PASIG PERFORMING ARTS
SPECIAL INTEREST GROUP

ORTHOPAEDIC SECTION
AMERICAN PHYSICAL THERAPY ASSOCIATION

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American Physical Therapy Association
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PASIG MONTHLY CITATION BLAST: No.38 **March 2009**

Dear PASIG and Orthopaedic Section members:

To all of those we met at this year's CSM, hello and welcome. The PASIG sends a warm thank you to our speakers and all those who attended our PASIG programming entitled "*Foot and Ankle in Performing Artists: From Show Girls in Heels and Skaters in Boots to Barefoot Dancers and Gymnasts. Measuring, Manual Therapy and Rehabilitation, and Footwear Modifications*." We had record-breaking turnout for this program. Cheers to our PASIG Vice President Tara Jo Manal for superb programming!

To those who presented your performing arts research as a platform or poster, thank you for your contributions. We hope to see your work published in the future. If I or any members of the Research Committee can be of any assistance, please don't hesitate to contact us.

And a warm welcome to all *Orthopaedic Section* members. We're mailing you this Citation BLAST as an example of what benefits the PASIG offers to its members. ***If you'd like to continue receiving these BLASTs, check us out and join the PASIG - it's free***. (To join, go to the Orthopaedic Section webpage: www.orthopt.org and you will find a membership application in the menu on the left).

The PASIG Research Committee initiated this monthly Citation BLAST on performing arts-related topics in June 2005 in the hopes of encouraging our members to stay current in the literature and, perhaps, consider conducting research themselves. Each month we send a new list of performing arts (PA) citations to members of the PASIG to further the pursuit of PA-related scholarship. Each month's citations are added to specific EndNote libraries: 1) Ice Skating, 2) Gymnastics, 3) Music, and 4) Dance. We've added additional EndNote libraries as our range of topics has grown. The BLASTS and updated libraries are posted on the PASIG webpage for our members to access and download. (Information about EndNote referencing software can be found at http://www.endnote.com, including a 30-day free trial).

For this March Citation BLAST, I've selected references on a topic recently in the press: *Platelet-rich Plasma Therapy*. The format is an annotated bibliography of articles on the selected topic from 1996 – 2008. Anyone interested in contributing a special topic citation blast, please volunteer.

The PASIG has new goals to provide members with content and to connect members together. To accomplish these goals, we have developed a survey to more fully understand our membership and to provide meaningful services that membership desires. Please click on the link below to complete the survey. It should take approximately 10 minutes. http://www.orthopt.org/surveys/pasig.php **Please complete the survey by March 31, 2009**.

As always, your comments and suggestions are welcome. Please drop me an e-mail anytime.

Regards,
Shaw

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Platelet-rich Plasma Therapy

I recently met a dancer who strained his adductor muscle while performing in Vail. He was seen at the Steadman-Hawkins Clinic and received platelet-rich plasma therapy (PRP). This subject seems topical with press attention about professional athletes receiving this treatment (NY Times, February 17, 2009, p1). One orthopaedic researcher, G. van Osch, calls it a "growth factor cocktail". Double blind studies are currently being conducted on Achilles tendon injuries, rotator cuff strains, partial knee ligament tears, and bone fractures. A patient's autologous plasma is centrifuged to deliver a high concentration of platelets within one to three injections. Use of anti-inflammatory medications is contraindicated following PCP treatment. Light exercise is permitted following an injection.

While the premise of using autologous bloods suggests it is a safe procedure, efficacy outcomes are necessary. Furthermore, for sports, PCP may violate anti-doping regulations that prohibit growth factor IGF-1. While 'doping' is not as much of an issue for performing artists, this treatment warrants monitoring. I know performing artists will be asking about it.

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Anitua E, Sanchez M, et al. (2006). New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol **24**(5): 227-34.

The therapeutic use of autologous platelet-rich plasma constitutes a relatively new biotechnology that has been a breakthrough in the stimulation and acceleration of soft-tissue and bone healing. The efficiency of this process lies in the local and continuous delivery of a wide range of growth factors and proteins, mimicking the needs of the physiological wound healing and reparative tissue processes. Consequently, the application of platelet-rich plasma has been extended to many different fields, including orthopedics, sports medicine, dentistry, cosmetic and periodontal medicine and cosmetic, plastic and maxillofacial surgery. This article highlights the use of this technology and discusses some of the obstacles and challenges that need to be addressed to maintain progress in this field.

