analog pain scores versus a 16% improvement (86 to 72) in control patients \((P = .001)\) (Figure 2). Platelet-rich plasma patients also had a 52% improvement (50.3 to 76.3) in their Mayo elbow scores versus 14% improvement (49.5 to 56.5) in control patients \((P = .008)\) at this time frame (Figure 3).

After 8 weeks, 60% (3 of 5) of the control patients had either sought treatment outside of the protocol or had formally withdrawn from the study. This factor limited further data evaluation to only the PRP-treated patients. At 6 months, the PRP-treated patients’ visual analog pain scores had improved a mean of 81% over baseline \((P = .0001)\) (Figure 4), and their Mayo elbow scores had improved 72% \((P = .0001)\) (Figure 5).

The 2 remaining control patients were also interviewed; one had a pain score of 0 of 100, and one had a pain score of 50 of 100. Their Mayo elbow scores were 100 and 70, respectively.

At final follow-up (mean, 25.6 months; range, 12-38 months), the PRP-treated patients reported a 93% reduction (mean, 5.7 of 100; range, 0-40) in pain when compared with before the treatment \((P < .001)\). Ninety-three percent of these patients were completely satisfied with the treatment, and 7% were partially satisfied. This same 93% were essentially pain free (10 or less of 100 on visual analog scale). One patient reported 40 of 100 on the scale at final follow-up but was still partially satisfied with her treatment. Overall, the patients reported engaging in a mean of 99% (range, 80%-100%) of the activities of daily living and 94% (range, 75%-100%) of their work or sporting activities.

### TABLE 1

<table>
<thead>
<tr>
<th>Modified Mayo Clinic Performance Index for the Elbow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter/findings</td>
</tr>
<tr>
<td>Pain</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Minimal</td>
</tr>
<tr>
<td>Mild</td>
</tr>
<tr>
<td>Moderate</td>
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<tr>
<td>Severe</td>
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<tr>
<td>Motion</td>
</tr>
<tr>
<td>Full motion</td>
</tr>
<tr>
<td>Stability</td>
</tr>
<tr>
<td>No clinical laxity</td>
</tr>
<tr>
<td>Daily Function/Performance</td>
</tr>
<tr>
<td>Combing hair</td>
</tr>
<tr>
<td>Able</td>
</tr>
<tr>
<td>Able with pain</td>
</tr>
<tr>
<td>Unable</td>
</tr>
<tr>
<td>Eating</td>
</tr>
<tr>
<td>Able</td>
</tr>
<tr>
<td>Able with pain</td>
</tr>
<tr>
<td>Unable</td>
</tr>
<tr>
<td>Hygiene</td>
</tr>
<tr>
<td>Able</td>
</tr>
<tr>
<td>Able with pain</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>Clothing</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>Unable</td>
</tr>
<tr>
<td>Shoes and socks</td>
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<tr>
<td>Unable</td>
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<td>Poor</td>
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</table>
DISCUSSION

Elbow epicondylar tendinosis is a common problem with many possible treatments. Quick cessation of symptoms is important to patients and is economically advantageous. If neither rest nor simple treatment provides a satisfactory remedy, a patient may pursue several other options. The most commonly recommended treatment is physical therapy. A recent meta-analysis of physical therapy, however, noted that there is insufficient evidence to conclude that it has any lasting value.\textsuperscript{18} We also surveyed owners of 8 physical therapy clinics within 15 miles of our office to determine the mean cost of treatment per patient per episode of elbow tendinosis. The typical treatment period consisted of an initial evaluation and 10 follow-up visits. The mean estimated cost was $1200. Corticosteroid injections have also been used extensively for this problem, but studies show that there is conflicting evidence about their efficacy.\textsuperscript{1,14} Jobe and Cicotti\textsuperscript{8} also concluded that superficial injection of corticosteroid may result in subcutaneous atrophy and that intratendinous injection may lead to permanent adverse changes within the ultrastructure of the tendon. Despite these issues, corticosteroid is still widely used. Hill et al\textsuperscript{6} surveyed 400 members of the American Academy of Orthopaedic Surgeons and found that 93% had given a corticosteroid injection for elbow epicondylitis. Extracorporeal shock wave therapy also has recently gained popularity. A recent randomized double-blind study, however, showed that this treatment is no better than placebo.\textsuperscript{13}

