AN ABSTRACT OF THE THESIS OF

Louisa K.K. Ho for the degree of Master of Science in Veterinary Science presented on June 19, 2014

Title: Is Platelet Rich Plasma (PRP) the Other Alternative? A Review and Current Research

Abstract approved:

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Wendy I. Baltzer

Platelet-rich plasma (PRP) has been applied as a hemostatic agent since the 1970s, and a potent source of autologous growth factors since the 1990s. PRP is rich in growth factors such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), fibroblast growth factor (FGF) and platelet factor-4 (PF-4). With these factors, PRP can enhance regenerative processes and facilitate tissue repair by significantly increasing cell migration, chemotaxis proliferation, differentiation and angiogenesis. PRP plays a strong role in orthopedic injuries, in particular sports medicine, in the enhancement of bone, muscle, ligament, tendon and cartilage healing.

The application of PRP in human musculotendinous injuries such as Achilles tendon and rotator cuff has had favorable clinical outcomes with very limited reports such usage in veterinary medicine. The first pilot study enrolled 10 dogs with supraspinatus tendinopathy to assess the short-term outcome of a single injection of
autologous PRP. Dogs were assessed at 2 weeks, 6 weeks and >16 weeks using both objective (gait analysis, ultrasound) and subjective (canine brief pain inventory score and lameness examination) measures. Results showed 40% of dogs had clinically resolved lameness by 6 weeks, with 50% returning to normal function at final evaluation. However, 6 dogs had persistent lameness, hence a single injection of PRP cannot consistently improve clinical lameness in dogs with supraspinatus tendinopathy.

Current research investigating alternative methods of simulating repair of damaged cartilage is an emerging field in both human and veterinary medicine. PRP has been promoted to enhance joint homeostasis, reduce synovial membrane hyperplasia and modulate synovial fluid cytokine levels. The second pilot study enrolled 10 dogs with coxofemoral osteoarthritis treated with a single intra-articular injection of autologous PRP. Objective measurements of lameness (radiographs, goniometry, accelerometry gait analysis) and subjective measures (canine brief pain inventory score and lameness examination) were assessed at 2 weeks and 6 weeks post-treatment. Both subjective and objective data did not find significant difference from pre-treatment to post-treatment evaluation at 2 and 6 weeks. Further research with a larger cohort of patients, longer follow-up times and with a series of PRP injections rather than a single dose is required to determine the clinical efficacy of PRP application in musculoskeletal disorders in the dog.
Is Platelet-Rich Plasma (PRP) the Other Alternative?  
A Review and Current Research

by  
Louisa K.K. Ho

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Major Professor, representing Veterinary Science

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Dean of the College of Veterinary Medicine

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Louisa K.K. Ho, Author
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CONTRIBUTION OF AUTHORS

Dr Sarah Nemanic and Dr Susanne Stieger-Vanegas assisted with data collection and interpretation.
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DEDICATION

To my mother, father and sister, for your unwavering support and guidance throughout the years. To Tom, my love, who knows me more than myself.
Chapter I

Introduction

1.1 Background and History

Regenerative biomedicine is a progressive, emerging branch of musculoskeletal and orthopedic medicine. This innovative field includes therapies such as platelet-rich plasma (PRP), mesenchymal stem cells (MSC), sclerosing agents, matrix metalloproteinase and growth factors. The goal of regenerative biomedicine is to enhance the body’s innate ability to repair and regenerate, accelerate the healing process after musculoskeletal injuries, and augment specific orthopedic and soft tissue conditions. PRP is an orthobiologic therapy defined as a portion of the plasma fraction of autologous blood with a platelet concentration above baseline used to treat or augment the treatment of specific orthopedic conditions. It involves a concentrated source of growth factors and cell signaling factors that play a significant role in tissue healing. The market for PRP in the United States alone was valued at $45 million in 2009, with an estimated projected worth of more than $120 million by 2016. To date, PRP has been used in the orthopaedic setting to treat Achilles tendinopathy, anterior cruciate ligament tears, plantar fasciitis and epicondylitis, as well as to augment spinal fusion, bone healing, rotator cuff repair, arthroplasty, and cartilage regeneration to name a few. Soft tissue applications of PRP have included use in wounds (such as for burns and ulcers), skin grafts, hair restoration, corneal ulcers, dental surgery and facial rejuvenation.

Platelet preparations have been used since the 1970s as a source of allogenic product for oral maxillofacial wounds. These preparations were in the form of fibrin glue, by polymerizing fibrinogen with thrombin and calcium. In 1985, the first patent use of PRP was
advocated for chronic wounds in diabetic patients. In 1987, PRP was used as an autologous transfusion support during cardiac surgery.\textsuperscript{11} The theoretical reduced risk of intolerance, immunogenic reactions, disease transmission and risk of adverse side effects commonly ascribed to medications and non-autologous substances, was viewed as an attractive method in treating patients with a product derived from their own blood. However, it was not until the 1990s, that commercial availability was instituted with the first clinical application performed for cancellous bone defects during mandibular reconstruction.\textsuperscript{2} The first use of PRP in sports injuries did not occur till the mid 2000s, specifically for treating elbow and rotator cuff tendinoses.\textsuperscript{12,13} Recent advancements have occurred over the last 2 decades in the use of PRP as it continues to emerge rapidly in multiple disciplines, including plastic surgery, dentistry, otolaryngology, spinal surgery, wound healing, orthopedics and in particular, sports medicine.\textsuperscript{1,14}

The preparation of platelet concentrates have evolved with different harvest techniques including conventional blood centrifugation, freezing and thawing cycles. With the variability in preparation of the platelet concentrate sample, there are inherently associated variability in the PRP formulation or cellular content (concentration and cellular counts), PRP storage modalities, PRP activation methods (collagen, calcium, thrombin) and PRP therapeutic protocols (amount or number of applications, targeting specific disease phases).\textsuperscript{15} Despite the increasing trend favoring the use of autologous platelet concentrates in both in vivo and in vitro research, recent meta-analyses concluded that a lack of standardization of study protocols exists.\textsuperscript{16} The variability in PRP harvest and outcomes, along with its application to different disease conditions, continues the extensive debate of its clinical benefit.
1.2. Platelet Rich Plasma Components

1.2.1. Platelets

Platelets are anucleate cytoplasmic fragments of multi-nucleated megakaryocytes formed in bone marrow. Their size range from 2 to 3μm and circulates in the circulatory system for 7 to 10 days at concentrations of 150 x 10^3/μL to 400 x 10^3/μL. Each platelet contains over 800 proteins with numerous post-translational modifications, resulting in over 1500 protein-based bioactive factors. Platelets contain procoagulant proteins important to their role in hemostasis, in addition to a milieu of growth factors contained within three types of granules (α, β, λ). Both endogenous and exogenous molecules can activate platelets to release their granular contents, including collagen, serotonin, calcium and magnesium. Exocytosis and degranulation of activated platelets release thromboxane-A_2_, adenosine diphosphate and thrombin, activating nearby platelets and provide an overall increase in platelet surface area. This interaction between growth factors and surface receptors on target cells activates intracellular signaling pathways that signals cellular proliferation, matrix formation, osteoid production and collagen synthesis.

Target cells, including mesenchymal stem cells, osteoblasts, fibroblasts, endothelial and epidermal cells express cell membrane receptors specific to growth factors in PRP. In vitro studies have shown that platelets actively secrete proteins within 10 minutes of clotting, with more than 95% of pre-synthesized growth factors secreted within 1 hour. After this initial burst of growth factors and cytokines, these bioactive proteins stimulate local stem cells, recruitment of inflammatory cytokines, followed by further sustained release of additional factors reported for up to 9 months. PRP proteins are therefore histopromotive factors that induce cellular chemotaxis, proliferation, differentiation, removal of tissue debris, angiogenesis
and extracellular matrix metabolism.\textsuperscript{3,18} Simultaneously, PRP inhibits excess inflammation and apoptotic metalloproteinase activity, whilst modulating the microvascular environment and altering efferent and afferent neural receptors.\textsuperscript{18}

1.2.2. Alpha Granules

Alpha granules are a source of cytokines and chemokines that give platelets their characteristic purple granular appearance. They include adhesive proteins (fibrinogen, von Willebrand factor), clotting factors (V, XI, XII, prothrombin), fibrinolytic factors (anti-thrombin, plasmin, plasminogen), glycoproteins and growth factors. These granules are formed during maturation of megakaryocytes, numbering 50-80 per formed platelet, with each granule containing more than 30 bioactive proteins. Their mode of action is to stimulate cellular chemotaxis, cell proliferation and maturation, modulation of inflammatory molecules and attract leukocytes.\textsuperscript{17}

1.2.3. Beta (Dense) Granules

Beta granules store adenosine diphosphate (ADP), adenosine triphosphate (ATP), calcium, histamine, serotonin and dopamine. Adenosine is a nucleoside involved in the transfer of energy and acts as a primary cytoprotective agent. Adenosine also activates macrophages to produce pro-inflammatory cytokines including interleukin-1, interleukin-8 and interleukin-10. Serotonin is a monoamine neurotransmitter. Its action is to increase capillary permeability, act as a chemoattractant for fibroblasts, induce influx of macrophages into tissue and suppress interferon-1α expression.\textsuperscript{18} Histamine is a biogenic amine and it acts as a vasodilator and increases endothelial membrane permeability, thus allowing inflammatory and immune cells to
marginate and enter the local area. The role of calcium is required in epidermal cell migration and regeneration.\textsuperscript{18}

1.2.4. Lambda (Lysosomal) Granules

Lysosomal granules secrete plasminogen activator, which converts plasminogen to plasmin in order to lyse blood clots. It also assists in removing infectious agents and cellular debris.\textsuperscript{18}

1.3. Growth Factors

Growth factors are a group of polypeptides involved in the regulation of growth and development of tissues. There are a multitude of growth factors, including: platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), platelet-derived endothelial growth factor (PDEGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF) and epidermal growth factor (EGF).\textsuperscript{1,17,18} The concentration of growth factors rise linearly with increasing platelet concentration.\textsuperscript{4} Once these cytokines are released, they are free to bind to transmembrane receptors on the surface of local or circulating cells. This initiates intracellular signaling, resulting in expression of proteins responsible for cellular chemotaxis, matrix synthesis and proliferation. Tissue regeneration can then occur through angiogenesis, extracellular matrix production, and collagen synthesis by autocrine and paracrine effects of growth factors.\textsuperscript{18}
1.4. Platelet-Rich Plasma Preparation

There are multiple PRP preparation techniques available commercially and in research. The PRP preparation method will influence the formulation and cellular content (concentration rates and cell counts), storage modalities, activation methods and therapeutic protocols (amount, number of injections, time interval between administrations). Different protocols between authors, institutions and study regimen introduce further confounding factors for comparison. A standardization of PRP preparation has yet to be proposed.

