Platelet Rich Plasma In Sports Medicine

by

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ABSTRACT OF THESIS

PLATELET RICH PLASMA IN SPORTS MEDICINE

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Platelet rich plasma has been an extremely hot topic in the sports medicine community in the recent years. In part due to high profile athletes partaking in PRP treatment instead of traditional treatment for their injuries. This paper examines current human PRP literature, some animal literature, and explores the science behind PRP.

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Chapter 1

Introduction

A plethora of high profile athletes have undergone platelet rich plasma therapy in the recent past couple of years. The list of superstar athletes include Los Angeles Lakers Kobe Bryant, Tiger Woods, Alex Rodriguez of the New York Yankees, Rafael Nadal, Hines Ward and Troy Polamalu of the Pittsburg Steelers. As a result, PRP has garnered a crowd of public attention. In addition, success for PRP has been found in maxillofacial, dental, and orthopaedic fields (Sundman et al., 2011). PRP has the potential to enhance tissue regeneration such as muscle, tendons, and bones. Despite the rising popularity among athletes, is the science sound and is platelet rich plasma therapy ready for public consumption?

1.1 Basic Science

Platelets, otherwise known as thrombocytes, are derived from fragments of their precursor megakaryocytes found in bone marrow (Sanchez et al., 2009). The normal platelet level count in blood ranges from 150,000 platelets/μL to 350,000 platelets/μL in human adults (Foster et al., 2009). Platelet rich plasma is defined as a volume of plasma that has a platelet count above baseline of whole blood (Arnoczky et al., 2011). Some papers suggest that PRP has no clinical effect until a concentration of 1,000,000 platelets/ μL in 5mL of plasma is reached (Ficek et al., 2011). This equates roughly to a three to five-fold increase in platelets.

1.2 Biological Active Factors and Growth Factors

A variety of growth factors and proteins are found in the alpha granules of platelets (See Table 1 for list and function). The main growth factors identified are EGF, PDGF, VEGF, IGF, FGF and TGF (Foster et al., 2009). The increases in concentration of multiple growth factors in platelets are responsible for the increased healing aspects of various tissues and actions such as cell proliferation, chemotaxis, cell differentiation, and angiogenesis. The dense granules in platelets also contain serotonin, adenosine, dopamine, histamine, and calcium (Foster et al., 2009). Adenosine is a nucleoside with the primary function of cytoprotection against tissue damage. Serotonin is a monoamine neurotransmitter which acts as a chemoattractant for fibroblasts and fibroblast proliferation. Histamine is a biogenic amine that is involved in local vasodilation Serotonin and histamine both are involved in macrophage response. Calcium is involved in keratinocyte proliferation and differentiation (Mishra et al., 2009).

The three phases of healing are inflammation, proliferation, and remodeling. Inflammation begins with tissue injury in which platelets release their growth factors and begin the clotting cascade. In the proliferation phase, after angiogenesis, production of extracellular matrix would be the main task to restore structure and function to damaged tissue (Sanchez et al., 2009).

Table 1 Platelet Growth Factors

Growth Factors	**Function**
PD-EGF	proliferation and chemoattractant of epithelial cells and fibroblasts,
platelet derived epidermal growth factor	influence extracellular matrix synthesis and metabolism,
	Stimulate increased differentiation of epithelial cells
PDGF A + B	myogenin of fibroblasts, smooth muscle cells of arteries, endothelial and epithelial cells
platelet derived growth factor	Mesenchymal stem cell replication, ostoid production, endothelial cell
	replication, collagen synthesis, collagen and protein synthesis. Synthesis of other
	factors (e.g IGF-1) resulting in fibroblast proliferation and differentiation,
	collagen deposition, and angiogenesis
VEGF, ECGF	angiogenesis
vascular endothelial growth factor	tendon cell proliferation
	Collagen I synthesis
	anti-apoptosis
IGF-1,2	Growth factor for normal fibroblasts, enhances synthesis of collagenase and
insulin like growth factor	PGE 2 in fibroblasts, regulates the metabolism of articular cartilage

	through
	an increased synthesis of collagen and matrix osteon.
TGF-α	Similar to EGF, binds to the same receptor, stimulates the growth of
transforming growth factor alpha	mesenchymal cells, endothelial and epithelial cells, but also factors in its
	development. Affects bone formation and regeneration
TGF-β	
transforming growth factor beta	
β-1	Cellular migration and proliferation; cell replication, collagen synthesis
	production of extracellular matrix reconstruction of basement membrane of
	damaged myofibres and satellite cells,
	Scar tissue formation such as in adult wounds
β-2	Increase of collagen production
	Scarless wound healing such as in fetal wounds
β-3	Reduction of scar tissue formation after healing like in fetal wounds more favorable
	ratio of Collagen 1 to Collagen 3 ratio
FGF	Stimulator of angiogenesis and regulator of cellular migration and proliferation.
Fibroblast growth factor	Influencing angiogenesis and satellite cell numbers

Sources: Foster, T. E., et al. (2009). "Platelet-Rich Plasma From Basic Science to Clinical Applications." American Journal of Sports Medicine **37**(11): 2259-2272.

Ficek, K., et al. (2011). "Application of platelet rich plasma in sports medicine." J Hum Kinet **30**: 85-97.

Schippinger, G., et al. (2012). "Does single intramuscular application of autologous conditioned plasma influence systemic circulating growth factors?" Journal of Sports Science and Medicine **11**(3): 551-556.

