WONDER WHY?

Platelet Rich Plasma Grafts In Musculoskeletal Medicine

Kristin S. Tate, MD, MPH & David M. Crane, MD

ABSTRACT

Platelet Rich Plasma (PRP) grafts are growing in popularity in the musculoskeletal arena. This article explains the risks and considerations for using PRP in the clinical setting, in addition to the authors' method of preparing a PRP graft. This article reviews the basic biology of platelets and growth factors, as well as the stages of healing. Lastly, the authors review the medical literature related to PRP and discuss their experience with PRP in their private practice.

Journal of Prolotherapy. 2010;2(2):371-376.
KEYWORDS: growth factors, platelet biology, Platelet Rich Plasma, PRP graft, ultrasound.

INTRODUCTION

The use of Platelet Rich Plasma (PRP) grafts in treating patients in the musculoskeletal arena has grown exponentially in the last few years. Since it first was introduced in 1987 by Ferrari et al in the cardiothoracic surgery arena,[1] PRP has been used and proven effective in multiple other medical specialties including cosmetic surgery, podiatry, ENT, neurosurgery, dentistry, maxillofacial surgery, urology, wound healing and ophthalmology.[2, 3] Although providers practicing musculoskeletal (MSK) medicine began using PRP for tendonosis and tendonitis in the early 1990s,[2] an informed patient population fueled by media attention has accelerated patient interest in this therapeutic alternative.

BASICS BIOLOGY OF THE PLATELET

Although this article aims to focus on clinical applications of PRP in musculoskeletal medicine, a brief discussion of blood components and growth factors is necessary. Blood is comprised of red blood cells, white blood cells, plasma, and platelets. Platelets have a lifespan of 7-10 days and aggregate at the site of an injury. The platelet is responsible for hemostasis, construction of new connective tissue, and revascularization.[4] The body's natural reparative mechanism relies on the ability to concentrate platelets and white cells within a fibrin clot at the injury site, which results in a controlled inflammatory response, predictably followed by a proliferative healing response. The proliferative healing response is dominated by platelets and white blood cells selectively time releasing growth factors, recruiting stem cells, and supporting tissue regeneration.[5]

Platelets are formed in bone marrow and contain many intracellular structures. For clinical application, the most notable of these components are two types of granules – the alpha granules and the dense granules. The alpha granules contain coagulation proteins, growth factors, cytokines, chemokines and various other proteins, including adhesion proteins. Platelets are known to contain at least six growth factors that are well known and have previously been proven to be vital to bone and soft tissue healing. *Table 1* summarizes these growth factors and their function.[2, 3] It is the rapid arrival of platelets to the area of injury and their ability to release these growth factors, that allow these tiny cells to play such a vital role in the healing process. The dense granules contain ATP, ADP, serotonin and calcium.[3] Thus, it is the dense granule that provides the factors necessary for platelet aggregation.

PLATELET ACTIVATION

It is necessary for platelets to be activated at the level of tissue injury in order for the PRP graft to be successful. It is during activation that the platelets successfully release their contents and begin the cascade of events that lead to the restoration and growth of normal collagen. The process of collagen repair can be separated into three separate phases or stages.

The three vital stages of healing are inflammation, proliferation, and remodeling.[6] All three of these stages are needed for successful return of a tissue to its

Table 1. Major growth factors in PRP and their roles.

Growth Factor	Role
Transforming Growth Factor-beta **TGF-β**	Regulates balance between fibrosis and myocyte regeneration; Stimulates undifferentiated mesenchymal cell proliferation; Regulates collagen synthesis; Stimulates angiogenesis; Stimulates endothelial chemotaxis; Inhibits macrophage proliferation; Regulates mitogenic effects of other growth factor.
Platelet Derived Growth Factor **PDGFa-b**	Stimulates cell replication; Stimulates angiogenesis; Regulates collagen synthesis; Mitogenic for fibroblast/glial/ smooth muscle cells; Mitogenetic for mesencymal cells and osteoblasts; Stimulates macrophage and neutrophil chemotaxis.
Vascular Endothelial Growth Factor **VEGF**	Stimulates angiogenesis; Increases vessel permeability; Mitogenetic for endothelial cells.
Basic Fibroblast Growth Factor **bFGF**	Stimulates angiogenesis; Stimulates proliferation of myoblasts; Mitogenic for mesenchymal cells, chondocytes and osteoblasts; Promotes growth/differentiation of chondrocytes and osteoblasts.
Epidermal Growth Factor **EGF**	Stimulates angiogenesis; Stimulates proliferation of myoblasts; Mitogenic for mesenchymal cells, chondocytes and osteoblasts; Promotes growth/differentiation of chondrocytes and osteoblasts.
Connective Tissue Growth Factor **CTGF**	Promotes angiogenesis and cartilage regeneration; Promotes fibrosis and platelet adhesion.