1.4.1. Platelet Count

According to the Red Cross Organization, a human PRP sample contains a minimum of 200,000 platelets/μL (normal range 150,000 – 400,000 platelets/μL). Marx et al determined clinical efficacy of PRP should have a minimum of 4 times above baseline (1million platelets/μL), while other reports of platelet concentration for a PRP sample indicate 200% or a 3 to 5 fold increase above baseline is the minimum necessary. More recently, there appears to be a ceiling threshold of benefit from high PRP concentrations. Torricelli et al showed that PRP concentrate of more than 750,000 platelets/μL (normal range 72,000 platelets/μL – 183,000 platelets/μL) leads to a shorter time to recovery when used in equine tendonitis. Other in vitro studies have also shown that a high PRP concentrate (>10% above baseline) suppressed, but that low PRP concentrate (between 1% to 5% above baseline) stimulated viability and proliferation of alveolar bone cells. Differences can also occur in platelet counting methods, hence consistency between laboratory standards is necessary. A critical aspect that requires more research is in the correlation between platelet concentration and clinical outcome. Individuals may also require different platelet concentration ratios to achieve a comparable biological effect.
1.4.2. Preparation

There are three techniques for preparation of the platelet concentrate: gravitational platelet sequestration, standard cell separators, and autologous selective filtration technology (or plateletpheresis). The two main commercial preparation methods are laboratory centrifuge and density gradient cell separator. Laboratory centrifugation requires a longer time for preparation, with more costs involved in equipment purchase and can be technician-dependent. There are many commercial cell separator devices available, mostly being closed-circuit, each with its own features and specifications. The Harvest SmartPrep System (Harvest Technologies Corp., Plymouth, MA) used at Oregon State University Veterinary Teaching Hospital utilizes a two spin speed centrifugation cycle resulting in a plasma supernatant with a gradient of different cell concentration based on its molecular weight. Erythrocytes are the most dense and they remain as a packed cell layer at the bottom of the collection cup (specific gravity, sp 1.09). The buffy coat of white blood cells is located at the top of the packed red blood cell layer (sp 1.06). Platelets are at its highest concentration in plasma just above the buffy coat, and decrease in concentration towards the top of the plasma layer (sp 1.03). The Harvest SmartPrep System has a two spin speed cycle that first reduces the number of erythrocytes in the plasma sample, then the second cycle concentrates the platelets. A single spin produces 1-3 times platelet concentrate above baseline with a lower leucocyte count. A double spin produces 4-8 times greater platelet concentrate above peripheral blood with a high leucocyte count (above peripheral blood).

The selective filtration technology depends on single use disposable proprietary filters. Platelets are captured on filter and are then harvested to provide a platelet-rich concentrate without the need for centrifugation. Most systems do not concentrate plasma proteins of the coagulation cascade unless an ultrafiltration system is used. Various systems will differ in
platelet collection efficiency, repeatability, final leukocyte count, platelet activation and ease of use (Table 1).

1.4.3. Activation and Anti-Coagulation

PRP can be used with or without platelet activation products. The addition of activation products can be chemical, biomaterial or physical agents, with the most common being calcium chloride and thrombin. Platelets can be activated endogenously or exogenously, with each type of activator exhibiting varying growth factor kinetics. Pre-clotted PRP is theorized to provide a more sustained growth factor release due to a slower elution of growth factor over several days. Exogenous activation results in rapid coagulation and quick clot formation – this is best applied to tissue manually rather than by injection. Exogenous activators include thrombin and calcium chloride that result in a rapid release of granular contents from the platelets, and requires immediate use of the PRP sample. Most commercial systems utilize 1000U of bovine thrombin per milliliter of 10% calcium chloride to the PRP concentrate. Caution should be taken with injection of thrombin since the systemic use of bovine thrombin (bovine factor V) has been shown to be associated with coagulopathies. Calcium chloride is another exogenous activator that provides formation of a fibrin matrix less dense than thrombin to provide a trapping mechanism for platelets. Injections with calcium chloride have a low pH that can cause pain and burning sensation on administration. The alteration in pH however, can affect the release of growth factors from PRP. A combination of calcium chloride and thrombin may be of benefit as it forms a coagulate causing fibrin to polymerize into an insoluble gel. This may result in a slower release of growth factors over a longer period of time. Furthermore, Harrison et al found that thrombin resulted in an immediate release pattern, whilst collagen provided a more sustained release pattern of growth factors. The use of collagen has also been shown to have
less clot retraction and stimulated equal release of PDGF and VEGF as compared to bovine thrombin. Endogenous tissue collagen can cause activation of platelets, by simple agitation of platelets (from centrifugation), or via needle-induced bleeding during PRP injection.

Endogenous activator such as tissue collagen can result in slower aggregation for platelets and release of growth factors, hence resulting in a more natural release pattern. An anti-coagulant is also added to the blood collected prior to PRP harvest, with the most common agent used being acid-citrate-dextrose anticoagulant – solution A (ACD-A). Dextrose supports platelet metabolism and viability. Lei et al showed that PRP quality (that is, maintaining platelet structure integrity, preventing platelet spontaneous activation, release of more TGF-β1 and enhanced proliferation of stromal cells) was significantly superior using acid-citrate-dextrose (ACD) compared to heparin or citrate alone. Other studies have assessed the use of ethylenediaminetetraacetic acid (EDTA) which has shown to be harmful, damaging large numbers of platelets, whereas trisodium citrate solution has been shown to have no negative cellular effects. Any additives may alter the content and effect of PRP, including the use of anti-coagulants which can be of clinical significance. The full significance of these different additives to PRP in dogs is currently unknown.

1.4.4. PRP Handling and Storage

Once the PRP sample is harvested, it remains stable in an anti-coagulated state for 8 hours or longer. However, if clotted, the PRP sample should be used within 10 minutes of clot initiation. Fresh administration after PRP preparation is recommended. The use of freeze-thawing storage methods have been shown to impair platelet function and lifespan, alter the growth factors’ release pattern, favor the accumulation of pyrogenic cytokines and increase the
risk of bacterial proliferation. In both studies reported here, PRP was administered within 20 minutes of preparation to all dogs.

1.4.5. Comparison of Different Commercial PRP Systems

There are many commercial PRP systems currently available: SmartPReP2 (Harvest Technologies, Plymouth MA, USA); GPS III (Biomet, Warsaw, IN, USA); E-PET (Pall Corporation, Port Washington, NY, USA); Magellan (Arteriocyte Medical Systems, Hopkinton, MA, USA); Secquire (PPAI Medical, Fort Myers, FL, USA); ACP Double Syringe System (Arthrex, Naples, FL, USA); Angel system (Cytomedix, Gaithersburg, MD, USA); ProTec (Pulse Veterinary Technologies, Alpharetta, GA, USA). Most systems produce approximately 7mls of PRP from 60mls of whole blood (Table 1). Selection of a particular system will require recognizing the ease of use in clinical or on-field practice, PRP production time, platelet yield, growth factor release, maintenance of sterility and cost effectiveness. To complicate comparisons, each commercially available PRP system varies with its spin protocol, platelet and leucocyte concentrations and delivery device. One study assessed the hematologic parameters of five different systems using equine blood, with results showing significant differences in platelets and growth factors between systems. Castillo et al found significant differences in the concentrations of white blood cells, PDGF and VEGF across three commercially available PRP concentration systems. The lack of standardization of PRP preparations therefore contributes to inconsistent results and outcomes in both clinical and in vitro studies.

Individual variation can also affect the PRP sample: hydration status, inflammation (leukocytosis, leukopenia), lipemia (diet), circadian rhythm, venous blood status, gender (this
affects the final PRP product less if a density-gradient system is used). A clinician’s awareness of exactly what each system constitutes and the literature available specific to the system will allow a better-informed decision regarding its therapeutic application.

1.5. Platelet-Rich Plasma Classification

With the emerging different systems and preparation methods available in harvesting PRP, a classification system needs to be addressed in order to standardize PRP samples. There are two current systems adapted: PAW (Platelets, Activation, White blood cells) classification system by DeLong et al. and a sports medicine system by Mishra et al. The PAW classification system is based on 3 components: 1) the absolute number of platelets, 2) the manner in which activation occurs, and 3) the presence or absence of white blood cells. This classification system utilizes four platelet concentrate levels (P1-P4); endogenous or exogenous activation method; a buffy coat (A) or plasma based system (B), and a subcategory of neutrophil content (α or β). Hence a PRP sample consisting of 900,000 platelets/μL with WBC and neutrophil content above baseline level using a plasma-based system will be classed as P3-Aα. A newer classification system by Mishra et al is utilized in sports medicine in humans. It is based on similar categories of WBC level, activation, and platelet concentration, categorized into Type 1 to Type 4. These systems are currently not widely adopted. Variability in the production of PRP has led to further classification (and more widely used) of platelet concentrates into: pure platelet-rich plasma (P-PRP), leucocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF) and leucocyte- and platelet-rich fibrin (L-PRF) (Table 2),
1.5.1. Controversy of Leucocyte Content

Although normal levels of white blood cells (WBC) may have positive immunomodulatory effects, literature suggests that excessive leucocytes, specifically neutrophils, may have negative effects. Leucocytes can release matrix metalloproteinase and reactive oxygen species capable of damaging articular tissue and having a catabolic effect.\(^{21}\) The role of WBC in PRP samples remains unclear and may depend on its indication and tissue application. PRP used for open wounds and for preventing infection may require supranormal WBC levels,\(^ {36} \) whereas PRP with low or absent WBC levels are more favorable to apply in areas that desire minimal scar formation.\(^ {37} \)

In vitro studies have shown that the WBC content in PRP may also have an antimicrobial effect. Bielecki et al showed that PRP inhibits the growth of *Staphylococcus aureus* and *Escherichia coli* in vitro, attributing to leucocytes that can be a source of cytokines and enzymes to help prevent infections.\(^ {38} \)

McCarrel et al showed that WBC in PRP contributes to inflammatory cytokine (TNF-α, IL-1β) production,\(^ {39} \) and that leukocyte-reduced PRP may be optimal for superior healing of tendons without scar tissue formation.\(^ {39} \) Platelet-derived mediators initially inhibit interleukin-1 (IL-1) production from macrophages and reduce their proliferation until day 4 when macrophage division occurs.\(^ {18} \) This initial suppression of macrophage activity may prevent excessive early inflammation that leads to dense scar tissue formation. Galliera et al believed that platelets can prevent excessive leukocyte recruitment by anti-inflammatory cytokines, such as TGF-β.\(^ {40} \) The role of leucocytes in PRP therefore remains controversial. It is recommended to have complete
blood count performed on each patient’s venous blood and PRP sample prior to administration, as individuals with active infections are contraindicated in receiving PRP.