A problem that the sports medicine field is facing is the lack of standardized definitions for PRP. A recent editorial stated that the definition of PRP was still not clearly established (Filardo and Kon, 2012). Some strides have been made to further specify the type of PRP in use. A paper by Ehrenfest et al. (2012) further breaks down PRP into six subcategories, including their activated forms. P-PRP, P-PRP gel, L-PRP, L-PRP gel, P-PRF, and L-PRF. Unactive PRP are in the liquid state, but when the clotting cascade is activated, the PRP becomes more solid and gel-like. P-PRP stands for pure platelet rich plasma which becomes P-PRP gel after activation. P-PRP is devoid of leukocytes and has lower platelet elevation. L-PRP is leukocyte and platelet rich plasma and becomes L-PRP gel after activation. L-PRP contains higher platelet elevation compared to P-PRP and contains higher growth factor amounts (Mazzocca et al., 2012a). P-PRF is pure platelet-rich fibrin and finally, L-PRF is leukocyte and platelet-rich fibrin (Ehrenfest et al., 2012). The potential advantages of platelet fibrin matrices are their ability to act as a conductive matrix for migration of cells and growth factor reservoir and slow release of growth factors rather than the quick release in PRP (Foster et al., 2009, Arnoczky et al., 2011). See Table 2 for available methods of production for PRP.

Table 2 Classification of Main Available Methods of Production for Platelet Concentration (Generic Names)

Platelet Concentrate Class and Terminology	Method of Production
P-PRP	**Cell Separator PRP**
	Vivostat PRF
	Anitua's PRGF
	Nahita PRP
L-PRP	**PCCS PRP**
	SmartPREeP PRP
	Magnellan PRP
	Angel PRP
	GPS PRP
	Friadent PRP
	Curasan PRP
	Regen PRP
	Plateltex PRP
	Ace PRP
P-PRF	**Fibrinet PRFM**
L-PRF	**Choukrun PRF**

Source: Ehrenfest DMD, Bielecki T, Mishra A, Borzini P, Inchingolo F, Sammartino G, Rasmusson L, Everts PA (2012) In Search of a Consensus Terminology in the Field of Platelet Concentrates for Surgical Use: Platelet-Rich Plasma (PRP), Platelet-Rich Fibrin (PRF), Fibrin Gel Polymerization and Leukocytes. Curr Pharm Biotechno 13:1131-1137.

1.3 General Procedure for Formulation of PRP

Whole blood is drawn from the patient in varying amounts depending on the method of production normally with an anticoagulant. The objective of the anticoagulant, usually acid-citrate dextrose or citrate phosphate dextrose, is to bind calcium which stops the clotting cascade by preventing the conversion of prothrombin to thrombin (Arnoczky et al., 2011).The blood is then centrifuged once or twice depending on the PRP preparation (Mazzocca et al., 2012a). The first spin is considered a "soft spin" which separates plasma and platelets from red blood cells and white blood cells (Arnoczky et al., 2011). A plasma supernatant is formed and can then be processed or alternatively, centrifuged a second time. The second centrifuge is a "hard spin" which further concentrates platelets and leukocytes into PPP and PRP. Calcium or thrombin is added to start the clotting cascade activating platelets and the precipitation of a fibrin scaffold (Arnoczky et al., 2011). Other times, the clotting cascade can be activated normally through contact with tendon derived collagen (Mishra et al., 2009). A local anaesthetic such as bupivacaine is usually applied to the area of injection. Depending on the area or tissue being treated, epinephrine can also be applied in conjunction with the anaesthetic. After injection, local pain occurs at the injection site for a week (Mazzocca et al., 2012a).

1.4 L-PRP and P-PRP differences

More does not necessarily mean better. Sundman et al. (2011) found a positive correlation with catabolic cytokines MMP-9(r^2 = .37, P<.001) and IL-1β(r^2 = .73, P<.001) and leukocyte count. In addition, a positive correlation with anabolic cytokines PDGF-

AB(r^2 = .60, P <0.001) and TGF-β(r^2 = .75, P<0.001) with regard to platelet counts (Sundman et al., 2011). With the catabolic cytokine correlation, leukocytes may not be the best candidate for all conditions despite the higher number of growth factors in L-PRP. A paper by Mazzocca et al. (2012b). somewhat confirm this suspicion by comparing, P-PRP, L-PRP, and a double spin procedure in various human tissue samples. Osteocyte proliferation was greatest in the double spin. The double spin was significantly higher than the L-PRP ($p<.05$), but not the P-PRP in osteocyte proliferation. Myocyte proliferation was greatest with P-PRP and was the only PRP to be significantly higher than controls ($p<.05$). Tenocyte formation proliferation was greatest with P-PRP, but L-PRP, and the double spin were significantly higher compared to controls as well ($p<.05$) (Mazzocca et al., 2012b). In addition, the L-PRP topped the various growth factor amounts for TGF PDGF, VEGF, HGF, FGF, EGF, IGF1, and TGF-β compared to the other PRP methods, affirming more is not always better.

1.5 Systemic Effects

In 2010, the World Anti-Doping Agency (WADA) banned platelet derived preparations such as PRP in intramuscular injections. The ban excluded other routes of administration as long as treatment was declared and in compliance with International Standard for Theurapetic and Use Exemptions However, the ban was lifted in 2011 later due to lack of evidence on systemic ergogenic effects (Wasterlain et al., 2013). In addition, systemic increase of growth factors may have some negative effects as well. For example, increased TGF-β may cause muscle fibrosis as seen in in vitro muscle tissue studies which may lead to higher re-injury chances (Schippinger et al., 2012).

Several studies have attempted to elucidate systemic growth factor increases. Wasterlain et al. (2013) discovered bFGF, IGF-1, VEGF, IGFB-3, and PDGF-BB increased over the time period of four days. They used a L-PRP setup and collected thirty to sixty mL of whole blood which yields three to six mL of L-PRP. Injection area was based on tendon injury area, so patients did not all have injuries in the same locations. Blood samples were collected by venipuncture at the time points before injection, .25 hours, 3 hours, 24 hours, 48 hours, 72 hours, and 96 hours. Blood was drawn the same time each morning and three hours after eating and exercising. Growth factors were quantified with Quantikine enzyme-linked immunosorbent assay (ELISA). bFGF had a steady increase from base to 2.29x base at forty eight hours and a huge drop to 1.61x base and rose again to 2.28x base at 96 hours. IGF-1 dropped slightly at .25 hours and slowly rose to 1.08x base at 24 hours and remained there throughout the four day period. VEGF increased sharply to 1.5x base at three hours and remained elevated at around 1.5x base throughout the four days. Insulin-like growth factor binding protein 3 (IGFB-3) is important because the product of IGF-1 and IGFB-3 is an indirect marker for human growth hormone. IGFB-3 increased to 1.19x base at three hours and slowly dropped to 1.09x at forty eight hours and sharply increased to 1.26 at ninety six hours.