normal function. From the time of platelet activation the inflammatory phase begins and can last up to three days. It is during this initial phase that the all-important growth factors are released. After the inflammatory phase, the influx of fibroblasts to the area of injury mark the beginning of the proliferative phase of healing. This second phase can last for weeks, during which time the fibroblasts differentiate and neo-vascularization occurs. The final stage of healing is the remodeling phase, during which the newly laid down collagen matures and strengthens. This final phase of healing can take up to one year to complete.[2, 6]

PLATELET RICH PLASMA MATRIX GRAFTS

Crane and Everts define PRP matrix graft as a "tissue graft incorporating autologous growth factors and/or autologous undifferentiated cells in a cellular matrix whose design depends on the receptor site."[2] PRP is the therapeutic outcome of the centrifugation, or pheresis, of an autologous blood sample in order to extract that portion of the plasma that contains the highest numbers of platelets.[7]

Normal platelet concentration in the blood is 200,000 platelets/ul. PRP has been found to contain up to 10 times the concentration of platelets found in whole blood. Individual patient factors and manufacturer's equipment leads to a degree of variability in final platelet numbers seen in a PRP graft. It is accepted that a PRP graft with a platelet count five to six fold greater than baseline value appears to be adequate to achieve significant outcomes. Many manufacturers promote a high platelet concentration as a reflection of the quality of their device. It must be kept in mind that there exists some data to support that PRP grafts with platelet concentrations greater than eight fold may have pro-inflammatory effects leading to inhibition and possible negative outcomes.[8]

PRP is obtained from a sample of the patient's venous blood drawn at the time of treatment. The blood draw occurs with the addition of an anticoagulant, such as citrate dextrose A to prevent platelet activation prior to use. This sample is then placed in a specialized "table-top" device that allows for automated separation of the PRP from the PPP (platelet poor plasma) and the RBC's (Red Blood Cells). The PRP contains a thin layer of concentrated platelets and a "buffy coat" layer containing an elevated level of leukocytes. Both the concentrated platelets and the "buffy coat" are suspended in a small amount of plasma for subsequent grafting. A 60cc venous blood draw will yield from 6-10cc of PRP depending on the device used and the technique employed. (*See Figure 1.*) This PRP graft is then activated at the time of injection with the addition of calcium and thrombin or when coming in contact with collagen.

Once it has been made, the PRP graft can be placed directly into the damaged tissue to initiate and accelerate repair and regeneration. The successful placement of the

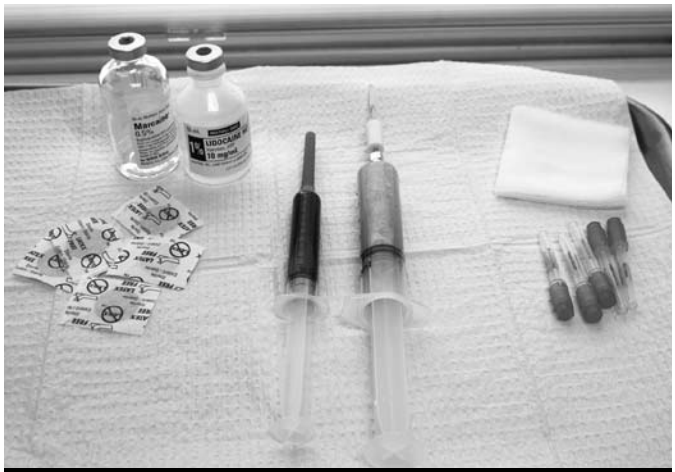

Figure 1. PRP graft ready for injection.

graft into the exact location of damage is necessary for optimal results. This application can be accomplished in the office setting by employing needle-guided radiological visualization of accurate placement (MSK ultrasound, fluoroscopy, CT, MRI), and in the operative setting via open or arthroscopic techniques.