1.5.2. PRP Delivery

Platelet delivery methods can vary depending on the anatomic location of treatment. Delivery as a liquid injection, spray, gel or clot matrix will require specific exogenous or endogenous activators and processing techniques. The anatomic location for delivery is another consideration, as PRP injection into the osseotendinous, mid-substance or myotendinous zone of a tendon may produce varying results.\textsuperscript{41} In the current literature available, there are wide inconsistencies with the methods of PRP applications, the timing of treatment, the number of injections per series, and the volume of injections.\textsuperscript{4,6,15,16} There are no current recommended PRP formulations to determine dosage and type. Hence, standardized dosing, composition and protocols will be required to compare results between studies.\textsuperscript{18}

1.6. Platelet Rich Plasma Now and Future Applications

The relative ease of preparation and application, relative safety of an autologous product and possible beneficial alternatives to musculoskeletal diseases makes PRP a promising therapeutic approach for future regenerative treatments. However, its future role as a sole treatment option versus augmentation with mesenchymal stem cells, fibrin scaffolds or bone marrow aspirates; or as its use as an adjuvant to standard treatment options remains to be seen and much continued research is needed in order to optimize the effects of PRP.
1.6.1. Literature Review

There is extensive documentation on the use of PRP in human studies and to a lesser degree on animal studies over the past 10 years. However, with the emerging literature, there remains a lack of ‘level of evidence 1’ studies and the non-standardized use of PRP has led to inconsistencies in efficacy and outcome. An example is a controlled laboratory study by Murray et al that augmented anterior cruciate ligament tears induced in pigs with or without PRP in two separate studies.\textsuperscript{42,43} There was improvement in the biomechanical properties of the ligaments at 4 weeks in the PRP treated group, however, in the other study (performed 2 years later), there was no improvement in maximum load and stiffness (these were however different pigs, with follow-up at 14 weeks). A recent meta-analysis by Sheth et al compiled all available randomized controlled trials or prospective cohort studies (n=23) up to year 2012 comparing autologous blood concentrates with a control therapy in human orthopedic injuries.\textsuperscript{44} The authors of that study found no significant benefit in the use of PRP up to 24 months post-treatment. There were wide confidence intervals but a small trend favoring PRP. A more promising meta-analysis reviewing prospective and randomized controlled studies up to year 2013 (n=13, 1543 cases) that used PRP to treat knee osteoarthritis found improved function compared to hyaluronic acid injections, especially for patients with lower degrees of degeneration.\textsuperscript{45} Additionally, a multicenter retrospective review of PRP as a treatment for chronic tendinopathies, showed 82% patients had a moderate (>50%) improvement in pain with a 85% client satisfaction.\textsuperscript{46} The clinical outcomes in the treatment of acute and chronic wounds using PRP were analyzed in a meta-analysis by Carter et al.\textsuperscript{47} A total of 24 randomized controlled trials and comparative group
studies found that PRP treated wounds demonstrated reduced presence of infection and improved healing over controls that did not receive PRP.

The following is a brief compilation of human musculoskeletal studies outlining the research that had positive and negative outcomes following the application of PRP.

**Positive PRP outcomes**

<table>
<thead>
<tr>
<th>Study (n)</th>
<th>Study Design</th>
<th>Diagnosis</th>
<th>Control group (n)</th>
<th>Outcome measures</th>
<th>Results</th>
<th>Critiques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mishra(^2),(2006) (15)</td>
<td>Prospective cohort, Level 2</td>
<td>Lateral epicondylitis</td>
<td>Local anesthetic (5)</td>
<td>VAS</td>
<td>PRP group: 60% improvement scores at 8wks, 81% at 6mths, 93% at &gt;1yr</td>
<td>Small sample size, not randomized, not blinded</td>
</tr>
<tr>
<td>Peerbooms(^4),(2010) (100)</td>
<td>Randomized controlled, Level 1</td>
<td>Lateral epicondylitis</td>
<td>Corticosteroid (49)</td>
<td>VAS, DASH</td>
<td>PRP group: 73% success Control: 49% success (&gt;25% reduction in pain)</td>
<td>Subjective measurements</td>
</tr>
<tr>
<td>Gosens(^9),(2011) (100)</td>
<td>Randomized controlled, Level 1</td>
<td>Lateral epicondylitis</td>
<td>Corticosteroid (49)</td>
<td>VAS, DASH</td>
<td>PRP reduced pain and increased pain than control even after 2yrs</td>
<td></td>
</tr>
<tr>
<td>Filardo(^3),(2013) (43)</td>
<td>Prospective</td>
<td>Patellar tendinopathy</td>
<td>--</td>
<td>VA, Tegner, client satisfaction, ultrasound</td>
<td>80% satisfied and returned to normal activities; no correlation with ultrasound and clinical findings</td>
<td>No control</td>
</tr>
<tr>
<td>Kon(^2),(2009) (20)</td>
<td>Prospective, pilot</td>
<td>Patellar tendinopathy</td>
<td>--</td>
<td>VAS, Tegner</td>
<td>80% satisfied and returned to normal activities; Improvement in 70% at 5mths</td>
<td>No control</td>
</tr>
<tr>
<td>Lyras(^3),(2009) (40 rabbits)</td>
<td>Prospective cohort</td>
<td>Patellar tendinopathy</td>
<td>Surgical defect, no treatment</td>
<td>Histology, mechanical test</td>
<td>PRP group: 72% increase force to failure, 39% increase ultimate stress, 53% increase stiffness at 14 days. No difference at 28days</td>
<td></td>
</tr>
<tr>
<td>Study (n)</td>
<td>Study Design</td>
<td>Diagnosis</td>
<td>Control group (n)</td>
<td>Outcome measures</td>
<td>Results</td>
<td>Critiques</td>
</tr>
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<tr>
<td>Sanchez(^5) (2007) (12)</td>
<td>Case control, Level 3</td>
<td>Achilles tendinopathy</td>
<td>Surgery alone</td>
<td>ROM, function, ultrasound, laboratory analysis</td>
<td>PRP had earlier ROM, function, less increase in tendon cross-section area</td>
<td></td>
</tr>
<tr>
<td>Aspenberg(^5) (2004) (x rats)</td>
<td>Controlled laboratory</td>
<td>Achilles tendinopathy</td>
<td>--</td>
<td>Mechanical properties, histology</td>
<td>30% increased tendon callus strength and stiffness</td>
<td></td>
</tr>
<tr>
<td>Sanchez(^5) (2008) (30)</td>
<td>Retrospective cohort</td>
<td>Knee osteoarthritis</td>
<td>Hyaluronan</td>
<td>Questionnaire</td>
<td>At 5wks, 33% PRP group had improved scores vs 10% controls</td>
<td>Small numbers</td>
</tr>
<tr>
<td>Randelli(^8) (2011) (55)</td>
<td>Prospective randomized controlled double blind, Level 1</td>
<td>Rotator cuff injury</td>
<td>No treatment (27)</td>
<td>Shoulder test scores, dynamometer MRI</td>
<td>PRP group: reduced pain in first few months, equivocal in long term &gt;6mths</td>
<td></td>
</tr>
</tbody>
</table>

VAS = visual analog scores  
DASH = disabilities of the arm, shoulder and hand outcome measure scores  
VISA = Victorian Institute of Sports Assessment

Evidence (Level) grade:
- I (High): high quality randomized controlled trials, prospective studies, with plausible described effect, precisely quantified and not vulnerable to bias.
- II (Intermediate): lesser quality randomized controlled trial, prospective comparative study or retrospective study, the described effect is plausible but is not quantified precisely, inconsistent results or may be vulnerable to bias.
- III (Low): case control study or retrospective comparative studies with concerns about plausibility or vulnerability to bias. Severely limit the value of the effect being described and quantified.
- IV: case series with poor reference standard
- V: expert opinion
## Negative PRP Outcomes

<table>
<thead>
<tr>
<th>Study (n)</th>
<th>Study Design</th>
<th>Diagnosis</th>
<th>Control group (n)</th>
<th>Outcome measures</th>
<th>Results</th>
<th>Critiques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krogh(^9) (2013) (60)</td>
<td>Randomized controlled, Level 1</td>
<td>Lateral epicondylitis</td>
<td>Corticosteroid (20), saline (20)</td>
<td>Questionnaire, Ultrasound + Doppler</td>
<td>All had pain reduction, steroid had short term pain reduction + tendon thickness at 1 month</td>
<td>No objective data</td>
</tr>
<tr>
<td>De Vos(^6) (2010) (54)</td>
<td>Stratified block, randomized controlled</td>
<td>Achilles tendinopathy</td>
<td>Saline(27)</td>
<td>VISA score, questionnaire</td>
<td>Both improved, no difference</td>
<td>No objective data</td>
</tr>
<tr>
<td>Valenti Nin(^5) (2009) (100)</td>
<td>Prospective randomized controlled double-blind, Level 1</td>
<td>Anterior cruciate ligament tear</td>
<td>Surgery only</td>
<td>Inflammatory parameters, MRI, VAS, arthrometer</td>
<td>No difference in allograft augmented with PRP group vs. control</td>
<td></td>
</tr>
<tr>
<td>Murray(^7) (2009) (6 pigs)</td>
<td>Controlled</td>
<td>Anterior cruciate ligament tear</td>
<td>surgery alone, contralateral limb</td>
<td>Mechanical properties</td>
<td>No difference in tensile strength, load at yield, stiffness,</td>
<td>Small case numbers, immature animals</td>
</tr>
<tr>
<td>Kon(^1) (2010) (115)</td>
<td>Prospective cohort</td>
<td>Knee osteoarthritis</td>
<td>--</td>
<td>VAS, objective, subjective</td>
<td>Improved at 6 months, Worse than pre-op at 12 months</td>
<td>No control</td>
</tr>
<tr>
<td>Kesikburun(^2) (2013) (40)</td>
<td>Randomized controlled, Level 1</td>
<td>Rotator cuff tendinopathy</td>
<td>Saline (20)</td>
<td>VAS, Rotator cuff index, range of motion</td>
<td>No difference between groups at 1yr post</td>
<td></td>
</tr>
</tbody>
</table>

BMSC = bone marrow stromal cells  
FDBA = freeze-dried bone allografts

**Evidence (Level) grade:**  
- I (High): high quality randomized controlled trials, prospective studies, with plausible described effect, precisely quantified and not vulnerable to bias.  
- II (Intermediate): lesser quality randomized controlled trial, prospective comparative study or retrospective study, the described effect is plausible but is not quantified precisely, inconsistent results or may be vulnerable to bias.
III (Low): case control study or retrospective comparative studies with concerns about plausibility or vulnerability to bias. Severely limit the value of the effect being described and quantified.