In another study, Schippinger et al. (2012) discovered a systematic increase only in the growth factor, TGF-β2, three hours and twenty four hours after intramuscular injection. The difference in results may be due to the different PRP methods of production tested as the Arthrex ACP system produces P-PRP compared to the GPS III system which produces L-PRP. In addition, the time period tested was twenty four hours against ninety six hours. These studies both did not contain a control group although do

show that PRP has the potential to boost performance when a systemic increase of growth factors occur. An improvement would be to include saline injection placebo groups.

Chapter 2

Clinical Application

2.1 Tendons

de Mos et al. (2008) performed an in vitro PRP study on human tenocytes. Human tenocytes were harvested from the hamstring of three children between thirteen and fifteen years old. Collagen was measured with a Hydroxyproline Assay. Gene expressions were measured with RT-PCR. DNA content was increased by twenty fold at day seven and by thirty fold at day 14. An increase in total collagen synthesis was found in a 20% volume PRP, roughly three times more than the control at day seven and fourteen. Collagen Iα1 and Collagen IIIα1 gene expression both decrease at day 7 and 14. However, the collagen III/I ratio remained the same. The ratio is important because ratio imbalances are seen in tendinosis, tendon repair, and tendon fibrosis (de Mos et al., 2008). Matrix metalloproteinase 1 (MMP1) showed upregulation at day seven and fourteen, while MMP3 only showed upregulation at day four. VEGF-A had increased gene expression during day four and day fourteen. TGF-β1 gene expression also increased on day four. Interesting to see that collagen expression decreased, but overall total collagen increased. The authors note the increased number of cells was the most likely reason. MMP 1 and MMP3 are involved in extracellular matrix remodeling. TGF-β1 was upregulated on the same day as MMP3 and is involved with extracellular basement membrane reconstruction. So, one possibility for the healing effects of PRP on tenocytes might involve an overall improved extracellular matrix and vascularization due to MMP, TGF-β1, and VEGF-A angiogenesis.

Tohidnezhad et al. (2011) tested Platelet Rich Growth Factors (PRGF), a form of P-PRP because the paper states the lack of leukocytes and erythrocytes, on the achilles tendon of post natal rats. Control of no PRGF, 2.5,% PRGF, 5% PRGF, and 10% PRGF groups were set up. CyQuant assay was utilized to measure cell proliferation and was found to be increasing with PRGF concentration with values of 1.31 for PRGF 2.5%, 1.49 for PRGF 5%, and 1.44 for PRGF 10%. The 4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST) assay measures the conversation of WST to formazin at 450nm by microplate spectrophotometry. The reaction measures the reductive capacity of cells. The WST assay measured cell viability and was increased with greater PRGF concentration with values of 1.47 for 2.5% PRGF, 1.54 for 5% PRGF, and 1.63 for 10% PRGF. A scratch test was performed to test migration of tenocytes which consists of a cell monolayer with a straight line or a "scratch". The 10% PRGF treated tenocytes were found to have passed the scratch boarder while the controls only move slightly. Immunnohistochemistry and immunofluorescent staining of cells cultured were performed to confirm tenocytic phenotype of cells incubated with PRGF. Immunohistochemistry revealed tenomodulin two days after isolation and remains eight weeks after isolation. Confocal laser scanning with immunoflourescent staining showed that tenomodulin was located in the cytoplasm and not the nucleus because they were located outside the DAPI staining which stains the nucleus.

Dual luciferase assay was used to measure the anti-oxidant response element (ARE) activity due to binding of Nrf2 (Tohidnezhad et al., 2011). First, a reporter construct of pNQO1-rARE was created by annealing two strands of NQO1 Gene with

Kpn1 and Nhe1. NQO1 ARE reporter plasmid contained the firefly Luciferase reporter gene and pRL-TK plasmid which has the *Renilla* Luciferase gene under the control of the herpes simplex virus thymidine kinase promoter as an internal control. Cells were transfected in a plate by lipotransfection. *Firefly* and *Renilla* luciferases were measured at forty eight hours. Values normalized to the *Renilla* luceriferase activity of the control. Relative ARE activity was 1.99 for 5% PRGF for six hours, 2.46 for 10% PRGF for six hours, and 2.38 for 20% PRGF for six hours (Tohidnezhad et al., 2011). ARE may be involved in the mechanism of cell proliferation from PRGF because of increased activity. However, the paper only proves PRGF increases ARE activity through NRF2 not anything more.

Elbow tendinosis/Lateral Epicondylitis

Lateral Epicondylitis is the most common elbow condition diagnosed. The cause of Lateral Epicondylitis is still unclear. Although, a combination of both mechanical overloads and abnormal microvascular responses are the most common suspects (Gosens et al., 2011). The main interventions for lateral epicondylitis for the elbow include conservative treatment such as physical therapy and corticosteroid injections. If all else fails, numerous surgical interventions can also be used. However recently, corticosteroid injection treatment has come under fire (Peerbooms et al., 2010a). The advantage of PRP in the case of elbow tendinosis would be another non-surgical alternative option after conservative treatment measures fail to improve patient symptoms.