RISKS AND CONTRAINDICATIONS

The natural acceleration of patient healing achieved with PRP has been proven to be inherently safe. The PRP graft is derived from autologous blood drawn at the time of treatment. Any allergic potential would be due to additive agents such as local anesthetics employed for patient comfort at the time of injection. Thorough screening should bring the risk of allergy effectively to zero. The autologous nature of the sample also eliminates concern over disease transfer. The application of the PRP graft should occur under sterile conditions. Under such conditions the risk of infection is the same as that of any percutaneous technique—1:50,000.[2] It has also been shown that due to the presence of white blood cells, PRP grafts are bacteriocidal, especially against Staphylococcus aureus and E. Coli.[9] Studies of autologous PRP grafts have shown no risk of carcinogenesis.

As seen with any needle-guided delivery method there is the possibility of hollow organ puncture. This risk is lessened when the practitioner utilizes and is skilled in radiologic methods of needle guidance such as MSK ultrasound or CT. The use of such guidance techniques also increases treatment success via ensuring accurate placement of the graft.

The most common patient complaint and the most notable drawback to PRP injections is their inherently painful nature. PRP injections can be painful both during the procedure itself as well as the ensuing acute inflammatory phase. The former can be minimized via appropriate local anesthetic placement prior to introduction of the graft itself. The practitioner can also mix the local anesthetic with PRP without reducing growth factor function or causing unwanted platelet activation.[10] Post-procedure discomfort can be managed with judicious use of topical ice application and analgesics (excluding NSAIDs). A narcotic analgesic is often necessary.

Also, many patients have a phobia of needles and those medical procedures that make use of percutaneous methods. This anxiety can be minimized by the use of an oral anxiolytic prior to the procedure, or conscious sedation measures for the procedure itself. These decisions must be made based on the facility where the procedure is being performed, as well as the practitioner's and patient's comfort level. It is better to address and treat the patient's fear than to risk the possibility of syncope at the time of the procedure.

Contraindications to the use of PRP grafts include septicemia, thrombocytopenia (platelet count < 105 / uL), platelet dysfunction syndrome, hypofibrinogenemia, history of a corticosteroid injection at the treatment site or systemic use of corticosteroid within two weeks of the procedure, the routine use of NSAIDs within 48 hours of the procedure, recent fever or illness, skin breakdown or rash at site of injection, history of active tumor, cancer or metastatic state, anemia (Hgb < 10 g/dL), and active infection with Pseudomonas, Enterococcus, or Klebsiella. [2, 3, 9, 11]

PRP GRAFTS AND MUSCULOSKELETAL MEDICINE

The rapid interest in PRP and its case-based success has led to widespread use of the technique in the treatment in the musculoskeletal arena. Orthopedists, physiatrists, primary care sports medicine physicians, rheumatologists, and pain management specialists are among the practitioners who are utilizing PRP grafts in their practices to manage and treat various tendon, ligament, muscle, bone, nerve, and cartilage injuries. It is important that physicians of all specialties realize the necessity of proper training in order to successfully perform PRP grafts in their practices. The practitioner must understand the science and basic cell biology of PRP since it differs greatly from other conventional treatment options. Also, the time required to train and become skilled in using a radiologic method to ensure successful percutaneous placement of the graft should not be underestimated. Again, it needs to be stressed that ensuring the exact placement of the PRP graft directly into the area injured is vital to successful outcomes. As with any emerging treatment regimen, we owe it to our patients to first understand the reason behind its use and then to become adept at performing the therapeutic technique prior to incorporating it into our practices.

PRP IN THE CLINICAL SETTING

PRP has been demonstrated for over 20 years to be a safe and effective treatment option in both human and animal studies. A plethora of animal studies have demonstrated

the effectiveness of PRP in treating injury to tendon, ligament, muscle, bone, and cartilage.[2, 3, 4, 12] As with many emerging new treatment options most of the evidence to support the use of PRP in the human musculoskeletal arena is case-based and anecdotal. Unfortunately, most human studies to date in the musculoskeletal arena are pilot studies or case reports with relatively small sample sizes. To date, very few clinical trials have been published. Despite the lack of controlled trials, the anecdotal evidence of PRP's efficacy is marked and patient satisfaction with this alternative option when faced with chronic pain or surgical intervention is high. The authors utilize PRP in the treatment of injuries to tendon, ligament, muscle, bone, nerve and joint/cartilage with great success at pain reduction and return to desired level of activity. (*See Figure 2.*)