IV: case series with poor reference standard

V: expert opinion

The use of PRP in the equine industry has exceeded its application to musculoskeletal disorders compared to canines. Bosch et al showed positive outcomes in horses treated with PRP for superficial digital flexor tendon lesions, with improved healing in 80% and improved neovascularization and higher collagen content in PRP treated tendons. Other authors have also found an earlier return to performance in horses treated with autologous platelet concentrates for various soft tissue musculoskeletal lesions. A pilot study using platelet concentrates for horses with osteoarthritis did not find a significant clinical effect compared to pre-treatment. However, one of the biggest limitations of these equine studies are the low sample numbers and lack of a control groups.

Although canine models have been used for human experimental studies, there are only two published clinical trials on the use of platelet concentrate in dogs to date. The experimental studies included the dog as a model to compare the healing of skin using PRP, and to assess the healing of mandibular and bone defects, both of which showed no significant advantage in the use of PRP compared to controls. The clinical trials published to date included a prospective study by Fahie et al that showed PRP used for osteoarthritis in dogs had a 55% decreased lameness score, 53% decreased pain score, and 12% increase in peak vertical force at 12 weeks post-treatment. Vilar et al combined the use of adipose mesenchymal stem cells with an autologous platelet concentrate in dogs with hip osteoarthritis and found a 10% increase in peak vertical force, and a 2% increase in vertical impulse at 6 months post-
Both of these studies have a control group, with a limitation of low sample size. To the author’s knowledge, there are no studies assessing the effects of PRP on canine tendinopathies, or as a sole application for the management of hip osteoarthritis in dogs.

1.7. Canine Tendinopathies

Tendon injuries can occur as an acute or chronic degeneration and as partial or complete tendon rupture. Repetitive trauma, metabolic disease, medications, genetic factors can all contribute to tendon degeneration. Damaged tendons never regain its full biomechanical strength. Healed tendons are therefore mechanically inferior to normal tendon tissue due to scar tissue formation. Recent studies indicate that poor vascularization around tendons prevents full healing capacity in these structures.

Tendon healing occurs through overlapping phases: hemostasis, inflammation, proliferation and remodeling. Immediately following injury, leaky capillaries allow for recruitment of hemostatic factors and inflammatory mediators. The coagulation cascade is activated with platelet aggregation, clot formation, and formation of an extracellular matrix construct. Platelets adhere to exposed collagen and extracellular matrix proteins. Platelets also release alpha granules in the form of bioactive cytokines, growth factors, and pro-inflammatory mediators such as serotonin, bradykinin, prostaglandins, thromboxane and histamine. In this initial inflammatory phase, neutrophils migrate to the injured site within 1-2 hours. At 48-72 hours, macrophages arrive at the injured site for wound debridement, regulation of inflammation, recruiting fibroblasts and endothelial cells. Woodall et al investigated the effects of PRP on the inflammatory phase on osteoblasts, fibroblasts and tenocytes, and showed
that there was a suppression of macrophages as early as 24 hours post treatment. Kajikawa et al found that PRP increased proliferation of macrophages at 3 and 7 days in patella tenocytes than in the control group.

During the cellular proliferation phase, fibroblasts synthesize collagen, with formation of granulation tissue and recruitment of further chemotactic, mitogenic and angiogenic growth factors. During the maturation and remodeling phase, growth factors such as PDGF and TGF-β and fibronectin, stimulate fibroblast proliferation, migration and synthesis of extracellular matrix. In turn, type I collagen combines with type III collagen to form a more robust matrix with increased tensile strength, as shown in rat supraspinatus tendon models. The application of PRP and its growth factors has been shown to augment revascularization, stimulate cell proliferation and total collagen production in tendon healing. De Mos et al showed that in vitro use of PRP in human tenocytes stimulated increased total collagen content, VEGF-A and TGF-β1 expression with slight increase in matrix metalloproteinase-3 (MMP3) production. Schnabel et al also supported these findings with PRP in cultured equine tenocytes that resulted in increased gene expression of matrix molecules of collagen type I and type III, without an increase in catabolic matrix metalloproteinases. These findings led to other authors suggesting that PRP may accelerate catabolic metabolism of traumatically injured tendons, whilst promoting angiogenesis and formation of a fibrovascular callus.

As the pathogenesis of tendinopathy is better understood as a degenerative process, the use of PRP becomes more promising as preclinical and in vitro studies have shown that PRP improves tendon fiber alignment, reduces fibrosis and increases angiogenesis in tendon defects. In a rabbit Achilles tendon rupture model, significant improvement in tendon regeneration was seen in PRP treated tendons compared to controls. The same authors also
found that PRP treated patellar tendon defects in rabbits showed significantly increased force at
failure, as well as ultimate stress and stiffness compared to controls in the early phase of tendon
healing. The use of autologous platelet concentrates were applied in both acute and chronic
tendinopathies in horses with ultrasonographic improvements in tendon structure and return to
pre-injury level of performance in all horses. Bosch et al performed a biomechanical,
biochemical and histologic analysis of the effects of intratendinous application of PRP in equine
tendon lesions. Their results showed higher collagen, glycosaminoglycan and cellularity in
PRP-treated tendons than controls, with higher strength at failure and elastic modulus. There
are no current published studies on the application for canine tendinopathies to the author’s
knowledge. Further in vivo and in vitro investigations are needed to clearly define the exact
mechanism by which PRP enhances tissue healing and its clinical effects in tendon healing in the
longterm.

1.8. Canine Supraspinatus Tendinopathy

The supraspinatus muscle arises from the supraspinous fossa of the scapula inserting as
an extremely strong tendon on the greater tubercle of the humerus. It extends the shoulder joint
and advances the limb. A comparative study across mammals showed that four muscles in the
animal model corresponded to the human rotator cuff: supraspinatus, infraspinatus, subscapularis
and teres minor muscle. Overuse injury, acute trauma and degeneration of the supraspinatus
tendon, in particular at its tendinous insertion, have been implicated as causes of supraspinatus
tendinopathy (ST). However, the exact etiology of ST causing lameness in dogs remains
unknown. A hypovascular zone located close to the enthesis of the supraspinatus tendon has
been implicated as a mechanism for tendon degeneration due to localized ischemia, reduced healing and subsequent chronic recurrent tears.\textsuperscript{91,92} PRP injections have been proposed as a promising alternative for treating rotator cuff tendinopathies in humans.\textsuperscript{7} A retrospective study of four human sports medicine centers across the United States evaluated intraslesional injection of PRP for chronic tendinopathies.\textsuperscript{46} Patients affected with Achilles tendon injury had 100% return to full function, whereas injury to the lateral epicondyle of the elbow, hamstring muscles and rotator cuff tendons had a 93%, 82% and 81% return to full function respectively.\textsuperscript{46} Several authors used PRP to augment rotator cuff repair and found decreased re-tear rates (20% in treated versus 55.6% controls)\textsuperscript{93} and higher strength in external rotation.\textsuperscript{58} Other studies did not find clinical difference in patients with PRP augmented rotator cuff repair.\textsuperscript{94-96} When PRP was used alone for rotator cuff tendinopathy, Scarpone \textit{et al} showed improved pain function and MRI appearance.\textsuperscript{97} There are currently no published studies on the effect of PRP intraslesional injection in dogs affected with supraspinatus tendinopathy according to the author’s knowledge. The following study (Chapter II) conducted at Oregon State University Veterinary Teaching Hospital is a pilot clinical trial utilizing autologous PRP as a treatment protocol for supraspinatus tendinopathy in dogs.

\textbf{1.9. Canine Osteoarthritis}

Osteoarthritis (OA, also known as degenerative joint disease, hypertrophic arthritis, degenerative arthritis and osteoarthrosis) is a disorder of progressive loss of articular cartilage and formation accompanied by variable degree of pain and debility.\textsuperscript{98,99} The degeneration of articular cartilage is characterized by changes in chondrocyte activity in favor of catabolic metabolism; alterations in synovial fluid, intermittent inflammation of the synovium, and
sclerosis and edema of underlying subchondral bone. OA is a complex condition divided into primary or secondary forms. Primary or idiopathic OA results from abnormal articular cartilage structure, metabolism and biosynthesis, and is considered uncommon in dogs. Secondary OA results from abnormal stress placed on normal articular cartilage (trauma, infection, immune-mediated arthropathy), or from normal forces acting on an abnormal joint (hip and elbow dysplasia, osteochondrosis). Multiple risk factors have been reported in the development of OA. These include weight, genetics, nutrition, gender, hormonal status, developmental abnormalities, muscle weakness and previous trauma.

1.9.1. The Synovial Joint: Joint Capsule, Synovial Fluid and Articular Cartilage

All synovial joints (also known as true joints) consist of a joint capsule, articular cartilage, subchondral bone and synovial fluid. The synovial membrane has an outer fibrous layer and inner layer that consists of nerves, blood vessels and lymphatic vessels. The synovium is lined with synoviocytes. Type A synoviocytes (macrophage-like) clears debris by phagocytosis, whilst type B synoviocytes (fibroblast-like) produce hyaluronic acid, proteins and pro-inflammatory cytokines including IL-6, IL-8, and matrix metalloproteinases. Synovial fluid is formed as a dialysate of plasma from type B synoviocytes containing hyaluronic acid, lubricin, proteinases and collagenases. It acts to lubricate the joint and provide shock absorption by changing its viscosity under moment shear. Synovial fluid also transports oxygen, nutrients and metabolic wastes from chondrocytes. Chondrocytes occupy less than 2% of articular cartilage by volume, with their nutritional supply derived from the synovial fluid and subchondral blood vessels. Articular cartilage is therefore composed of chondrocytes embedded
in a matrix comprising mainly of water, collagen and proteoglycans. Adult articular cartilage itself is relatively avascular.

Proteoglycan aggregates are made up of multiple mucopolysaccharides and glycosaminoglycans (GAGs) that form complexes with hyaluronic acid to act as osmotic traps holding water between collagen strands. These GAGs include chondroitin-4-sulphate (composed of repeating disaccharide units of glucosamine and galactosamine), chondroitin-6-sulphate, hyaluronic acid, keratin sulphate, dermatan sulphate and heparin sulphate. Proteoglycan and water aggregates thus act as shock absorbers that enable cartilage to withstand loading forces.