Cancedda R, Giannoni P, et al. (2007). A tissue engineering approach to bone repair in large animal models and in clinical practice. Biomaterials **28**(29): 4240-50.

The repair of large segmental bone defects due to trauma, inflammation and tumor surgery remains a major clinical problem. Animal models were developed to test bone repair by tissue engineering approaches, mimicking real clinical situations. Studies differed with regard to animals (dog, sheep, goat), treated bone (femur, tibia, mandible), chemistry and structure of the scaffolds. Still, an advantage in the bone formation and in the healing of the segmental defect was always observed when scaffolds were seeded with bone marrow derived stromal cells (BMSCs). In the year 1998 was performed the first implantation of a porous ceramic construct in a bone segmental defect of a patient; it was the first construct seeded with cultured autologous osteogenic cells. Since then, only few other similar cases were treated by the same approach. However, in other fields, such as oral and maxillofacial surgery, injectable cells/platelet-rich plasma composites have been used as grafting materials for maxillary sinus floor augmentation and/or onlay plasty. More recently, the reconstruction of a human mandible was also reported by means of a bone-muscle-flap in vivo prefabrication technique, where the patient served as his own bioreactor. Indeed continuous implementations test and provide new means of defects treatment and cure. However, based on results so far obtained in animal models and pilot clinical studies, one can affirm that the bone tissue engineering approaches, although successful in most cases, need further validation before a wide application in clinics. In particular, the supply of oxygen and nutrients to the cells in the inner part of the implanted scaffolds remains a major concern, requiring additional investigations.

Cara DC, Ebbert KV, et al. (2004). Mast cell-independent mechanisms of immediate hypersensitivity: a role for platelets. J Immunol **172**(8): 4964-71.

Mast cells have been implicated as the central effectors in allergic responses, yet a fatal anaphylactic response can be induced in mast cell-deficient mice. In this study, we examined the immediate hypersensitivity response in wild-type (WT) and mast cell-deficient mice (W/W(v)) in two different tissues (skin and skeletal muscle). Vascular permeability and leukocyte recruitment were studied after immediate challenge or 4 h postchallenge in OVA-sensitized mice. In skin, immediate challenge induced a significant increase in vascular permeability (75%) within 30 min and was accompanied by increased leukocyte adhesion 4 h postchallenge. In the absence of mast cells, no changes in vascular permeability or leukocyte recruitment were observed in skin. In WT skeletal muscle, immediate challenge induced a rapid increase (80%) in vascular permeability within 5 min and significant leukocyte recruitment after 4 h. Surprisingly, in W/W(v), a gradual increase in vascular permeability was observed, reaching a maximum (50%) within 30 min. Despite the absence of mast cells, subsequent leukocyte emigration was similar to that observed in WT mice. Pretreatment with anti-platelet serum in W/W(v) returned Ag-induced vascular permeability and leukocyte recruitment to baseline. Platelets were shown to interact with endothelium in skeletal muscle, but not dermal microvasculature. These data illustrate that mast cells play a prominent role in vascular permeability and leukocyte recruitment in skin in response to Ag, however, in skeletal muscle; these changes can occur in the absence of mast cells, and are mediated, in part, by the presence of platelets.

Creaney L, Hamilton B (2008). Growth factor delivery methods in the management of sports injuries: the state of play. Br J Sports Med **42**(5): 314-20.

In recent years there have been rapid developments in the use of growth factors for accelerated healing of injury. Growth factors have been used in maxillo-facial and plastic surgery with success and the technology is now being developed for orthopaedics and sports medicine applications. Growth factors mediate the biological processes necessary for

repair of soft tissues such as muscle, tendon and ligament following acute traumatic or overuse injury, and animal studies have demonstrated clear benefits in terms of accelerated healing. There are various ways of delivering higher doses of growth factors to injured tissue, but each has in common a reliance on release of growth factors from blood platelets. Platelets contain growth factors in their alpha-granules (insulin-like growth factor-1, basic fibroblast growth factor, platelet-derived growth factor, epidermal growth factor, vascular endothelial growth factor, transforming growth factor-beta(1)) and these are released upon injection at the site of an injury. Three commonly utilised techniques are known as platelet-rich plasma, autologous blood injections and autologous conditioned serum. Each of these techniques has been studied clinically in humans to a very limited degree so far, but results are promising in terms of earlier return to play following muscle and particularly tendon injury. The use of growth factors in sports medicine is restricted under the terms of the World Anti-Doping Agency (WADA) anti-doping code, particularly because of concerns regarding the insulin-like growth factor-1 content of such preparations, and the potential for abuse as performance-enhancing agents. The basic science and clinical trials related to the technology are reviewed, and the use of such agents in relation to the WADA code is discussed.