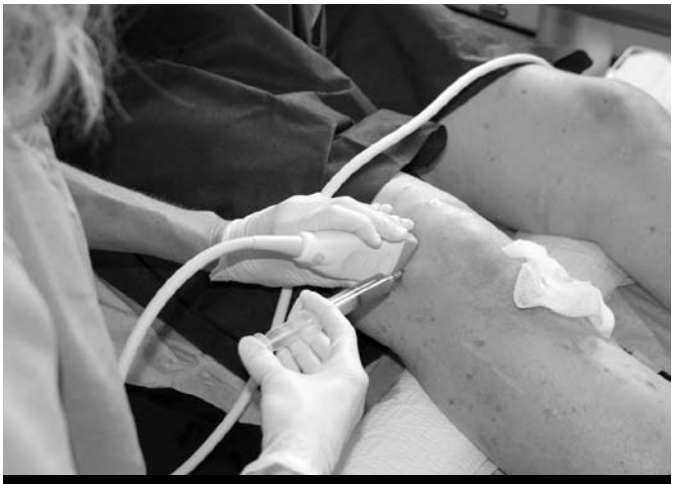

Figure 2. Dr. Tate performing PRP treatment to a knee using ultrasound guidance.

Most current published data demonstrating the effectiveness of PRP in musculoskeletal applications has been its use in tendon injury and tendon pathology. It is well accepted that tendonosis is an intrinsic degenerative disorder as evidenced by surgical biopsies. These samples show a lack of inflammatory cells and disorganize collagen matrices.[13, 14] It has also been established that chronic mechanical overuse is not the main etiologic factor in the development of tendonosis.[15, 16] A controlled, but adequate, inflammatory response followed by a proliferative healing phase is needed to treat this type of chronic tendon pathology. When directly applied to the area of tendonosis, PRP provides this exact cascade of events and leads to healing of the abnormal tendon.

A few human studies on the application of PRP on Achilles tendon rupture have been published. Sanchez[17] reported on a case control study of 12 athletes with complete Achilles tendon rupture who underwent surgical repair. The group treated with PRP had statistically significant improvement in time to functional recovery. In a follow-up report, Sanchez[18] carried out a case study of open suture repair of Achilles tendon rupture both with and without PRP. The PRP treated group recovered their range of motion (ROM) sooner, had no wound related complications, and took less time to return to running and full activities.

De Vos[19] and a group from the Netherlands recently published a study on treating Achilles tendon injury with PRP versus saline injections under ultrasound guidance. The study was a single-center randomized trial performed over a six month period on 54 subjects. The researchers concluded that among patients with chronic Achilles tendinopathy who were treated with eccentric exercises, a PRP injection compared with a saline injection did not result in greater improvement in pain and activity. This study does have some limitations. Both the PRP treatment and the placebo group underwent an eccentric exercise program. It is well known that eccentric exercise alone can lead to improved outcomes in patients with chronic Achilles tendinopathy. The study should have either included a third control group to isolate the effects of eccentric exercise or chosen only subjects who had already undergone an eccentric exercise program and failed to improve. Also, the diagnosis of tendinopathy was based upon subjective complaints and physical exam findings. If the researchers had access to ultrasound for needle guidance they should also have employed ultrasound to provide further objective evidence of chronic Achilles tendinopathy before including patients in the study group. Thirdly, there was no laboratory platelet count performed to verify that adequate concentrations of PRP were achieved prior to injection. It is well known that the concentration of platelets & leukocytes produced varies between the numerous platelet separation devices available on the market. Thus, having a known cell count is necessary in order to ensure an adequately concentrated PRP graft has been obtained. Fourthly, there was only a single injection of PRP in small amounts without noting peppering of the teno-osseous junction. In the authors' experience performing a series of two to three injections every five to six weeks provides optimum results in return to full activity without limitation or discomfort.

Looking at tendonosis in the patient with elbow pain, Mishra et al.[20] studied the use of PRP in 20 patients whose chronic (mean of 25 months) lateral epicondylitis failed surgical intervention. The treatment groups were partially randomized as follows: 15 of these patients were injected with PRP, while the remaining five received a local anesthetic. At the study's final follow-up (two years), he found a near 93% improvement in the patient's perception of pain, and that 94% had returned to full sporting or work activities. Although an excellent pilot study, the small sample size and retrospective nature of this research limits its overall power.[21]

In 2004, Barnett et al.[22] conducted a small pilot study using PRP to treat patients with plantar fasciitis diagnosed on the basis of clinical findings and confirmed with musculoskeletal ultrasound. This study was a case series of nine plantar fascia patients. All the patients underwent a diagnostic ultrasound that confirmed the presence of a thickened and hypoechoic plantar fascia. He then utilized ultrasound to inject PRP graft to both the medial and central bands of the plantar fascia. Six of nine patients had complete resolution of pain at two months and one of the three remaining patients received an additional PRP injection with subsequent symptomatic relief. At their one year follow up visit, all nine patients had ultrasound evidence of improvement in the appearance of the plantar fascia, and 77.9% of the patients were pain-free, one of whom requires a second PRP injection to reach this pain-free state.