Proteoglycans consist of glycosaminoglycan chains contained within a meshwork formed by collagen fibrils that limits their ability to expand to within 20% of its potential. Proteoglycans also have a strong affinity to water and can occupy up to 50 times their dry weight volume when hydrated. This combination imparts elasticity and turgidity to the cartilage, and helps resist deformation when a compressive load is applied. A major proteoglycan is aggrecan which attaches to hyaluronic acid to form an aggrecan aggregate, composed of a central core protein with many glycosaminoglycan side chains (up to 100 chondroitin sulfate side chains). Hyaluronan is a highly hydrated glycosaminoglycan that is produced by chondrocytes and type B synoviocytes. When aggrecan monomers bind to hyaluronan in the presence of link proteins, highly negatively charged aggregates form from the glycosaminoglycan side-chains. These aggregates imbibe water by electrostatic and osmotic forces. The proteoglycan swells and contributes to the resilience, elasticity and resistance to compression of cartilage. Proteoglycans are therefore responsible for the fluid dynamics of joint lubrication, along with other factors including loading, motion and ultrastructural damage to the cartilage matrix.
Articular cartilage morphology is divided into zones that are based on chondrocyte organization, collagen fiber orientation and proteoglycan distribution. Zone 1 is the superficial layer with low cellularity and low proteoglycan content. Collagen fibers are oriented parallel to the articular surface. These collagen fibrils can withstand high tensile stress, resist deformation and distribute load more evenly. Zone 2 is the transitional layer. The collagen fibers are obliquely oriented which may alter under compression. This zone has increased resistance to fluid flow and provides maximal resistance against load. Zone 3 is the radial layer and contains the major portion of the cartilage matrix. The high proteoglycan concentration of zone 3 can withstand compressive loads well. Zone 4 is the calcified cartilage layer. The collagen fibers are radially oriented with little proteoglycan. It is separated from the subchondral bone by a cement line at the osteochondral junction. This mineralized cartilage has undulating contours that allow shear stresses to be converted to compressive force on the subchondral bone thus providing some protection to the bone.

The extracellular matrix distributes force over the underlying subchondral bone and is composed predominantly of water (65-80%) as synovial fluid, collagen (20-30%) and proteoglycans (5-10%). Collagen imparts tensile strength to articular cartilage and provides a framework for proteoglycans and chondrocytes. Type II collagen is the predominant form (90%) in articular cartilage. Type VI collagen is found in pericellular region of chondrocytes to help form the chondron (a chondrocyte and its surrounding pericellular matrix). Type IX collagen links type II collagen together.
1.9.2. Development of Osteoarthritis

When a compressive load is applied to cartilage, synovial fluid moves out of the cartilage until equilibrium is reached between the osmotic force generated by the proteoglycans and the compressive force applied. The fluid flow is slow and dependent on the collagen matrix and density of proteoglycan molecules. When the load is removed, the negative charge attracts the water component of synovial fluid, and cartilage regains its pre-compressed thickness. The flow of synovial fluid under pressure allows normal articular cartilage to compress under load without permanent damage to its matrix. The movement of synovial fluid also aids in removal of metabolic waste and acquiring nutrition and oxygen. Cellular deformation can occur with repetitive mechanical stress and is thought to incite release of factors that activate degradative enzymes leading to development of OA. Articular cartilage is therefore a dynamic tissue, with chondrocytes constantly regenerating in an anabolic state. OA is thus a condition whereby the catabolic process exceeds the anabolic process, resulting in ineffective regeneration of cartilage. This leads to a decrease in proteoglycan and hyaluronic acid content.

The release of degradation enzymes from the extracellular matrix, chondrocytes and synovial cells initiates an inflammatory response that alters the normal balance of cartilage matrix degradation and repair/synthesis. The major enzymes involved in cartilage degradation include proteinases (cathepsin), metalloproteinases (collagenase, caseinase), prostaglandin E₂, and pro-inflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-α, interferon-γ and nitric oxide). The decreased synthesis of inhibitors to these pro-inflammatory mediators and cytokines causes further damage to articular cartilage resulting in a vicious cycle of inflammation and cartilage destruction.
Hypoxia results in lactate accumulation in the synovia and a decreased pH.\textsuperscript{117} Interleukin-1 triggers production of cyclooxygenase-2 (COX-2) in chondrocytes as well as inhibits the synthesis of type II collagen, and instead stimulates type I and type III collagen leading to fibrosis.\textsuperscript{118} Nitric oxide activates catabolic enzymes, metalloproteinases (MMP-1, collagenase; MMP-3, stromelysin, MMP-8, gelatinase), decreases collagen and proteoglycan synthesis and induces chondrocyte apoptosis.\textsuperscript{118} Tumor necrosis factor (TNF) may increase leucocyte adhesion on synovial vascular endothelium – this influx of leucocytes can contribute to subsequent release of more inflammatory substances. Fibrillation and fissuring of degenerative articular cartilage alters the compliance of the underlying subchondral bone and zone of calcified cartilage.\textsuperscript{119} The subchondral bone acts as a shock absorber to transform shear forces into tensile and compressive forces.\textsuperscript{120} Deep fissuring, thinning and constant inflammation of the synovia leads to sclerosis of the subchondral bone resulting in pain and lameness. In addition, ensuing synovitis is associated with increased capillary permeability with subsequent leakage of serum proteins leading to synovial edema and alteration of synovial fluid volume and viscosity.\textsuperscript{121} Osteophyte formation from chronic inflammation and eventual calcification is considered an attempt to limit movement, inflammation and the resulting pain. The maintenance of normal cartilage homeostasis requires the coordinated synthesis and degradation of articular cartilage matrix molecules. Ongoing, repetitive injury shifts the balance between proinflammatory (IL1\(\alpha\), IL1\(\beta\), TNF\(\alpha\)) and anti-inflammatory cytokines.\textsuperscript{122}
1.9.3. Growth Factors and Cytokines Involved in Osteoarthritis

Transforming Growth Factor – β (TGF-β)

In cartilage repair, the four most investigated members of the TGF-β superfamily includes TGF-β1, bone morphogenetic protein-2 (BMP-2), BMP-7 (also known as osteogenic protein-1) and cartilage-derived morphogenetic protein (CDMP-1). TGF-β is widely considered a promoter of chondrocyte anabolism in vitro (enhances matrix production, cell proliferation and osteochondrogenic differentiation), and intra-articular injections to increase bone formation in vivo. TGF-β1 stimulates chondrogenesis and decrease catabolic activity of IL1. However, overexpression from prolonged TGF-β1 activity can induce osteophyte formation. TGF-β3 stimulates extracellular matrix synthesis, and has been shown in rabbits to improve chondrogenesis and in vivo, hyaline cartilage regeneration.

Bone Morphogenetic Protein (BMP)

In vitro investigations have recently identified that BMP-2 stimulates matrix synthesis, enhances cartilage matrix turnover, and increases collagen type II and aggrecan expression. BMP-7 induces hyaline cartilage-like regeneration of full-thickness osteochondral defects in a dog model. BMP-7 also decreased cartilage degeneration, decreased expression of MMPs and inhibits progression of OA in rabbit cranial cruciate-deficient stifles.

Insulin-like Growth Factor-1 (IGF-1)

IGF-1 has been shown to induce chondrogenic differentiation of MSCs and protect the synovial membrane from chronic inflammation. IGF-1 enhances its anabolic effects when combined with TGF-β1 or BMP-7.
Fibroblast Growth Factor (FGF)

FGF-2 is found in pericellular matrix of cartilage, with studies showing enhanced proteoglycan synthesis and cell proliferation in equine models, but resulting in inflammation and osteophyte formation in rabbit stifles. It has become better understood that growth factors work synergistically to enhance cartilage matrix synthesis and modulate their regulatory functions. Based on the concept that a combination of bioactive growth factors is likely necessary for cartilage repair, recent attention has been focused on PRP, autologous conditioned serum (ACS) and bone marrow concentrate (BMC). A further advantage of PRP and BMC is that on clotting, they form 3-D scaffolds to fill the cartilage defect and act as a guide for neochondrogenesis in situ.

1.9.4. Treatment Options for Osteoarthritis

As there is no current known cure for OA, the main treatment goal is to alleviate pain in order to gain strength and mobility, and to delay the progression of the disease. The treatment is often described as multimodal – this relies on a combination of physical therapy, anti-inflammatory medications, analgesics, nutraceuticals and alternative therapies. However, treatment is mainly palliative providing variable symptomatic relief. Surgical intervention, such as microfracture, osteochondral auto- or allograft, to total or partial joint replacement, rarely restores full function. Traditional use of viscosupplementations, such as intra-articular hyaluronic acid (HA), polysulfated glycosaminoglycans, oral glucosamine, and chondroitin sulfate is also seen as palliative. Intra-articular hyaluronic acid achieved 6 months of symptom control in human knee OA. Franklin et al assessed hyaluronan with corticosteroids
compared to autologous conditioned plasma in canine elbow OA and found no difference between the groups for up to 6 months following treatment. The use of HA in canine knee OA did not find any difference in synovial fluid composition weather treated with HA or saline. A review of clinical trials available in the use of hyaluronan for dog OA, found an extremely low level of comfort supporting its use. The use of oral glucosamine/chondroitin sulfate has shown conflicting results with positive clinical effects compared to carprofen, in contrast, other authors have found no significant difference in arthritic dogs supplemented with glucosamine and chondroitin compared to controls.

The widespread use of non-steroidal anti-inflammatories as an analgesic for canine OA has shown a moderate to high level of comfort across multiple clinical trials. Non-steroidal anti-inflammatory drugs (NSAIDs) decrease prostaglandin synthesis by inhibiting the cyclooxygenase (COX) enzymes that are active in arachidonic acid metabolism. However, their major weakness is adverse effects/toxicity and an inability to slow down the progression of osteoarthritis. NSAIDs that selectively inhibit COX2 and spare COX1 receptors will allow analgesia whilst reducing the common side effects of COX1 inhibition, which includes altered gastrointestinal and thrombocyte function. However, a systematic review of NSAIDs in dogs found adverse effects in 55% of studies, 5% with adverse effects of ≥ 80%, and other studies that did not find statistical differences between treated and control dogs. The potential for long term adverse effects of NSAIDs and inconsistencies of nutraceuticals has therefore led to an increase in current research efforts towards ‘biotherapeutics’ for restoring metabolic balance within the capsular joint. These treatment options include cytokine inhibitors, gene therapy and growth factors to potentially preserve normal joint homeostasis or reverse structural damage in degenerative joints.
The use of bioactive agents has been of recent interest, including the use of intra-articular PRP for management of osteoarthritis. The rationale is that the multifaceted roles of PRP are contributed by a wide variety of growth factors that can enhance anabolic, bone remodeling, proliferation, angiogenesis, inflammation, coagulation and cell differentiation. Platelets can be activated by both native and exogenous molecules including, calcium, thrombin, ADP, collagen and magnesium to release an initial burst of growth factors (GF) followed by a sustained release. Platelet activation can also increase the inflammatory cytokines due to the presence of hepatocyte GF. This GF stimulates cartilage matrix synthesis, and affects catabolic cytokines such as IL-1, IL-4, IGF-1, osteogenetic protein and functions in bone remodeling. There are numerous anabolic growth factors in PRP that stimulate chondrocyte synthesis of proteoglycans, aggrecan, type II collagen; induce synoviocyte and mesenchymal stem cell proliferation; drive chondrogenic differentiation of MSCs; and decrease the catabolic effects of cytokines such as IL-1 and MMPs.