Mishra and Pavelko (2006) performed a cohort human study on chronic elbow tendinosis. One hundred forty applicants were screened for chronic elbow epicondylar

pain for over three months with additional exclusion criteria. Only twenty patients, fifteen percent, in the study met the all the inclusion criteria. Fifteen of the patients were PRP treated and the other five patients were used as controls. Fifty five mL of whole blood was drawn from patients with five mL of anticoagulant in a sixty mL syringe. The whole blood was processed with the L-PRP Gravitational Platelet Separation (GPS) System and five mL of PRP was obtained. Two to three mL of PRP was injected into the common extensor tendon with a 22 gauge needle while the remaining PRP was sent to labs for platelet counting. Primary outcomes were analyzed using a Visual Analog pain scale (VAS) and a modified Mayo Elbow Performance Index. The Visual Analog pain scale ranged from zero, no pain, to one hundred, most painful. Eight weeks after the treatment, the PRP- treated patients had 60% lower Mean Visual Analog pain scores compared to the 16% for control patients. In addition, the PRP- treated patients had 52% lower Mayo elbow scores compared to the control scores which only dropped by 11%.

Unfortunately after the eight week follow up, three of the five control patients withdrew from the study. As a result, comparisons between PRP treated and control patients were dropped past the eight week follow up due to lack of data and only data pertinent to PRP treated patients were evaluated. The next follow up at six months, PRP treated patients continued to improve with an 81% mean visual analog pain score decrease compared to the initial scores and mayo elbow scores improved 72% from the initial scores. Finally, the final follow up occurred after roughly twenty four months where 93% of the fifteen patients were satisfied with the PRP treatment. These patients were rated ten or less out of 100 in terms of the visual analog pain scoring (Mishra and Pavelko, 2006). However, a negative for the study was the extremely low sample size.

Also, the study lacked a control group to compare with the PRP treatment group halfway through the study which was another negative.

Although another study performed by Peerbooms et al. (2010a), affirms the results from the Mishra and Pavelko (2006) paper. The double blind randomized study included one hundred and six patients with inclusion criteria of lateral epicondylitis for at least six months and minimum of fifty out of a hundred on a visual analog score for pain. The objective of the research was to compare and contrast L-PRP injection with corticosteroid injection treatment. Fifty one patients were assigned to the PRP group and the other forty nine were assigned to the corticosteroid treatment control group. Twenty seven mL of whole blood was drawn from the patient and mixed with three mL of sodium citrate anticoagulant. Three mL of PRP was prepared with the Recovery System from Biomet Biologics. The PRP was then buffered to physiological pH with 8.4% bicarbonate. Bupivacaine hydrochloride 0.5% and epinephrine were added and one mL of PRP or corticosteroid was injected into the highest tenderness area and the other two mL were injected into the common extensor tendon. Visual Analog pain Scale (VAS) and Disabilities of Arm, Shoulder, Hand scores (DASH) were used to determine primary outcomes of pain and daily use of the elbow. The primary endpoint of the study was to have a 25% reduction in VAS or DASH scores within a year without a need for reintervention. Three patients from each group were lost to follow up. In addition, five members of the PRP group choose surgery or reinjection reintervention after 5 months. Similarly, thirteen members of the corticosteroid group also opted for same decision. At the end of the one year period, only 49% (24/49 patients) of the corticosteroid group was successfully treated in terms of VAS score versus 73% (37/51 patients) of the PRP group.

The results were significant with a p value of less than .001. A similar trend was also seen with the DASH scores with 51% (25/49 patients) in the corticosteroid group against 73% (37/51 patients) of the PRP group for successful treatment with a p value of less than .005 (Peerbooms et al., 2010a).

Gosens et al. (2011) released a follow up paper of Peerbooms et al. (2010a), which included an extended follow up to patients at two years. VAS scores for the PRP group steadily improved the whole duration compared to the lack of improvement of corticosteroid after twelve weeks (Gosens et al., 2011). Mean base starting VAS score for PRP was 69. The PRP VAS scores dropped 14 points after 4 weeks and another 8 points after eight weeks. The trend of dropping seven to eight points continues for PRP until the one year to two year mark where only a 4 point decrease in VAS score was seen ending in a mean average VAS score of 21.3 at two years. Corticosteroid treatment on the other hand, started at a mean 66.2 mean VAS score and at week 4 sharply decreased by twenty two points to 44.3. However, the corticosteroid group then fails to improve much and even regresses to 55.8 after twenty six weeks. The corticosteroid group ended up with a mean VAS score of 42.4 after one hundred four weeks, not much better than the week four score.

DASH score trends were analogous with VAS trends. The PRP group again steadily improved throughout the study during follow ups. On the other hand, the corticosteroid group stopped improving after the twelve week. The data supports that corticosteroids only treat short term and not long term even when extended to two years. The PRP group started with a mean DASH score of 54.3 which was reduced to 43.1 at four weeks. The PRP group continued to improve their DASH scores by six or seven

after each time period except for twenty six weeks where they regressed by six points. At one hundred four weeks, the PRP group ended with a mean DASH score of 17.6. The corticosteroid group again has a huge improvement jump from a mean baseline of 43.3 to 31.2 after four weeks. Regression to 37.6 occurred at twenty six weeks and at one hundred four weeks the mean DASH value was 36.5, worse than the four week DASH value. A flaw of the study is the groups being compared because the comparisons are between a known treatment and an experimental treatment.

However, not all studies pertaining to chronic lateral epicondylitis with PRP treatment contained positive results. Some recent studies comparing autologous blood injection (ABI or whole blood) and platelet rich plasma found very little difference in results between the two. Thanasas et al. (2011) performed a single blind randomized study. Patient outcome was measured with VAS (from 0 lowest- 100 agonizing pain) for pain and Liverpool elbow scores (0 worst-10 best). Liverpool elbow score evaluated range of motion, daily activities, and ulnar nerve function (Thanasas et al., 2011). A L-PRP method was utilized which increased mean platelet concentration by 5.5 times base platelet concentration. Twenty eight patients were split into two groups of fourteen, one for PRP and the other for ABI. Patient follow ups were performed at six weeks, three months, and six months. VAS mean improvement was found to have p value of less than .05 at the six weeks time point in which the PRP group had a mean improvement of 3.8 against the ABI group mean improvement of 2.5. However, no statistical significance for mean VAS improvement difference was found at other timepoints. As for Liverpool elbow score differences, no statistical significance was found at any of the three follow up time intervals. A huge problem with the study was that the patients were not blinded

and knew which treatment was performed. The researchers claim that the procedures were too different and patients would easily be able to identify which treatment was injected. Also, the study had a very low sample size making it difficult to draw a clear conclusion.