A prospective study done by Scarpone[23] on patients with shoulder pain who had partial thickness rotator cuff tendon tears in the absence of acromioclavicular (AC) joint narrowing, was performed in 2004. The patients enrolled had all failed traditional conservative measures including NSAIDs, physical therapy and steroid injections. Twelve of 14 patients studied had statistically significant improvement in pain (using two separate pain scales), strength, and endurance at eight weeks.

Other soft tissue applications of PRP that are being used and studied are in the treatment of acute and chronic muscle tears. Sanchez[18] in 2005 published a study of 20 athletes with small intra-substance muscle tears whose underwent ultrasound guided percutaneous injection of PRP. He reported that the patients recovered up to two times faster than would be expected with other conservative treatment regimens, and none of the athletes had resultant excessive fibrosis or adverse affects.

The application of PRP intraoperatively is growing in popularity as well. Hee et al.[24] performed a controlled trial on a group of patients undergoing instrumented transforaminal lumbar interbody fusion. Twenty-three patients were randomized to the group receiving PRP intraoperatively. This study showed accelerated bony healing in the PRP group. Interestingly, there was no increase in overall fusion rate versus control.

Jenis et al.[25] studied the use of PRP in anterior interbody lumbar fusions. Twenty-two patients underwent iliac crest bone autographs and 15 received PRP and an allograft. Radiographic evaluation at six, 12, 24 months demonstrated an 85% fusion rate for the autograft group and an 89% fusion rate for the PRP plus allograft group.

Gardner[26] performed a retrospective case series in a group of patients undergoing total knee arthroplasty (TKA) who received platelet gel intraoperatively. He found that these patients that were treated with platelet gel at the time of surgery had a lower blood loss, improved ROM and less need for narcotic pain control.

Everts et al.[27] also studied the use of PRP in patients undergoing total knee replacement. This study was controlled and involved 160 patients. Eighty-five of these patients were treated intraoperatively with platelet gel/fibrin sealants. This group of patients who received the autologous platelet product had a lower post-op wound complication rate, reduced need for blood transfusion, fewer post-procedure wound infections and shorter overall hospital stays.

CONCLUSION

In summary, for over 20 years, PRP has been used safely in many medical specialties and in numerous dental applications. PRP matrix grafts are rapidly gaining popularity in the management of patients with musculoskeletal complaints driven by consumer demand and physician interest. These autologous grafts have been proven in many studies to be very safe. In the musculoskeletal arena, PRP has a host of anecdotal evidence of efficacy, and a few studies performed to date support these practice based findings. In addition, this biological alternative is a relatively low cost option to patients with a variety of tendon, ligament, bone, nerve, cartilage, and muscle pathology.

First line treatment methods such as rest, bracing or kinesiotaping, evaluation of kinetic chain abnormalities and physical therapy should be considered before pursuing the application of a PRP graft. Should these first line treatments fail, application of a PRP graft should be considered and discussion of this alternative approach needs to be shared with the patient. Once the PRP has successfully turned off the pain generators, restoration of the normal kinetic chain via physical therapy, Pilates therapy, etc…can be pursued.

The authors have been using PRP grafts in the outpatient setting with great success over the past five years. Despite such anecdotal success, there exists a desperate need for randomized placebo controlled trials to support the clinical evidence put forth in the literature to date. We have also begun using other biologic treatment options such as bone marrow aspirate stem cell grafts and other autologous matrices for those cases where a greater autologous stem cell load is needed. Future studies using validated clinical measures, and radiological, biomechanical and tissue injury/healing-responsive biomarkers as secondary outcome measures are needed to determine whether PRP and other autologous biologic grafts can play a definitive role in a cure for musculoskeletal injuries. ■