de Mos M, van der Windt AE, et al. (2008). Can platelet-rich plasma enhance tendon repair? A cell culture study. Am J Sports Med **36**(6): 1171-8.

BACKGROUND: Autologous platelet-rich plasma (PRP) application appears to improve tendon healing in traumatic tendon injuries, but basic knowledge of how PRP promotes tendon repair is needed. HYPOTHESIS: Platelet-rich plasma has a positive effect on cell proliferation and collagen production and induces the production of matrix-degrading enzymes and endogenous growth factors by human tenocytes. STUDY DESIGN: Controlled laboratory study. METHODS: Human tenocytes were cultured 14 days in 2% fetal calf serum medium complemented with 0%, 10%, or 20% vol/vol platelet-rich clot releasate ([PRCR] the active releasate of PRP) or platelet-poor clot releasate (PPCR). At day 4, 7, and 14, cell amount, total collagen, and gene expression of collagen I alpha 1 (COL1) and III alpha 1 (COL3), matrix metalloproteinases ([MMPs] MMP1, MMP3, and MMP13), vascular endothelial-derived growth factor (VEGF)-A, and transforming growth factor (TGF)-beta1 were analyzed. RESULTS: Platelet numbers in PRP increased to 2.55 times baseline. Growth-factor concentrations of VEGF and platelet-derived growth factor (PDGF)-BB were higher in PRCR than PPCR. Both PRCR and PPCR increased cell number and total collagen, whereas they decreased gene expression of COL1 and COL3 without affecting the COL3/COL1 ratio. PRCR, but not PPCR, showed upregulation of MMP1 and MMP3 expression. Matrix metalloproteinase 13 expression was not altered by either treatment. PRCR increased VEGF-A expression at all time points and TGF-beta1 expression at day 4. CONCLUSION: In human tenocyte cultures, PRCR, but also PPCR, stimulates cell proliferation and total collagen production. PRCR, but not PPCR, slightly increases the expression of matrix-degrading enzymes and endogenous growth factors. CLINICAL RELEVANCE: In vivo use of PRP, but also of PPP to a certain extent, in tendon injuries might accelerate the catabolic demarcation of traumatically injured tendon matrices and promote angiogenesis and formation of a fibrovascular callus. Whether this will also be beneficial for degenerative tendinopathies remains to be elucidated.

Intini G, Andreana S, et al. (2007). Calcium sulfate and platelet-rich plasma make a novel osteoinductive biomaterial for bone regeneration. J Transl Med **5**: 13.

BACKGROUND: With the present study we introduce a novel and simple biomaterial able to induce regeneration of bone. We theorized that nourishing a bone defect with calcium and with a large amount of activated platelets may initiate a series of biological processes that

culminate in bone regeneration. Thus, we engineered CS-Platelet, a biomaterial based on the combination of Calcium Sulfate and Platelet-Rich Plasma in which Calcium Sulfate also acts as an activator of the platelets, therefore avoiding the need to activate the platelets with an agonist. METHODS: First, we tested CS-Platelet in heterotopic (muscle) and orthotopic (bone) bone regeneration bioassays. We then utilized CS-Platelet in a variety of dental and craniofacial clinical cases, where regeneration of bone was needed. RESULTS: The heterotopic bioassay showed formation of bone within the muscular tissue at the site of the implantation of CS-Platelet. Results of a quantitative orthotopic bioassay based on the rat calvaria critical size defect showed that only CS-Platelet and recombinant human BMP2 were able to induce a significant regeneration of bone. A non-human primate orthotopic bioassay also showed that CS-Platelet is completely resorbable. In all human clinical cases where CS-Platelet was used, a complete bone repair was achieved. CONCLUSION: This study showed that CS-Platelet is a novel biomaterial able to induce formation of bone in heterotopic and orthotopic sites, in orthotopic critical size bone defects, and in various clinical situations. The discovery of CS-Platelet may represent a cost-effective breakthrough in bone regenerative therapy and an alternative or an adjuvant to the current treatments.