Creaney et al. (2011) performed a similar single blind randomized study comparing autologous blood injection with PRP in resistant elbow tedinopathy. Patient related tennis elbow evaluation (PRTEE) score was used to evaluate pain and physical function in patients in which a 25 point reduction (roughly 50% reduction) was considered a successful treatment. A key difference to note compared to the Thanasas et al. (2011) paper was that all the patients selected had already failed conservative treatment options such as physical therapy. In addition, a P-PRP method was used in this study rather than L-PRP. The P-PRP techninque yielded a 2.8 higher platelet concentration compared to baseline platelet concetration.

One hundred and fifty patients were split into eighty patients for the PRP and seventy for ABI. Twenty patients were lost to follow up, ten from each group. Two injections were given, one at the beginning of the trial and the other a month later. The mean PRTEE improvement at six months was 35.8 in the PRP group to 46.8 in the whole blood group, a difference of over ten which was considered clinically significant. The mean PRTEE differences at the one month and 3 month follow ups were not significantly different between groups. The authors warn that the results may be skewed at the end due to the greater percentage of whole blood patient failures who opted for surgery and as result, were removed from the data set analysis. The side effect of the data set removals create an artificial inflation of the remaining patients. Overall at the end of six months,

66% of patients in the PRP group and 72% of patients on the whole blood group had a 25 point reduction in PRTEE score or more (Creaney et al., 2011).

However, the and Creaney et al. (2011) clinical study had some limitations. For example, there was a lack of a placebo group. The ethics committee decided that having a unactive placebo group was considered unethical. In addition, only the patients were blinded, but not the investigator (Creaney et al., 2011). Despite the flaws of the study, the inclusion criteria of only patients who failed conservative therapy was a nice twist. A better route to go about may have been similar to other studies in which patients are given a physical therapy regiment before injection.

The results from both Creaney et al. (2011) and Thanasas et al. (2011) have similar results which provide some evidence that PRP and whole blood in the treatment of chronic lateral epicondylitis have very little difference in terms of patient improvement. Which raises some questions for future research such as why is the higher platelet count from PRP not having an increased effect on the scores compared to ABI and what are the mechanisms of action that are causing these results.

Plantar Fasciitis

The plantar fascia is a thickened fibrous aponeurousis that originates in the medial calcaneal tubercle (Ragab and Othman, 2012). Plantar fasciitis is the most common cause of heel pain. It is thought to be caused by degeneration of collagen fibers rather than inflammation with the most common nonsurgical intervention being corticosteroid injection (Aksahin et al., 2012).

Ragab and Othman (2012) performed a study of chronic plantar fasciitis with twenty five patients. Fifty mL of whole blood was drawn from the patient and combined with 5 mL sodium citrate. Blood was centrifuged for fifteen minutes at three thousand rounds per minute. The PRP was injected into the tender area of the plantar fascia with a 22 gauge needle using a peppering technique. A peppering technique involves creating four or five penetrations and a skin portal to the fascia (Ragab and Othman, 2012). Patient outcome was measured with VAS for pain and thickness of plantar fascia bands via sonography Thickness of plantar fascia was measured where the plantar fascia crosses the anterior aspect of the inferior border of the calcaneus. A plantar fascia chicness of more than 4mm was considered abnormal. Average VAS scores dropped from 9.1 pre-injection to 1.6 post injection ($P<.001$). After three months, the medial and central bands of the plantar fascia dropped from 7.1mm to 4.8mm and 6.6mm to 5.4mm ($P<.001$) (Ragab and Othman, 2012). However, no effect was seen on the lateral bands which remained at a constant 4.6mm throughout the three months.

A serious flaw in the study was the complete lack of a control or placebo group except a before and after comparison of lateral, central, medial band values. A more prudent approach would be to have a control group of physical therapy and check if similar physiological changes occur with the bands of the plantar fascia. Without a control group, it is extremely difficult to draw real conclusion from the results. The utilization of sonographic imaging was a good idea because it allowed for an additional objective measurement.

Another study was performed by Aksahin et al. (2012), contrasting corticosteroid injection with PRP. Sixty patients were analyzed through VAS scores and Roles and

Maudley scores. Roles and Muadley scores were rated as excellent, good, acceptable, and poor in terms of patient satisfaction with symptom reduction and pain while walking. Results were similar for both PRP and corticosteroid groups at six months for VAS scores. PRP group had a reduction from 7.333 to 3.93($P<.001$) and the corticosteroid group had a reduction from 6.2 to 3.4 ($P<.001$) (Aksahin et al., 2012). Roles and Maudley scores between groups were similar as well. Another double blind-randomized performed by Peerbooms et al. (2010b) comparing corticosteroid treatment and PRP is currently in progress with a similar experimental setup to their lateral epicondylitis research. However, results have not been released yet (Peerbooms et al., 2010b). An additional set of comparison data would be important to support the data found in the Aksahin et al. (2012) paper.

Patellar Tendinopathy

Patellar tendinopathy, also commonly known as "Jumper's Knee", is caused by the degeneration of collagen fibers of the patellar tendon (Gosens et al., 2012). In a study by Gosens et al. (2012) on patellar tendinopathy., thirty six patients were split into two groups, one group of fourteen, who were already treated with cortisone, sclerosing ethoxyscerol, or surgical intervention and another group of twenty two who were untreated. All patients already exhausted conservative treatment such as eccentric exercises. PRP preparation and injection technique were the same as Peerbooms et al. (2010a) except injection was in the patellar tendon origin. Endpoints were measured with VAS (0 lowest pain-10 highest pain) scores for pain and Victorian Institute of Sports Assessment (VISA-P) scores. VISA-P measures pain symptoms, function, and ability to play sports and ranges from 0(worst health) to 100 (best health). The untreated PRP

group recovered better than the group who already had been treated with other interventions in their VISA-P score. However, the difference was not clinically significant in the VAS scores. The main idea of Gosens et al. (2012) was to attempt to establish link between other non-conservative interventions and reduced PRP effectiveness. Only the VISA-P scores support this notion with the PRP group having a change from 39.1 to 58.6(P=.003), but without other evidence quite difficult to clearly conclude even a correlation.Gosens et al. (2012) mention that they were able to MRI some patients, but not enough. An improvement would be to MRI both groups and include a control group.