The application of PRP in cartilage repair is an emerging field in the use of protein biotherapeutics for restoring metabolic balance within the capsular joint. PRP may also influence overall joint homeostasis, reducing synovial membrane hyperplasia and modulating cytokine levels, thus leading to an improvement in the clinical outcome, even if only temporarily, without affecting cartilage tissue structure and joint degenerative progression. The use of intra-articular PRP at 3 weekly injections in 40 human patients with hip OA showed 57.5% had reduced pain with 40% excellent response sustained to 6 months. Other authors have also shown decreased pain levels from 6-12 months after intra-articular injection of PRP into OA knees. On comparison of the use of PRP versus a viscoelastic substance such as hyaluronic acid, PRP had improved clinical performance and reduction in pain in the
PRP treated group for patients with knee OA. A recent publication assessing the use of autologous platelet concentrate for single intra-articular injection of OA in dogs showed a significant improvement in lameness score, pain score and peak vertical force (PVF). This was supported by Vilar et al that single intra-articular injection of platelet growth factor (with adipose mesenchymal stem cells) decreased pain, and improved PVF and vertical impulse compared to controls. Another pilot study used autologous platelet concentrate to improve functional outcome after intra-articular cranial cruciate ligament repair and found that PVF was equal to the contralateral limbs compared to controls on the 90th day. However, the effect of platelet concentrates warrants additional studies as Murray et al did not detect biomechanical improvement in the use of intra-articular PRP post cranial cruciate ligament repair in porcine models. Autologous platelet concentrates were also injected into horses with OA and found significantly improved lameness and joint effusion, with most marked improvement at 2 months post-treatment and persisting for up to 8 months. Osteoarthritis management is therefore focused on controlling pain, improving joint function and slowing the degenerative process within the joint.

1.10. Canine Coxofemoral Osteoarthritis (Hip Dysplasia)

Osteoarthritis is one of the leading causes of death or reason for euthanasia attributed to degenerative joint disease, affecting up to 20% in military working dogs. OA of the coxofemoral joint secondary to hip dysplasia is particularly common, with the prevalence of hip dysplasia (HD) ranging from 41 to 73% within the Labrador and Rottweiler population alone. A meta-analysis of the prevalence of inherited disorders found that hip dysplasia was 7%
amongst pure-bred and mixed breeds. Several risk factors have been evaluated attributing to the pathogenesis of OA, one of which is excessive body weight leading to increased stress on joints inducing the transformation of passive hip joint laxity to functional hip joint laxity. In a landmark study of littermate hip-dysplasia predisposed Labrador retrievers, the onset of OA was delayed among restricted-fed dogs, compared to control-fed dogs; with 25% control-fed dogs having radiographic evidence of hip OA at 2 years, compared to 5% in restricted-fed dogs. Radiographic and clinical evidence of coxofemoral OA is a common sequel of canine hip dysplasia, especially in older dogs.

To date, there are two published studies on the use of PRP as a treatment option for osteoarthritis in dogs. One assessed the use of intra-articular platelet therapy in single joints affected with osteoarthritis (excluding hips), the other, combined platelet therapy with adipose-derived mesenchymal stem cells (AD-MSC) for hip OA. The use of autologous PRP for treatment of human patients with OA of the hip resulted in pain relief and improved function for up to 6 months. A recent review on the clinical application of intra-articular PRP showed an overall support that PRP does not just target cartilage, but that it can also influence the entire joint environment, leading to a short-term clinical improvement. There are currently no published studies on PRP as a treatment option for coxofemoral OA in the dog. Chapter III details an undergoing preliminary non-controlled prospective study at Oregon State University Veterinary Teaching Hospital, assessing the outcome and efficacy of a single intra-articular PRP injection for adult dogs affected with coxofemoral OA.
Chapter II

Single ultrasound-guided platelet-rich plasma injection for treatment of supraspinatus tendinopathy in dogs

2.1. Abstract:

The effect of a single platelet-rich plasma (PRP) injection on gait, owner perceived outcome and ultrasonographic tendon defects in dogs with supraspinatus tendinopathy (ST) was determined in a prospective, pilot study of 10 dogs. Pressure mat gait analysis, ultrasound appearance, and canine brief pain inventory score data were collected pre- and post-treatment (2, 6 and > 16 weeks). PRP platelet concentration from the Harvest SmartPReP® system ranged from 5.2 to 8-fold greater than peripheral blood. Ultrasound showed overall improvement in heterogeneity and echogenicity in 60% of dogs. Vertical impulse, stride length, and stance time did not change following PRP injection. Despite an initial improvement, peak vertical force (PVF) significantly declined 16-weeks following injection (p=0.022, repeated measures ANOVA). Single PRP injection may transiently improve lameness in dogs with ST, however, lameness and declining PVF occurred by 16 weeks following injection. Single PRP injection for ST cannot be recommended in dogs.
2.2. Background

Supraspinatus tendinopathy (ST) is characterized by degeneration of the tendon fibers and occurs in young to middle aged, large breed dogs with no sex predilection (1-3). Clinical signs, when present, may include intermittent weight-bearing lameness that worsens after exercise and is refractory to rest and non-steroidal anti-inflammatories (NSAIDs) (1, 4, 5). The etiology of ST remains unknown but may involve overuse injury as a result of repetitive stress loading, traumatic fiber tears, and chronic inflammatory fibrosis (6-8). Following resolution of lameness, there is a lifelong increased susceptibility to re-injury due to the reduced strength of fibrotic scar tissue compared to the original tendon (9). Magnetic resonance imaging (MRI) is the gold standard for ST diagnosis in dogs and humans, but radiology and computed tomography (CT) can identify mineralized ST lesions and ultrasound is both sensitive and accurate in identifying ST in dogs with or without the presence of mineralization (2, 4, 10, 11).

Surgical management (mineralized foci/tendon resection, transverse humeral ligament release) of ST results in excellent functional outcome in up to 50% of non-mineralized-ST and 64% of mineralized ST (1, 4, 12). Medical management of ST includes rest, analgesics, physical therapy, steroid injection, and extracorporeal shock wave therapy (10). Unfortunately, persistence or recurrence of lameness has been reported in 55% of surgically-treated dogs and in up to 33% of non-surgically treated dogs (3-5, 13). Autologous platelet concentrate or platelet-rich plasma (PRP) used in human tendonopathies has demonstrated improved recovery, earlier range of motion, earlier return to activity and reduced pain (14-16). PRP is rich in growth factors such as platelet derived growth factor, vascular endothelial growth factor, transforming growth factor-β, and fibroblast growth factor which stimulate primary tendon healing (17, 18). In
horses, PRP treated tendon lesions had improved function, higher collagen, glycosaminoglycan and cellularity than controls (19-21).

The purpose of the study reported here was to assess the short-term efficacy of single intralesional PRP treatment using kinetic gait analysis and owner assessment for the management of ST. Our hypothesis was that ultrasonographic features and clinical lameness in dogs with ST are improved following single autologous intralesional PRP injection.

2.3. Materials and Methods

A prospective, pilot study (approved by the Institution’s Animal Care and Use Committee) was performed on 10 client-owned dogs presented for thoracic limb lameness and diagnosed with ST from January 2011 to October 2013 at Oregon State University Veterinary Teaching Hospital. Dogs were excluded if they had concomitant ipsilateral thoracic limb pathology (elbow dysplasia, medial shoulder instability, osteochondrosis or previous surgery of the forelimb) diagnosed either with radiographs, CT or MRI. Dogs were examined and the lameness graded as: grade 0 – no detectable gait abnormalities, grade 1 – subtle lameness only at a trot, grade 2 – subtle lameness at a walk, obvious at trot; grade 3 – obvious lameness at walk or trot; grade 4 – non-weight-bearing lameness (2,4). All dogs underwent ultrasound examination of the shoulder, with or without additional CT, radiographs, arthroscopy or MRI. Pre- and post-treatment pressure platform gait analysis was performed (2 meter, High Resolution Mat, Tekscan, Inc., San Diego, CA) with each dog walked at a velocity of 1 to 1.5m/s with an acceleration tolerance of ± 0.5 m/s. Data for at least 3-5 valid trials were collected at each time point and peak vertical force (PVF, % body weight), vertical impulse (VI, %body
weight*seconds), stride length (SL, centimeters), and stance time (StT, seconds) were
determined. Autologous PRP was prepared using a commercially available Harvest SmartPReP
unit (Harvest Technologies Corp., Plymouth, MA) (22) and the PRP injected into the ST lesion
under ultrasound guidance. Owners were instructed to rest their dogs for 6 weeks following
injection. Objective data (PVF, VI, SL, StT, ultrasonographic lesion size and appearance), and
subjective data (lameness, Canine Brief Pain Inventory, University of Pennsylvania) (23, 24),
were performed before and at 2, 6 and >16 weeks post-injection. All analyses of outcome
measures, including gait, CBPI, and physical examination, were evaluated separately for dogs
with unilateral versus bilateral ST.

All dogs were sedated with dexmedetomidine (5μg/kg intravenously) and butorphanol
(0.2mg/kg intravenously) for ultrasound, blood collection for PRP and ultrasound-guided
injection, then reversed with atipamezole (50μg/kg intramuscularly). Tramadol at 3mg/kg q8hrs
by mouth was prescribed for 5 days post-injection. NSAIDs, steroids and joint supplements were
prohibited during the study period (6 months). Using sterile technique, 30 milliliters (mls) of
venous blood (60 mls in bilateral dogs) was collected and immediately processed for PRP (22).
The processed PRP concentrate (3.0 mls) was immediately, steriley injected into each lesion
under ultrasound guidance.

Ultrasound examinations were performed with a linear broadband transducer (12-
14MHz), using a Phillips IU22 ultrasound machine (Philips Healthcare, Andover, MA) with the
dog in lateral or dorsal recumbency with mild supination of the elbow. The supraspinatus was
evaluated from proximal until its insertion at the greater tubercle of the humerus, and the biceps
tendon from the origin at the supraglenoid tubercle to the muscular belly using a parasagittal
plane and in both long and short axes. The supraspinatus tendon, biceps tendon, bicipital bursa,
greater tubercle of the humerus, infraspinatus tendon and articular surfaces were and assessed for echogenicity, heterogeneity, size and appearance. PRP was injected intralesionally within the supraspinatus tendon lesion until high resistance was reached (using a 22-gauge needle, 1.5-inch length), with remaining PRP sample injected into the bicipital bursa and glenohumeral joint.

**Statistical Analysis**

Pressure mat data were analyzed using the statistical programming environment ‘R’. Data sets from pressure mat studies were tested for normality with the Shapiro-Wilks test. Data were first analyzed using mixed linear models to assess the effects of treatment (affected versus unaffected limb), time post-treatment, and the interaction (if any) between treatment and time.