Kasemkijwattana C, Menetrey J, et al. (2000). Use of growth factors to improve muscle healing after strain injury. Clin Orthop Relat Res(370): 272-85.

Muscle injuries represent a large number of professional and recreational sports injuries. Muscle strains habitually occur after an eccentric contraction, which often leads to an injury located in the myotendinous junction. Treatment varies widely, depending on the severity of the trauma, but has remained limited mostly to rest, ice, compression, elevation, antiinflammatory drugs, and mobilization. The authors' research group aims to develop new biologic approaches to improve muscle healing after injuries, including muscle strains. To achieve this goal, the authors investigated several parameters that will lead to the development of new strategies to enhance muscle healing. The authors first evaluated natural muscle healing after strain injuries and showed that muscle regeneration occurs in the early phase of healing but becomes impaired with time by the development of tissue fibrosis. Several growth factors capable of improving muscle regeneration were investigated; basic fibroblast growth factor, insulin-like growth factor, and nerve growth factors were identified as substances capable of enhancing muscle regeneration and improving muscle force in the strained injured muscle. The current study should aid in the development of strategies to promote efficient muscle healing and complete recovery after strain injury.

Kasemkijwattana C, Menetrey J, et al. (1998). Development of approaches to improve the healing following muscle contusion. Cell Transplant **7**(6): 585-98.

Muscle injuries are a challenging problem in traumatology, and the most frequent occurrence in sports medicine. Muscle contusions are among the most common muscle injuries. Although this injury is capable of healing, an incomplete functional recovery often occurs, depending on the severity of the blunt trauma. We have developed an animal model of muscle contusion in mice (high energy blunt trauma) and characterized the muscle's ability to heal following this injury using histology and immunohistochemistry to determine the level of muscle regeneration and the development of scar tissue. We have observed a massive muscle regeneration occurring in the first 2 wks post-injury that is subsequently followed by the development of muscle fibrosis. Based on these observations, we propose that the enhancement of muscle growth and regeneration, as well as the prevention of fibrotic development, could be used as approach(es) to improve the healing of muscle injuries. In fact, we have identified three growth factors (bFGF, IGF-1, and NGF) capable of enhancing myoblast proliferation and differentiation in vitro and improving the healing of the injured muscle in vivo. Furthermore, the ability of adenovirus to mediate direct and ex vivo

gene transfer of beta-galactosidase into the injured site opens possibilities of delivering an efficient and persistent expression of these growth factors in the injured muscle. These studies should help in the development of strategies to promote efficient muscle healing with complete functional recovery following muscle contusion.

Menetrey J, Kasemkijwattana C, et al. (2000). Growth factors improve muscle healing in vivo. J Bone Joint Surg Br **82**(1): 131-7.

Injury to muscles is very common. We have previously observed that basic fibroblast growth factor (b-FGF), insulin growth factor type 1 (IGF-1) and nerve growth factor (NGF) are potent stimulators of the proliferation and fusion of myoblasts in vitro. We therefore injected these growth factors into mice with lacerations of the gastrocnemius muscle. The muscle regeneration was evaluated at one week by histological staining and quantitative histology. Muscle healing was assessed histologically and the contractile properties were measured one month after injury. Our findings showed that b-FGF, IGF and to a less extent NGF enhanced muscle regeneration in vivo compared with control muscle. At one month, muscles treated with IGF-1 and b-FGF showed improved healing and significantly increased fast-twitch and tetanus strengths. Our results suggest that b-FGF and IGF-1 stimulated muscle healing and may have a considerable effect on the treatment of muscle injuries.

Mishra A, Pavelko T (2006). Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med **34**(11): 1774-8.

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 noted 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.

Mishra A, Tummala P, et al. (2009). Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. Tissue Eng Part C Methods.