Achilles Tendinopathy

Achilles Tendinopathy results from the degeneration and disorganization of collagen fibers rather than inflammation. Compared to other tendinopathies, a key difference is that the Achilles tendon seems to be a non-self-limiting injury (de Jonge et al., 2011). In a study performed by de Jonge et al. (2011), fifty four patients were randomly split into two groups of twenty seven. Outcomes were measured with VISA-A, UTC, and modified Öhberg scoring system for neovascularization.. One group was the PRP treatment group and the other was the placebo group which received saline injection instead. The end results find that there is a lack of clinical difference between PRP and saline placebo groups. VISA-A scores improved an average of thirty one points for PRP and twenty five points for the saline control group. The UTC patterns were analogous for both groups as well. Echotype I increased while echotype II remained the same

(Echotypes I and II added together represent organized tendon bundles). Echotypes III and IV(Echotypes III and IV represent more amorphous or fibrillar structures (de Jonge et al., 2011) decreased slightly compared to base. Anterior-posterior diameters were not significantly different between PRP and saline control groups with a .8mm mean improvement in PRP and a 1.2mm improvement in the saline control group. Neovascularization scores for PRP and saline control groups increased until twelve weeks and continued to steadily decline (de Jonge et al., 2011). The authors speculate that the reason for the saline placebo group improvement could possibly be due to needle technique because needle trauma can initiate a healing response.

Although the results of the paper are quite disappointing for PRP results, the experimental design of the study is quite good. Unlike many other experiments, the placebo consists of a saline control group, a negative control, rather than a conservative treatment group. Although in this case, the saline injection actually resulted in a positive result. In addition, the ultrasonographic tissue characterization and color Doppler ultrasonography imaging allowed for more physiological changes to be monitored and compared.

Anterior Cruciate Ligament Reconstruction

de Almeida et al. (2012) tested whether applying PRP to the patellar tendon harvest site would improve tendon healing and clinical outcome at 6 months after ACL reconstruction with a patellar tendon graft. A PRP group of twelve and a control group without PRP of fifteen were created. ACL reconstruction with patellar tendon graft was performed for both groups. Patient outcome was measured with a MRI of the gap area of

the patellar harvest site and VAS scores. Results found the gap area in the PRP group was only 4.9mm compared to the control group of 9.4mm (P=.046). VAS scores also were much better in the PRP group compared to the control with scores of 3.8(PRP with 5.1(control) (P=.02)(de Almeida et al., 2012).

Hamstring

A retrospective study was performed by Wetzel et al. (2013) for proximal hamstring injuries. The proximal hamstring injuries include tendinopathy, strain, or partial tearing excluding full proximal hamstring tears and ischial tuberosity. Injection of PRP was located at the ischial tuberosity. Fifteen patients were selected with seventeen proximal hamstring injuries. A regiment of conservative treatment consisting of six weeks to twelve weeks of physical therapy and non-steroidal inflammatory drugs (NSAID) for one week was given to patients. Ten patients that failed to improve after the conservative treatment were placed in the PRP group and the other five who improved after the physical therapy were placed in the conservative treatment control group. VAS (0-10 scale) scores were used to measure pain and the Nirschl Phase Rating Scale (See Table 3) was used to score level of disability for hamstring injuries. Other subjective information gathered were return to work status, return to preinjury sports status, and overall satisfaction.

VAS results for the PRP group changed from an average pretreatment score of 8.2 to an average post treatment .7 (P<.01). NPRS results were similar with VAS results in the PRP group with a mean score of 5.5 pretreatment which was reduced to 1.5 post treatment (P<.01). On the other hand, the conservative treatment group had a VAS

average of 7.4 (P=.06) reduced to 1.2 (P=.06) and NPRS average of 4.4 reduced to 2 (Wetzel et al., 2013). Comparison between the difference in VAS and NRPS scores yielded no statistical difference. Overall, the PRP group improved more than the conservative treatment control group. The study included eight ex-collegiate or competitive athletes in the PRP group and two in the Conservative Treatment Control group. The athletes in both groups surveyed a return to their desired competitive level. However, results were still very inconclusive due to low sample size and some biases were evident. NPRS follow up data was collected via phone interview creating recall bias for patients.

Table 3: Nirschl Phase Rating Scale

Phase	Level of Disability
1	Mild stiffness or soreness after activity with resolution of symptoms within 24 hours
2	Mild stiffness or soreness prior to activity that is relieved by warm-up; symptoms are not present during activity but return afterward and resolve within 48 hours
3	Pain that is present during activity without causing activity modification.
4	Pain with activity that causes modification.
5	Pain that is present during all activities and occurs with activities of daily living.
6	Intermittent rest pain that does not disturb sleep.
7	Constant rest pain that disturbs sleep.

Source: Wetzel RJ, Patel RM, Terry MA (2013) Platelet-rich Plasma as an Effective Treatment for Proximal Hamstring Injuries. Orthopedics 36:E64-E70.

2.2 Muscle

Muscles are the least studied PRP topic (Mazzocca et al., 2012b). .No clinical human studies have been performed (Mishra et al., 2009). Foster et al. found that bFGF and IGF-1 improved healing and increased fast twitch and tetanus strength.in rat muscles (Foster et al., 2009). In addition, PRP injection into a gastrocnemius contusion of a mouse recovered with increased myofiber diameter (Mazzocca et al., 2012b).