Following initial analysis using mixed linear models, repeated measures ANOVA followed by Tukey’s Honest Significant Difference Test was used to assess between-time point changes for Peak Vertical Force. Stride Length Difference was inconsistent with a normal distribution, thus a nonparametric method (Friedman Test followed by Wilcoxon Paired Ranks test with Bonferroni correction) was used assess the effects of time on this parameter. Log transformation was used to achieve a normal distribution of data for Stride Length and Stance Time in the forelimbs. Data for lameness score, ultrasound lesion size and CBPI were analyzed using statistical software (Prism 5.0, GraphPad Software, Inc., La Jolla, Ca) to test for normality with the D’Agostino & Pearson omnibus normality test. Data were then analyzed with a repeated measures ANOVA followed by Dunnett’s Multiple Comparison post-test to compare time points to pretreatment data. For all statistical analyses, a calculated value of P<0.05 was set as the threshold for statistical significance.
2.4. Results

Ten dogs met the inclusion criteria. The breeds in the study population were Labrador (n=3), Boxer (n=2), English Mastiff, whippet, Golden Retriever, Belgian sheepdog and Borzoi. Sex distribution included intact male (n=3), intact female (n=1), neutered male (n=3), spayed female (n=3). The median age was 4 years (mean 5.3, range 2-12) and body weight was 33kgs (mean 35.3, range 13.5-80.5, Table 3). The duration of forelimb lameness prior to PRP ranged from 4-60 months (median 7.5, mean 12.9 ± 16.8). Previously prescribed treatments included: rest, NSAIDs; analgesics (tramadol 2/10 dogs, gabapentin 2/10 dogs); rehabilitation (laser therapy 1/10 dogs, prolotherapy 1/10 dogs, hydrotherapy 1/10 dogs); shoulder intra-articular injection (depomedrol 1/10 dogs) and nutraceuticals (glucosamine 2/10 dogs, fish oil 1/10 dogs, Adequan 1/10 dogs).

In a pilot study (n=4), PRP samples were compared to corresponding peripheral blood in healthy dogs. Platelet count was 5.2 to 8 times above peripheral blood (mean 6.82 ± 1.09). White blood cell count (WBC) was 1.8 to 5.5 times greater than peripheral blood (mean 3.72 ± 1.49), similar to the manufacturer’s reports of 8.2 ± 2.3-fold platelet concentration and 4.0 ± 1.2-fold WBC concentration.

The median severity of lameness at presentation was 2/4 with 4 right forelimb lamenesses, 3 left, and 3 bilateral. Initial examination identified pain on limb manipulation (n=4), pain on extension (n=5), flexion (n=3), and muscle atrophy over the scapula (n=3). Diagnosis of ST by ultrasound identified 2 tendons on the right, 3 on the left, and 5 bilateral ST. Ultrasound and radiographs were performed in all dogs, with additional MRI performed in 2 dogs. In total, 15 supraspinatus tendons were treated with PRP injection (5 unilateral, 5
bilateral). Pre-treatment ultrasound revealed disruption of normal fiber patterns of the supraspinatus muscle with a collection of hyperechoic core lesions (n=4/15), hypoechoic regions (8/15), and areas of mixed echogenicity (n=3/15). All dogs included in the study had minimal to no ST mineralization (lesions with > 1mm diameter mineral were excluded). Multiple pinpoint hyperechoic areas within the ST were present in 5 tendons. Areas of ‘core’ lesions (hypo- to anechoic heterogeneous areas within the supraspinatus muscle or at its insertion) were seen in 13/15 tendons. No statistically significant improvement in lesion size was seen following PRP treatment (P>0.05). Ultrasonographic resolution of lesions within the supraspinatus tendon was seen at 2weeks (n=1/15), 6 weeks (n=4/15) and 8 months (n=1/15). At final evaluation, only 2 previously resolved core lesions remained healed (homogenous tendon, unable to detect disrupted tendon fibers). Overall improvement in heterogeneity and echogenicity were seen in 9/15 tendons. There was a 50% reduction in core lesion size in 1/15, slight reduction (within 0.2cm) or similar in size in 5/15 tendons and increased core lesion size in 6/15 tendons at >16-weeks.

Involvement of the biceps tendon occurred in 4 joints (with hypoechoic core lesions 3/15, hyperechoic areas 1/15) with resolution of biceps core lesions seen in 2 dogs at final examination. Six dogs were diagnosed at the final evaluation with: medial shoulder instability (1 dog), tear of biceps tendon (1 dog), and continuation of ST (4 dogs). Ultrasonographic resolution of core lesions within the supraspinatus tendon was documented in only 5 ST at last follow-up. MRI findings were comparable to ultrasound findings, except for joint capsule thickening as well as infraspinatus and subscapularis muscle architecture, which were better appreciated on MRI than ultrasound imaging.

Gait data is reported as the mean ± standard deviation. For all dogs, gait velocity was not
significantly different between dogs or over time. Mean absolute difference in velocity between pre-treatment to 6-weeks: 0.62m/s (median 0.16m/s), and pre-treatment to final evaluation: 0.42m/s (median 0.55m/s, P>0.05). Gait data was lost in 4 dogs at final follow-up at >16 weeks (2 unilaterally and 2 bilaterally affected dogs).

In unilaterally affected dogs, PVF was significantly less in the affected limb compared to the contralateral limb (p=0.0019) pre-treatment, and in affected limbs, decreased after PRP injection over time (p=0.0222, repeated measures ANOVA followed by Tukey’s Honest Significant Difference Test, Figure 1). Affected limb PVF pre-injection (% body weight) was 57.4 ± 9.8, at two weeks post was 25.6 ± 13.5, and at >16 weeks post was 44.7 ± 41.6. In the unaffected limb PVF was 57.8 ±13.8, at two weeks post was 59.3 ± 11.6, and at >16 weeks post was 60.9 ± 5.7.

VI (%body weight*sec) differed significantly between limbs (affected versus unaffected, p=0.00047) and as a result of time (p=0.0050), however there was no significant interaction between time and treatment. VI was significantly lower in the affected limb (pre-injection: 18.5 ± 7.53, 2 weeks post: 16.6 ± 5.54, >16 weeks post: 12.47 ± 11.00)) compared to the unaffected limb (pre-injection 20.8 ± 6.69, 2 weeks post: 16.9 ± 2.95, >16 weeks post: 19.0 ± 1.75, Figure 2).

Stride length (centimeters) was variable between dogs due to the size variation in the study population. In the affected limb, SL at pre-injection was 67.6 ± 14.8, 2 weeks post: 67.6 ± 13.2, and >16 weeks post was 70.8 ± 11.5. In the unaffected limb SL was 71.7 ± 21.0 pre-injection, 63.2 ± 15.4 at 2 weeks post, and 67.2 ± 11.2 at >16 weeks post-injection. SL was log-transformed before analysis to achieve normality and there was no significant difference between
limbs, or effect of time, on log SL (two way, repeated measures ANOVA). The difference in SL was calculated as a percentage of length (affected)/length (unaffected) to account for the variable-sized dogs in the study. SL difference (%) showed no significant effect of time (Friedman Test, p=0.4303).

Stance time (seconds) in the affected limb was 0.47 ± 0.14 at pre-injection, 0.41 ± 0.12 at 2 weeks post, and 0.42 ± 0.37 >16 weeks following injection. In the unaffected limb StT pre-injection was 0.48 ± 0.13, at 2 weeks post 0.40 ± 0.09, and at >16 weeks post was 0.43 ± 0.09. StT was log-transformed before analysis to achieve normality. There was no significant difference between limbs, or effect of time, on log StT (Two Way, Repeated Measures ANOVA, p>0.05).

Bilaterally affected dogs (n=5) showed no significant improvement in PVF at 2 weeks following PRP treatment (54.5% ± 4%), 6 weeks (52.1% ± 3.1%) or >16 weeks (56.5% ± 4.6%) compared to pre-treatment (52.6% ± 3.3%, P>0.05). The VI at pre-treatment (18.7% ± 1.7%) was also not significantly different from any subsequent gait evaluations (2 weeks post: 16.6% ± 0.7%; 6 weeks post: 20.5% ± 2.5%, final evaluation 18.9% ± 2.1%, P>0.05). At 2 weeks following bilateral PRP treatment, SL increased slightly but not significantly (mean 91.9cm ± 9.6cm, versus pre-treatment 88cm ± 4.2cm) and StT remained unchanged (mean 0.45secs ± 0.01secs, versus pre-treatment 0.47secs ± 0.02secs).

Clinical signs of lameness (researcher assessment) resolved by 6 weeks in 3 bilaterally affected and 1 unilaterally affected dog. There was a mean lameness score of 2.2/4 at pre-treatment, 1.6/4 at 2 weeks post, 0.9/4 at 6 weeks post and 0.9/4 at >16 weeks post-treatment (P<0.05). Pain severity score improved significantly between pre-treatment and at final follow-
up (P= 0.03). Owner assessment from the CBPI showed the baseline pain severity score decreased from 5.08±0.49 at pre-treatment to 3.2 ± 3.2 at 6 weeks evaluation, and 0.84 ± 1.89 at >16-weeks. The baseline pain interference score decreased from 6.58 ± 1.36 at pre-treatment to 6.2 ±4.5 at 6 weeks post-treatment, and 1.03 ± 2.14 at final follow-up (P= 0.035). At the final follow-up, three dogs had an ‘excellent’ and 1 dog had a ‘very good’ overall quality of life.

2.5. Discussion

The findings of this pilot study showed that a single PRP application into supraspinatus tendinopathy lesions may have very mild positive clinical improvement in the short-term (<6wks), but few long-term positive effects. PRP has been used commercially to treat knee injuries, tendon and muscle injuries, osteoarthritis and chondropathies in human and equine medicine with some success (17, 18). It is important to note that all dogs in this report had a chronic lameness ranging from 4 months to 5 years that was refractory to conservative management prior to presentation, with 4/10 (40%) dogs having clinically resolved lameness by 6 weeks and with 5/10 (50%) returning to normal function and activity levels by their final evaluation greater than 16 weeks later. The effectiveness of PRP in the management of chronic tendon injuries in dogs is unknown. PRP may have been more effective if it had been administered during the acute phase of tendon injury as with Achilles tendon injuries in humans (25-27). There were 6 dogs that had persistent lameness despite treatment and in the unilaterally affected dogs, PVF actually worsened over time; hence the authors reject the null hypothesis that a single injection of PRP can consistently improve clinical lameness in dogs with ST. Similar to
this report, meta-analysis of the literature in humans has provided insufficient evidence to support PRP use in musculoskeletal soft tissue injuries (28).