The success of tissue engineering applications can potentially be dramatically improved with the addition of adjuncts that increase the proliferation and differentiation of progenitor or stem cells. Platelet-rich plasma (PRP) has recently emerged as a potential biologic tool to

treat acute and chronic tendon disorders. The regenerative potential of PRP is based on the release of growth factors that occurs with platelet rupture. Its autologous nature gives it a significant advantage in tissue engineering applications. To test whether PRP may be useful specifically for cartilage regeneration, a cell culture experiment was devised in which mesenchymal stem cells (MSCs) were grown in control media or media enhanced with inactivated, buffered PRP. Proliferation 7 days after PRP treatment was increased: 1.041 versus 0.199 for the control media cells ($p < 0.001$). The messenger RNA (mRNA) level of the osteogenic marker RUNX2 was 52.84 versus 26.88 for the control group ($p < 0.005$). Likewise the mRNA level of the chondrogenic markers Sox-9 and aggrecan was 29.74 versus 2.29 for the control group ($p < 0.001$) and 21.04 versus 1.93 ($p < 0.001$), respectively. These results confirm that PRP enhances MSC proliferation and suggest that PRP causes chondrogenic differentiation of MSC in vitro.

Mishra A, Woodall A, et al. (2009). Treatment of tendon and muscle using platelet-rich plasma. Clin Sports Med **28**(1): 113-25.

Tendon and muscle injuries are common in elite and weekend athletes. Treatment of these injuries in both groups is rapidly evolving. Sports medicine patients are demanding better and less invasive solutions for all types of musculoskeletal disorders. In this context, platelet-rich plasma (PRP) has emerged as a potential solution. PRP is a fraction of whole blood containing concentrated growth factors and proteins. These cytokines direct tissue healing through autocrine and paracrine effects. The number of basic science, animal, and human investigations of PRP for tendon and muscle injuries worldwide has risen sharply in recent years. These studies are helping clinicians better understand the mechanisms of PRP and are guiding novel treatment protocols. In this paper, the value of PRP as a treatment for acute or chronic tendon and muscle disorders is explored.

Randelli PS, Arrigoni P, et al. (2008). Autologous platelet rich plasma for arthroscopic rotator cuff repair. A pilot study. Disabil Rehabil **30**(20-22): 1584-9.

BACKGROUND AND PURPOSE: Arthroscopic repair of rotator cuff tears can produce excellent results. The application of platelet rich plasma during arthroscopic rotator cuff repair is safe, and produces results which do not deteriorate over time. METHODS: A total of 14 patients undergoing arthroscopic repair of a rotator cuff tear received an intra-operative application of autologous platelet rich plasma in combination with an autologous thrombin component after tear repair. Following the procedure, patients were given a standardized rehabilitation protocol, and followed for 24 months. Outcome measures included a pain score (VAS) as well as functional scoring (UCLA and Constant scores). RESULTS: Of the original 14 patients, 13 were seen at a final follow-up appointment 24 months after the index operation. Patients demonstrated a significant decrease in VAS scores and significant increases in the UCLA and Constant scores at 6, 12 and 24-month follow-ups compared to a pre-operative score. CONCLUSION: No adverse events related to this application were noted during the procedure. The application of platelet rich plasma during arthroscopic rotator cuff repair is safe and effective, and produces results which seem to be stable with time. A prospective randomized investigation will be necessary to ascertain the efficacy of platelet rich plasma application to improve or expedite the surgical outcome following arthroscopic rotator cuff repair.

Sanchez M, Anitua E, et al. (2007). Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. Am J Sports Med **35**(2): 245-51.

BACKGROUND: Platelet-rich fibrin matrices release a natural mixture of growth factors that play central roles in the complex processes of tendon healing. HYPOTHESIS: Application of autologous platelet-rich matrices during Achilles tendon surgery may promote healing and