One study on mice showed that PRP is more effective with acute small sprains rather than one acute large sprain that require myogenesis in the tibialis anterior (Hammond et al., 2009). Acute small sprains were simulated by forty five lengthening contractions of the tibialis anterior (TA) with a sixty degree arc. Twenty mL of whole blood was drawn from Sprague-Dawley inbred rats. PRP was conditioned with ten seconds of high-frequency ultrasound to lyse platelets and release growth factors. One hundred microliters of PRP was injected into the TA at day 0,3,5, and 7, going with the multi injection route. RTPCR was performed for the TA on day 7 and MyoD and Myog muscle transcription factors were elevated compared to the Platelet Poor Plasma (PPP) ($P<.001$). GAPDH was used as a control and did not change in transcription levels after muscle injury. Densitometry of bands quantified the MyoD and Myog normalized to GAPDH levels and found that the mRNA transcription of MyoD and Myog were higher than their PPP counterparts. A western blot was performed to assess semiquantitative changes in the levels of MyoD and myogenin proteins. TA muscles were frozen in liquid nitrogen and homogenized with PowerGen 125 homogenizer in addition to protease inhibitors. The samples were boiled, centrifuged, and protein concentration was ascertained with Bradford assay. Sodium doecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the samples and transferred to

nitrocellulose. Nictrocellulose was blocked in milk-PTA and incubated with anti-myoD or anti-myogenin polyclonal antibodies for six hours. Nitrocellulose was combined with an anti-rabbit secondary anti bodies, allowing bands to be viewed through chemiluminescent assay. The immunoblot confirmed increase protein expression of myoD and myogenenin ($P<.05$). More research is a definitely necessary for PRP and muscle interaction.

2.3 Bone

In animal studies, TGF-β, FGF, IGF I and IGF II, VEGF, and PDGF were found to have positive effects on bone regeneration. TGF-β stimulates migration of osteoprogenitor cells and is a potent regulator of cell proliferation, cell differentiation, and extracellular matrix synthesis (Kempen et al., 2010). Studies have shown TGF-β to have both a stimulatory and inhibitory effect on bone growth (Kempen et al., 2010). Exogenous FGF enhances callous formation. IGF has an anti-apoptotic effect on osteoblasts and enhances bone matrix synthesis (Kempen et al., 2010). VEGF is beneficial to bones through its angiogenesis.

Bone grafting animal studies have also given mixed results. Kurikchy et al. (2013) examined histologically xenographic bone grafts with rabbit femur bones. Five mL of autologous blood was drawn from each of the sixteen New Zealand rabbits. The blood was then centrifuged for twenty minutes at 1,200 rpm. PRP and PPP portions were then centrifuged again at 2,000 rpm to separate the PRP from PPP. Platelet count was performed and counted manually under the microscope which averaged to a two to three-fold increase.. PRP was then activated with 10% calcium chloride solution and mixed

with organic bovine bone, once the PRP became gel-like. Organic bovine bone was added in a ratio of 0.5mL of PRP with 30mg of organic bovine bone. Three bone defects of 3mm were created on the femur with a 4mm distance between them. The holes were treated with either organic bovine bone or organic bovine bone with PRP except for the last hole which was left untreated as a control. Experimental areas were then covered with a soft tissue flap and rabbits were given pain medication and antibiotics. Four rabbits were sacrificed each week. Bone piece with defect and soft tissue were removed and fixed in phosphate buffered formaldehyde for forty eight hours. Tissue blocks were decalcified with formic acid and sodium citrate for four weeks. They were then dehydrated with graded alcohol and embedded in paraffin. Histological sections between four and six μm were stained with hematoxylin and eosin and Van Gieson stains. Osteoblasts, osteoclasts, and osteocytes were counted during the fourth week through selection of five random sites of each section in 40x magnification.

During the first week, the control group had newly formed granulation tissue with a large blood clot filling the defect site. The organic bovine bone group had dense granulation tissue around multiple blood clots. The organic bovine group with PRP had a little bit of new bone formation on edge of defect. During the second week, the control group contained formations of fibrocartilaginous callus and hyaline cartilage. Bone spicules were formed around edge of defect. The organic bovine bone group was similar to the control group, but more mature and organized. The organic bovine bone group with PRP contained vascularized bone marrow and an increase of bone spicules and their distribution were seen. The third week had the control group form new bone trabeculae from edge of defect and diffused along the callus. The organic bovine bone group

contained more bone trabeculae with primary osteon formations and active osteoblasts. The organic bovine bone group with PRP had better bridging of new bone and increased surface area of bone trabeculae. During the fourth week, the control group had newly formed osseous tissue, but with irregular distribution of osteoblasts, osteocytes, and few number of osteoclasts. Only a small number of primary osteons were formed. The organic bovine bone group had more mature bone tissue, but also had only a small number of primary osteons. However, osteoblasts were in much higher numbers. The organic bovine bone group with PRP had well-formed osseous tissue, but also had an irregular distribution of bone cells. Osteocytes were increased and diameter of osteon with lamellar thickness was increased compared to the organic bovine bone groups and control groups. Cell count during the fourth week revealed that the organic bovine bone group had the highest osteoblast count with a mean count of 24.6 osteoblasts per unit area compared to 13.6 (control) and 13.2 (organic bovine with PRP). The organic bovine bone with PRP group contained the greatest osteocyte amount with a meant count of 98.67 osteocytes per unit area compared to 61.67 (control) and 77 (organic bovine group). In addition, the organic bovine group with PRP had the greatest osteon diameter and lamellar thickness ($P<.05$).