BIBLIOGRAPHY

1. Ferrari M, et al. A new technique for hemodilution, preparation of autologous platelet-rich plasma and intraoperative blood salvage in cardiac surgery. *Int J Artif Org*. 1987;10:47–50.
2. Crane D, et al. Platelet rich plasma (PRP) matrix grafts. *PPM*. 2008;8:12-27.
3. Everts P, et al. Platelet-rich plasma and platelet gel: a review. *JECT*. 2006;38:174-187.
4. Sampson, et al. Platelet rich plasma injection grafts for musculoskeletal injuries: a review. *Curr Rev Musculoskelet Med*. 2008;1(3-4):165-174.
5. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent*. 2001;10:225-228.
6. Kumar, et al. *Pathologic basis of disease*, 7th ed. Chapter 2- Acute and chronic inflammation. Copyright 2005 Saunders. Available from: www.mdconsult.com.
7. Marx RE, et al. *Dental and craniofacial applications of platelet-rich plasma*. Chapter 1. Quintessence Publishing Co Ltd. ISBN: 0867154322.
8. Hesham E, et al. Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. *J Periodontol*. April 2007;78:661-669.
9. Bielecki TM, et al. Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances; an in vitro study. *The Journal of Bone and Joint Surgery*. March 2007;89-B(No. 3):417-420.
10. Creaney L, et al. Growth factor delivery methods in the management of sports injuries: the state of play. *Br. J. Sports Med*. Published online 5 Nov 2007;10.1136/bjsm.2007.1-16.
11. Kevy SV, et al. Comparison of methods for point of care preparation of autologous platelet gel. *J Extra Corpor Technol*. 2004;36:28-35.
12. Gamradt, et al. Platelet rich plasma in rotator cuff repair. *Techniques in Orthopaedics*. 22(1):26-33.
13. Almekinders LC, et al. Etiology, diagnosis and treatment of tendonitis: an analysis of the literature. *Med Sci Sports Exerc*. 1998 Aug;30(8):1183-90.
14. Aström M, et al. No effect of piroxicam on achilles tendinopathy: a randomized study of 70 patients. *Acta Orthop Scand*. 1992 Dec;63(6):631-4.
15. Cook JL, et al. Asymptomatic hypoechoic regions on patellar tendon ultrasound: A 4-year clinical and ultrasound followup of 46 tendons. *Scand J Med Sci Sports*. 2001 Dec;11(6):321-7.
16. Shalaby M, et al. Patellar tendinitis: the significance of magnetic resonance imaging findings. *Am J Sports Med*. May 1999; 27(3):345-349.
17. Sanchez M, et al. Comparison of surgically repaired achilles tendon tears using platelet-rich fibrin matrices. *Am J Sp Med*. 2007.
18. Sanchez M, et al. Application of autologous growth factors on skeletal muscle healing. *2nd World Congress on Regenerative Medicine*. Podium Presentation. May 2005.
19. De Vos RJ, et al. Platelet-rich plasma injection for chronic achilles tendinopathy – a randomized controlled trial. *JAMA*. 2010;303(2):144-149.
20. Mishra, et al. Treatment of chronic elbow tendinosis with buffered platelet rich plasma. *AM J Sports Med*. 2006;10(10):1-5.
21. Mishra A, et al. Human skin fibroblast proliferation in buffered platelet rich plasma. American Academy of Orthopaedic Surgeons Poster Presentation. March 2006.
22. Barett S. Growth factors for chronic plantar fasciitis? *Podiatry Today*. 2004.17:36-42.
23. Scarpone MA, et al. PRP as a treatment alternative for symptomatic rotator cuff tendinopathy for patients failing conservative treatment. 2005. Available from: http://www.treatingpain.com/pages/ int_pain/ScarponeRotatorCuffstudy.pdf. Last accessed 1/2/08. 35(2):245-251.
24. Hee, et al. Do autologous growth factors enhance transforaminal lumbar interbody fusion? *Eur Spine J*. 2003;12(12):400-7.
25. Jenis, et al. A prospective study of autologous growth factors in lumbar interbody fusion. *Spine J*. 2006;6(1):14-21.
26. Gardner, et al. The efficacy of autologous platelet gel in pain control and blood loss in total knee arthroplasty: an analysis of the hemoglobin, narcotic requirement and range of motion. *Int Orthop*. 2006;31:309-13.
27. Everts, et al. Platelet gel and fibrin sealant reduce allogeneic blood transfusions in total knee arthroplasty. *Acta Anaesthesiol Scand*. 2006;50:593-19.