functional recovery. STUDY DESIGN: Case-control study and descriptive laboratory study; Level of evidence, 3. METHODS: Twelve athletes underwent open suture repair after complete Achilles tendon tear. Open suture repair in conjunction with a preparation rich in growth factors (PRGF) was performed in 6 athletes and retrospectively compared with a matched group that followed conventional surgical procedure. The outcomes were evaluated on the basis of range of motion, functional recovery, and complications. Achilles tendons were examined by ultrasound at 50 +/- 11 months in retrospective controls and 32 +/- 10 months in the PRGF group. In the laboratory portion of the study, PRGF treatment was characterized by the number of platelets and concentration of insulin (IGF-I), transformed (TGF-beta1), platelet-derived (PDGF-AB), vascular endothelial (VEGF), hepatocyte (HGF), and epidermal (EGF) growth factors in patients affected by musculoskeletal traumatic injuries. RESULTS: Athletes receiving PRGF recovered their range of motion earlier (7 +/- 2 weeks vs 11 +/- 3 weeks, P = .025), showed no wound complication, and took less time to take up gentle running (11 +/- 1 weeks vs 18 +/- 3 weeks, P = .042) and to resume training activities (14 +/- 0.8 weeks vs 21 +/- 3 weeks, P = .004). The cross-sectional area of the PRGF-treated tendons increased less (t = 3.44, P = .009). TGF-beta1 (74.99 +/- 32.84 ng/mL), PDGF-AB (35.62 +/- 14.57 ng/mL), VEGF (383.9 +/- 374.9 pg/mL), EGF (481.5 +/- 187.5 pg/mL), and HGF (593.87 +/- 155.76 pg/mL) significantly correlated with the number of platelets (677 +/- 217 platelets/microL, P < .05). CONCLUSION: The operative management of tendons combined with the application of autologous PRGF may present new possibilities for enhanced healing and functional recovery. This needs to be evaluated in a randomized clinical trial.

Sanchez M, Azofra J, et al. (2003). Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. Med Sci Sports Exerc **35**(10): 1648-52.

INTRODUCTION: The application of an autologous plasma rich in growth factors is beneficial in restoring connective tissues, as shown by clinical evidence in oral surgery and more recently in arthroscopic anterior cruciate ligament reconstruction and two cases of ruptured Achilles tendon in professional athletes. This is attributed to the slow delivery of growth factors from harvested platelets that have been activated by endogenous thrombin promoted by the addition of calcium chloride. PURPOSE: This case report describes a new application of this therapy in the arthroscopic treatment of a large, nontraumatic avulsion of articular cartilage in the knee of an adolescent soccer player. METHODS: After arthroscopic reattachment of the large (>2 cm) loose chondral body in its crater in the medial femoral condyle, autologous plasma rich in growth factors was injected into the area between the crater and the fixed fragment. RESULTS AND CONCLUSION: Despite the extremely poor prognosis of the case, complete articular cartilage healing was considerably accelerated, and the functional outcome was excellent, allowing a rapid resumption of symptom-free athletic activity. This technique opens new perspectives for human tissue regeneration.

Sariguney Y, Yavuzer R, et al. (2008). Effect of platelet-rich plasma on peripheral nerve regeneration. J Reconstr Microsurg **24**(3): 159-67.

Activated platelets release various growth factors, some of which are recognized to improve nerve regeneration. This study evaluated the effect of platelet-rich plasma (PRP) in end-to-end neurorrhaphy. A total of 45 Wistar rats were used, with the initial five used for PRP preparation. The right hind limbs were used as experimental, with the left as control. The animals were treated in five groups. Group A (n = 4): The right sciatic nerve was dissected only from the sciatic notch to the bifurcation. In all other groups, the nerve was sharply transected and repaired with: group B (n = 8): two sutures; group C (n = 8): six sutures; group D (n = 10): two sutures and PRP; and group E (n = 10): six sutures and PRP. Groups D and E were compared with groups B and C, respectively. Group E had a shorter latency

time in electromyography (P < 0.01) and a thicker myelin layer in the histological evaluation (P < 0.003) in comparison with group C. These positive effects of PRP were not detected in the nerves were repaired with two sutures. In this animal model, the application of PRP to the repair site helped to improve remyelinization of the sciatic nerve in rats when the epineural repair was done with six sutures.

Sato K, Li Y, et al. (2003). Improvement of muscle healing through enhancement of muscle regeneration and prevention of fibrosis. Muscle Nerve **28**(3): 365-72.

Skeletal muscle is able to repair itself through regeneration. However, an injured muscle often does not fully recover its strength because complete muscle regeneration is hindered by the development of fibrosis. Biological approaches to improve muscle healing by enhancing muscle regeneration and reducing the formation of fibrosis are being investigated. Previously, we have determined that insulin-like growth factor-1 (IGF-1) can improve muscle regeneration in injured muscle. We also have investigated the use of an antifibrotic agent, decorin, to reduce muscle fibrosis following injury. The aim of this study was to combine these two therapeutic methods in an attempt to develop a new biological approach to promote efficient healing and recovery of strength after muscle injuries. Our findings indicate that further improvement in the healing of muscle lacerations is attained histologically by the combined administration of IGF-1 to enhance muscle regeneration and decorin to reduce the formation of fibrosis. This improvement was not associated with improved responses to physiological testing, at least at the time-points tested in this study.