Biologic treatments in orthopaedics are just beginning to evolve. Bone morphogenic proteins and other growth factors have been extensively studied in vitro. These important cytokines are now being tested in vivo.\textsuperscript{4} Marx et al\textsuperscript{12} confirmed the value of adding PRP to bone graft in maxillofacial surgery. They showed that PRP-enhanced autografts were 50% more dense than were autografts alone at 6 months in a mandibular defect model.\textsuperscript{12} Data were recently presented that noted PRP-enhanced allograft had significantly increased shear strength and energy absorption when compared to allograft alone.\textsuperscript{7} Platelet-rich plasma may also be helpful for wound healing.\textsuperscript{5} An animal study, however, found that PRP does not enhance demineralized bone matrix’s osteoinductive capacity and may actually inhibit it.\textsuperscript{16} Platelet-rich plasma injections have also been used to

\begin{figure}
\centering
\includegraphics[width=\textwidth]{pain_scores.png}
\caption{Visual analog pain scores for the group treated with platelet-rich plasma (PRP) and the control group.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mayo_scores.png}
\caption{Mayo elbow scores for the group treated with platelet-rich plasma (PRP) and the control group.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mean_pain_scores.png}
\caption{Mean pain scores of patients treated with platelet-rich plasma (PRP).}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mayo_mean_scores.png}
\caption{Mean Mayo elbow scores for patients treated with platelet-rich plasma (PRP).}
\end{figure}
treat recalcitrant plantar fasciitis. Barrett and Erredge\(^2\) reported a 78% success rate with 1-year follow-up. This same study also documented a decrease in plantar fascia thickness over time when treated with PRP.\(^3\) Edwards and Calandrumlio,\(^3\) using whole blood, noted a 79% success rate when treating lateral epicondylitis. The follow-up time, however, was short (9.5 months), and 32% of the time, multiple injections were required.

We postulate that the concentrated growth factors within PRP work in concert to initiate a healing response within a damaged tendon. This hypothesis is supported by in vitro data from Klein et al.\(^10\) They reported that transforming growth factor beta significantly increases type I collagen production in tendon sheath fibroblasts. This same mechanism may be at work in our in vivo model of chronic severe elbow tendinosis. It may also be possible that PRP helps recruit bone marrow–derived stem cells to the site of injection or somehow modulates the microvascular environment. Further study into the mechanisms by which PRP works is needed. We chose to study elbow tendinosis because it is clinically very common and does not involve a weightbearing limb. We have, however, also treated several cases of plantar fasciitis and quadriceps and patellar tendinosis with these methods with good results.

The patients in this study were vigorously screened before enrollment. We evaluated 140 patients for this study and enrolled only 15%. This left the study with the most severe tendinosis patients and eliminated any patient who had improved with time or nonoperative treatment. In the present investigation, the patients treated with buffered PRP did significantly better than control patients did at 4 weeks and 8 weeks. After 8 weeks, 60% of the control patients either formally withdrew from the study or sought treatment outside of the protocol. This factor, unfortunately, limited further direct comparison. We were unable to blind the patients because our institutional review board refused to allow the drawing and discarding of a small amount of blood that would be required to fully blind the patients. This factor may have influenced the control group and may explain why this group did not respond to the needling technique. At 6 months after treatment, however, the PRP-treated patients improved a mean of 81% in their visual analog pain scores and 72% in their Mayo elbow scores. Two years after treatment, the PRP-treated patients reported a 93% reduction in pain. This finding is similar to the 95% reduction in pain reported in patients treated with suture anchor repair by Thornton et al.\(^20\) Of importance, no PRP-treated patient was worse after treatment, and there were no complications in this study.

The limitations of this study include lack of a randomized control group and the small number of patients. This study, however, was designed only as a pilot investigation. A double-blind, placebo-controlled, prospective multicenter trial has now been approved, which should help better evaluate PRP as a treatment for elbow tendinosis. Further study of PRP versus whole blood or cortisone should also be performed in the future.

This report outlines the first in vivo human investigation of autologous growth factors as a treatment for chronic severe elbow tendinosis in patients who have failed nonoperative treatment. The data suggest buffered PRP may be an alternative to surgery in patients with this disorder. In the present investigation, the PRP-treated patients demonstrated significant improvement with a single injection that was sustained over time with no reported complications.

ACKNOWLEDGMENT

We thank James Cox for his help in the preparation of the PRP and his contributions to this research.

REFERENCES