An observational prospective study for medial high tibial osteonomy with an allograft was performed by Peerbooms et al. (2012) with forty one patients. Primary endpoint was measured through bone density above and below the wedge via CT scan in Hounsfield values. The PRP group had lower bone density both above and below the wedge compared to the control group. At the one week postop time point, the PRP group had significantly lower bone density above (77.1 vs 132.8) and below (50.8 vs 119) the

wedge (p=.02) in both cases. At twelve weeks, the PRP group had significantly lower bone density below the wedge with values of 68.5 for PRP against 129.1 for the control group (Peerbooms et al., 2012). From the results, PRP seemed to make the healing process worse with lowered bone density. The results are somewhat in line with the findings of Schlegel et al. (2004) which found that in a autologous bone graft with PRP on pig forehead bone defects, the PRP enhanced bone graft had superior mineralization rate early, but as the weeks went on, the non PRP enhanced bone graft leveled out after twelve weeks. However, in this case, Peerbooms et al. (2012), has the opposite with PRP being worse and normalizing at the end.

However, a possible culprit was the introduction of the non-steroidal inflammatory tablets (NSAID), paracetmol and diclofenae, for post operation pain in the study adds another unnecessary and perhaps, controversial variable. NSAIDs inhibit prostaglandins, which are involved in bone repair and homeostasis, by lowering cox 1 and cox 2 activity and therefore reducing bone healing (Quaile, 2012). A similar study should be performed without the NSAIDs to confirm if the PRP results would remain the same.

Chapter 3

Discussion

Experimental Design

Clinical experimental trials for human PRP studies are difficult. The general consensus for tendinopathy treatment seems to reflect PRP as an option after conservative treatment failure and before surgical operation intervention. Most studies compare experimental with a standard treatment such as corticosteroids because these studies are tied down by ethics boards. Besides theMishra and Pavelko (2006), none of the other studies contained a control group with absolutely no treatment. Of course, the untreated patients did not improve and left on their own accord. In another study, such a group was considered unethical (Creaney et al., 2011). Conservative treatment control groups also run into other dilemmas. For example, the creation of a conservative treatment regiment for patients before splitting experimental groups to control and PRP experimental groups has its pros and cons. A benefit of using this method is that all the patients are normalized and follow the same routine pretreatment. However, a very huge con is the destruction of randomization because patients who improve from the conservative treatment would be placed in the control group while the patients who do not improve are placed in the PRP group. The plan of attack here would be to inject the conservative group with a placebo such as saline and the experimental with PRP and compare results at the end.

de Jonge et al. (2011). argued that PRP does not work alone rather PRP works in conjunction with the conservative exercises. All studies at least have postop rehab

exercises for around four weeks so the point made is quite valid. The advantage of De Jonge et al.'s route would be that the random and double blind would be intact. However, the other method better tests the statement that PRP should be tried after conservative treatment has failed and before surgical intervention despite the loss of double blind and randomization. Regardless, the choice is rather difficult.

Several recent papers have begun utilization of imaging technology. Imaging technology is extremely useful because it adds another objective variable to be measured and contrasted. Hopefully, these imaging techniques become more popular and standardized if possible.

Cost issues

Most papers state that PRP is relatively cheap. However, an interesting note was the estimate cost analysis of corticosteroid treatment against PRP for the consumer. The estimated cost of corticosteroid treatment in the Netherlands as of November 2009 was about too hundred euros (US $300 at the time). On the other hand, although PRP treatment costs the same amount as corticosteroid treatment, an additional Diagnosis Treatment Combination (DBC) fee of three hundred and sixty euros(roughly US 540$) is also required in the Netherlands, increasing the total cost to five hundred and sixty euros(roughly US 840$) (Peerbooms et al., 2010a). As a result, not everyone will be able to afford PRP treatment due to cost.

PRP treatment in the United States is even more expensive. A New York Times article in January 2011, stated that a single PRP injection costs around a thousand dollars and was unlikely to be covered by insurance (Reynolds, 2011). Although comparatively,

Mishra and Pavelko (2006) surveyed eight physical therapy clinics within fifteen miles of their clinic and found the average cost for an initial checkup and subsequent ten follow up sessions to be around one thousand and two hundred dollars. Despite the cost, however, physical therapy treatment has the possibility of being covered by certain insurance policies for lateral epicondylitis. In conclusion, with current health insurance policies considering PRP to be still be in the experimental stage, PRP treatment in the United States will also be inaccessible to everyone due to cost.

Lack of Standardization

As an emerging topic in sports medicine, one of the problems that plague the scientific community is the lack of consensus on standard procedures (Filardo and Kon, 2012). As seen in Table 2, most PRP categories have many different methods of production. All the variations of PRP creation give different amount of platelets, leukocytes, and growth factor amounts. The lack of a standard makes comparison between different studies very difficult. In fact, the optimal type of PRP for each situation has yet to be determined such as when is L-PRP more beneficial than P-PRP. In addition, the differences in methods of production may be the key reason one study fails while another succeeds. Other issues that should be sorted out include volume of injection, buffering/activation, injection technique, pros and cons of single injection against multiple injections, and postop rehabilitation protocol (Gosens et al., 2012).

Chapter 4

Conclusion

Benefits

One of the best benefits of PRP is its relative safety and simplicity of use. Because the blood is autologous, an immune response will not be activated by the body. As a result, the risk of disease transmission and tissue rejection are no longer applicable. Besides local pain at the injection site, rarely are there complications. Although, there are minor concerns of systemic increases of growth factors due to PRP injection which could lead to cancerous growth, but no studies have yet proven the concerns (Aksahin et al., 2012). Other side effect complications seen rarely in some studies were syncope, dizziness, headache, nausea, gastritis, sweating, and tachycardia (Patel et al., 2013).

On the other hand, PRP still needs some more work before it can be proven sound. Although a plethora of areas have been touched. The majority of the studies concentrate on chronic problems with little research being performed on acute injuries. Perhaps, the reason for the lack of acute injury study is because the timing of PRP injection matters compared to chronic conditions (Mishra et al., 2009). In addition, more thorough random, double blind clinical studies need to be performed. Many of the current studies are small cohort studies which lack of placebo groups, high sample sizes, or include other biases which skew or muddle some of the current results. As a result, it is hard to take the results at face value. Of course, many of the studies are pilot studies, due to the relatively new application of PRP in sports medicine. PRP simply needs more time to mature so an open mind should be kept in terms of the soundness of PRP treatment.

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