Schnabel LV, Mohammed HO, et al. (2007). Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. J Orthop Res **25**(2): 230-40.

Platelet rich plasma (PRP) has recently been investigated for use in tissue regeneration studies that seek to utilize the numerous growth factors released from platelet alpha-granules. This study examined gene expression patterns, DNA, and collagen content of equine flexor digitorum superficialis tendon (SDFT) explants cultured in media consisting of PRP and other blood products. Blood and bone marrow aspirate (BMA) were collected from horses and processed to obtain plasma, PRP, and platelet poor plasma (PPP). IGF-I, TGF-beta1, and PDGF-BB were quantified in all blood products using ELISA. Tendons were cultured in explant fashion with blood, plasma, PRP, PPP, or BMA at concentrations of 100%, 50%, or 10% in serum-free DMEM with amino acids. Quantitative RT-PCR for expression of collagen type I (COL1A1), collagen type III (COL3A1), cartilage oligomeric matrix protein (COMP), decorin, matrix metalloproteinase-3 (MMP-3), and matrix metalloproteinase-13 (MMP-13) was performed as were DNA and total soluble collagen assays. TGF-beta1 and PDGF-BB concentrations were higher in PRP compared to all other blood products tested. Tendons cultured in 100% PRP showed enhanced gene expression of the matrix molecules COL1A1, COL3A1, and COMP with no concomitant increase in the catabolic molecules MMP-3 and MMP-13. These findings support in vivo investigation of PRP as an autogenous, patient-side treatment for tendonitis.

Virchenko O, Aspenberg P (2006). How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation. Acta Orthop **77**(5): 806-12.

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.

Wehling P, Moser C, et al. (2007). Autologous conditioned serum in the treatment of orthopedic diseases: the orthokine therapy. BioDrugs **21**(5): 323-32.

The common strategies for the treatment of patients with orthopedic diseases do not address the underlying pathogenesis. Several biologically based, local therapies aiming to influence the cytokine imbalance are either in development or in the initial stages of clinical use. A method based on exposure of blood leukocytes to pyrogen-free surfaces (e.g. glass spheres) elicits an accumulation of anti-inflammatory cytokines, including interleukin-1 receptor antagonist, and several growth factors, including insulin-like growth factor-1, platelet-derived growth factor, and transforming growth factor-beta(1), in the liquid blood phase. Based on these observations, a new therapy using cell-free, autologous conditioned serum (ACS) from the incubation of whole blood with glass spheres was developed. The injection of ACS into affected tissue(s) has shown clinical effectiveness and safety in animal models and studies, as well as in human clinical studies, for the treatment of osteoarthritis, lumbar stenosis, disc prolapse, and muscle injuries.

Woodall J, Tucci M, et al. (2008). Cellular effects of platelet rich plasmainterleukin1 release from prp treated macrophages. Biomed Sci Instrum **44**: 489-94.

The therapeutic use of Platelet Rich Plasma (PRP) as a biological tool to enhance soft tissue and bone healing has recently yielded encouraging results in many areas of clinical medicine. PRP is a specific portion of whole blood that contains a high concentration of platelets. The local treatment of bone and soft tissue injuries with this autologous blood product has become increasingly common in recent years. There is still little known about the mechanism by which PRP acts at the cellular level. The macrophage cell has been shown to be critical in the healing of tissues. In this study the macrophage release of a specific pro-inflammatory factor, interleukin-1 (IL-1), was evaluated in macrophage cells activated and treated with platelets as compared to control macrophages in culture. The results show that platelets caused an initial suppression of IL-1 release from activated macrophage compared to controls. The initial suppression was followed by an increase in IL-1 release at day seven over control and activated macrophages that had ceased release of IL-1 at day seven. The initial suppression of the inflammatory response to activation during days 1, 2 and 3 could have broad implications in the explanation of a mechanism by which PRP acts. If PRP can truly be used as a transient anti-inflammatory agent that initially suppresses inflammation and then stimulates a late healing response, then indications for use of PRP may expand beyond the current scope of treatment.

Wright-Carpenter T, Klein P, et al. (2004). Treatment of muscle injuries by local administration of autologous conditioned serum: a pilot study on sportsmen with muscle strains. Int J Sports Med **25**(8): 588-93.

Muscle injuries represent a major part of sports injuries and are a challenging problem in traumatology. Strain injuries are the most common muscle injuries after contusions. These injuries can lead to significant pain and disability causing time to be lost to training and competition. Despite the frequency of strain injuries the treatment available is limited and is generally not sufficient to enhance muscle regeneration efficiently when fast resumption of sport activity is a primary target. A number of growth factors play a specific role in regeneration and it has been proven that a previously described method of physically and chemically stimulating whole blood (to produce autologous conditioned serum) induces concentration increases in FGF-2, HGF, and TGF-beta1. A preliminary study was conducted on muscle strain injuries in professional sportsmen receiving either: 1. autologous conditioned serum (ACS) or 2. Actovegin/Traumeel treatment as control. Assessment of recovery from injury was done by: 1. Sport professional's ability to participate to 100 % under competition conditions in their respective sport and 2. MRI analysis. A significant difference in the recovery time from injury was demonstrated: 16.6 +/- 0.9 in the ACS treated instead of 22.3 +/- 1.2 (mean +/- SEM) days in the Actovegin/Traumeel control group (p = 0.001). MRI analysis supported the observed acceleration of the lesion recovery time. We conclude that ACS injection is a promising approach to reduce the time to recovery from muscle injury.

Wright-Carpenter T, Opolon P, et al. (2004). Treatment of muscle injuries by local administration of autologous conditioned serum: animal experiments using a muscle contusion model. Int J Sports Med **25**(8): 582-7.

Muscle contusions represent a major part of sports injuries. The suggested treatments are generally sufficient to support muscle healing, but require a relatively long period of time. Given that autologous blood products are safe treatments, we have used a technique which stimulates the release of certain growth factors in the autologous conditioned serum (ACS). Those growth factors are known to improve the proliferative activity of myogenic precursor cells. Mice were subjected to an experimental contusion injury to their gastrocnemius muscle; one group received local injections of ACS at 2 hrs, 24 hrs, and 48 hrs after injury, a control group received saline injections. The histology results showed that satellite cell activation at 30/48 hrs post injury was accelerated and the diameter of the regenerating myofibers was increased compared to the controls within the first week after injury. ELISA results on the ACS have shown that the elevations in FGF-2 (460 %) and TGF-beta1 (82 %) could be partly responsible for the accelerating effects on regeneration due to proliferative and chemotactic properties. We conclude that ACS injection is a promising approach to reduce the time of recovery from muscle injury. In terms of clinical targets, this new approach could be used in the treatment of sports injuries and may also be interesting in postoperative situations.

Yuasa T, Kakuhata R, et al. (2004). Platelet-derived growth factor stimulates glucose transport in skeletal muscles of transgenic mice specifically expressing platelet-derived growth factor receptor in the muscle, but it does not affect blood glucose levels. Diabetes **53**(11): 2776-86.

Insulin stimulates the disposal of blood glucose into skeletal muscle and adipose tissues by the translocation of GLUT4 from intracellular pools to the plasma membrane, and consequently the concentration of blood glucose levels decreases rapidly in vivo. Phosphatidylinositol (PI) 3-kinase and Akt play a pivotal role in the stimulation of glucose transport by insulin, but detailed mechanisms are unknown. We and others reported that not only insulin but also platelet-derived growth factor (PDGF) and epidermal growth factor facilitate glucose uptake through GLUT4 translocation by activation of PI 3-kinase and Akt in cultured cells. However, opposite results were also reported. We generated transgenic mice that specifically express the PDGF receptor in skeletal muscle. In these mice, PDGF

stimulated glucose transport into skeletal muscle in vitro and in vivo. Thus, PDGF apparently shares with insulin some of the signaling molecules needed for the stimulation of glucose transport. The degree of glucose uptake in vivo reached approximately 60% of that by insulin injection in skeletal muscle, but blood glucose levels were not decreased by PDGF in these mice. Therefore, PDGF-induced disposal of blood glucose into skeletal muscle is insufficient for rapid decrease of blood glucose levels.