Further research is required with a larger cohort of patients, comparing different PRP systems, and with multiple administrations of different platelet concentrations to determine the efficacy and protocol of PRP intralesional injection that would definitively characterize clinical outcome in dogs with ST. Although single PRP injections are used in human tendonopathies as an augmentation to surgical repair, other studies used a series of injections (29, 30). The treatment of ST with intralesional PRP injection alone in dogs has not been reported prior to this study to the authors’ knowledge. PRP has been used in combination with adipose derived cultured cells and found to improve lameness and ultrasonographic lesion size in dogs with unilateral ST (31). The addition of progenitor cells to PRP may be required in dogs in order to stimulate regeneration of the tendon tissue. Further work with prospective, placebo controlled clinical trials is warranted. The most effective concentration of platelets administered intralesionally in tendons in dogs has also not been definitively determined. In this study, the concentration of platelets was 5 to 8 fold greater than peripheral blood, which may have not been an ideal concentration. The literature reports variable platelet concentrations in PRP, however, many studies state that platelet concentration for a PRP sample should be 200% (32) or 3 to 5 fold greater than baseline peripheral platelet count (33-36).

The effect of WBCs and their concentration in the PRP may also have affected the outcome in this study. Platelet and WBC counts were performed in only 4 dogs, however the researchers have previously determined that the system used in all dogs in this report have consistent results in platelet concentrate and WBC concentration within manufacturer’s guidelines (unpublished data). The role of leukocyte activity within PRP is controversial. The
initial suppression of macrophage activity by leukocytes may prevent excessive early inflammation that can lead to dense scar tissue formation, and leukocyte-reduced PRP may be optimal for superior healing of tendons without scar tissue formation (18, 37).

Diagnostic ultrasound has been reported as an excellent technique to evaluate calcifying and non-calcifying ST in dogs (2). ‘Core’ lesions on ultrasound were visualized as ovoid or round, ill- or irregularly delineated heterogenic structures within the tendon itself. Hypoechoic areas mottled within the lesion or the musculature may indicate inflammation or effusion from acute tendonitis (2). Hyperechoic regions, as pinpoint or larger structures within the tendon, were observed as dystrophic mineralization or fibrosis. Ideally, all dogs would have had a MRI of both glenohumeral joints and surrounding structures (gold standard for ST diagnosis) as well as arthroscopy of the shoulders and elbows to rule out any underlying causes of lameness (38). Radiographs, CT or MRI of the shoulders and elbows were performed in all dogs in this report and any dogs with underlying shoulder joint or elbow joint pathology were excluded from the study.

A significant limitation of this study is the small sample size and lack of control group. There were no owners of affected dogs willing to enroll in a blinded, placebo-controlled clinical trial, however, such a study would be warranted. It is interesting to note that owners’ perception of pain severity and pain interference to quality of life differed from the recorded objective outcome measures. Improvement in functional lameness was observed in all dogs, however, gait and ultrasound lesion size did not significantly change following PRP injection. In fact, PVF and VI declined by the >16 week evaluation in unilaterally affected dogs, likely indicating progression of the ST in many dogs. Up to 39.7% and 44.8% of owner and veterinarian bias,
respectively, may be due to a placebo effect, therefore interpretation of these results in such a
small cohort of dogs must be interpreted with caution (39).

The results reported here indicate that single injection of autologous PRP in dogs with ST
does not resolve pathologic ultrasound features or overall clinical lameness. However, the use of
ultrasound for diagnosis of ST and for guidance of intralesional injection is feasible and
effective.

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Chapter III

Short-Term Outcomes Following an Intra-articular Autologous Platelet-Rich Plasma Injection in Adult Dogs with Coxofemoral Osteoarthritis

3.1. Abstract

The effect of a single platelet-rich plasma (PRP) injection on gait, owner perceived outcome and radiographic findings in dogs with coxofemoral osteoarthritis (OA) was determined in a prospective, pilot study of 10 dogs. Pressure mat gait analysis, radiographic OA score, accelerometry counts, goniometer measurements and canine brief pain inventory score data were collected pre- and post-treatment (2 and 6 weeks). Both subjective and objective data showed a lack of significant findings (P>0.05). Larger case numbers with longer follow-up times are required to determine if single intra-articular PRP injection is a viable treatment option of coxofemoral OA in dogs.

3.2. Background

Osteoarthritis (OA) of the coxofemoral joint is a significant and debilitating cause of lameness and chronic pain in adult dogs (1). Management of primary or secondary OA involves a multimodal approach oriented toward alleviating joint pain, improving joint health and slow its disease progression. Several recent reviews have focused on the efficacy of different therapies used in the management of OA in dogs, with agreement that restoration of diseased articular cartilage remains a challenge for researchers and clinicians (2-4). Current research investigating
different methods in stimulating repair of damaged cartilage is an emerging field in both human and veterinary medicine (4-8). Platelet rich plasma (PRP) is one such ‘biotherapeutic’ method aimed to regenerate progressive loss of cartilage with widespread application in multiple disciplines including musculotendinous injuries, maxillofacial surgeries, skin wounds and joint repair (9-11).

PRP has been promoted to enhance joint homeostasis, reduce synovial membrane hyperplasia and modulate synovial fluid cytokine levels (12). It is defined as a portion of the plasma fraction of autologous blood with a platelet concentration above baseline (9, 13). Platelets contain alpha granules that release growth factors and cytokines to stimulate extracellular matrix gene expression and inhibit metalloproteinase activity, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β); vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) (14). Synovial fluid in chronic OA joints have elevated levels of interleukin-1β (IL-1β), prostaglandin E2 (PGE-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) (7). These pro-inflammatory cytokines and tissue inhibitors contribute to cartilage destruction by inhibiting chondrocyte proteoglycan and synovial fluid synthesis. The rationale for the use of PRP is to stimulate collagen deposition, promoting angiogenesis and extracellular matrix synthesis by a ‘supra-physiological’ release of PDGFs directly at the site of treatment (15).

The use of intra-articular PRP in human and equine OA has shown to reduce pain and improve function sustained up to 6 months (8, 16, 17). A combination of autologous platelet concentrate and adipose-derived mesenchymal stem cells was injected into arthritic joints in 8 dogs with significant improvement in ground reaction forces by 6 months (4). Fahie et al demonstrated the use of intra-articular autologous platelet concentrate injection for OA in dogs
had a significant improvement in lameness score, pain score and peak vertical force (5). There is currently no published report of the efficacy of PRP in dogs affected with coxofemoral OA. The hypothesis of this study was that adult dogs with coxofemoral OA treated with single intra-articular autologous PRP will have improved objective measurements of lameness (radiography, goniometry, accelerometry and kinetic gait analysis) and improved subjective measurements of lameness (questionnaire and visual lameness score).

3.3. Materials and Methods

Dogs with osteoarthritis of the hip joint were enrolled in a prospective, pilot clinical trial. The study was approved by the Institutional Animal Care and Use Committee (IACUC). Ten client-owned dogs with a history of lameness attributable to coxofemoral OA were examined at Oregon State University Veterinary Teaching Hospital. Dogs were enrolled into the study if they met the following criteria: more than 18 months of age and over 15 kilograms, radiographic evidence of coxofemoral OA, lameness attributable to the hip and no previous history of joint, muscle, tendon or ligament disease or injury of either pelvic limbs. Dogs were excluded from the study if they were unable to discontinue the use of non-steroidal anti-inflammatories (NSAIDs), use of immunomodulatory drugs for co-existing conditions, active infection, malignancy or inability to return for follow-up evaluations. All dogs had to discontinue use of NSAIDs, nutraceuticals and dietary supplements at least 1 week prior to enrolment.

Dogs fitting the inclusion criteria were enrolled in the study. An initial orthopedic and neurologic examination, goniometry measurements, gait analysis and standard two-view radiographs of the pelvis were performed. In addition, a canine brief pain inventory
questionnaire (CBPI, University of Pennsylvania) (18, 19), lameness score and surgeon evaluation score were obtained.

Dogs were sedated for PRP harvest and injected steriley into the affected hip joint under ultrasound guidance by a board-certified radiologist. If dogs were bilaterally affected, the limb that was more clinically lame or had worse gait parameters was selected for treatment. Complete blood count was performed on the patient’s peripheral blood and the PRP sample. Follow-up examinations were performed at 2 and 6 weeks post-procedure. Each follow-up evaluation underwent gait analysis, goniometry measurements, accelerometer data collection, CBPI questionnaire, lameness score and surgeon evaluation. Repeat pelvic radiographs were performed at the 6 week evaluation. The contralateral coxofemoral joint was treated with intra-articular PRP at the end of the 6 week study period depending on owner preference. A rest protocol for 6 weeks was prescribed post-procedure which included leash walks only up to 5 minutes and restriction from jumping, running or excessive play.

Hip range of motion measurements were performed using a transparent plastic goniometer with 1 degree increments centered over the axis of rotation of the joint. The fixed arm of the goniometer was placed on a line from the tuber sacrale to the ischiatic tuberosity, with the mobile arm placed along a line between greater trochanter and lateral femoral condyle (20). The angle at maximum flexion and extension was recorded on both hips whilst the patient was awake.

Each dog was walked across a 2 meter-long pressure platform system (High Resolution Mat, Tekscan, Inc., San Diego, CA). Gait analysis determined peak vertical force (PVF, % body weight, BW), vertical impulse (VI, %BW*seconds), stride length (SL, distance from paw strike
to paw strike of same leg, centimeters) and stance time (StT, seconds). Data for at least 3 valid trials were collected at each time point.

Dogs were sedated with dexmedetomidine (5μg/kg intravenously) and butorphanol (0.2mg/kg intravenously) for pelvic radiographs (all time points), blood collection for PRP and ultrasound-guided injection (first time point only). Atipamezole (50μg/kg intramuscularly) was given for reversal of sedation post procedure. Tramadol at 3mg/kg q8hrs was prescribed for 1 week beginning the day following PRP injection, with usage thereafter as needed.

Radiographs were performed in hip extended ventrodorsal and lateral recumbency at pre- and 6 week post-treatment in accordance with the guidelines stated by the Orthopedic Foundation for Animals (OFA). Digital radiographs were performed using either a Bennett x-ray machine (450mA, 125kVp) or Sedecal x-ray machine (20mA, 80kVp). Radiographic interpretation and OA severity grading was performed by a board-certified radiologist using the OFA grading scheme.(21, 22) Ultrasound examinations were performed with a linear broadband transducer (12-14MHz), using a Phillips IU22 ultrasound machine (Philips Healthcare, Andover, MA). The lateral or medial aspect of the affected coxofemoral joint space was visualized under radiologist preference for intra-articular injection of autologous PRP under sedation.

PRP was harvested from 30 milliliters of autologous venous blood from a jugular venipuncture using the Harvest SmartPReP unit system (Harvest Technologies Corp., Plymouth, MA). All preparation was performed under manufacturer’s instructions. In brief, 1ml of anticoagulant-citrate-dextrose solution, solution A, USP 2.13% free citrate ion (ACD-A) was prepared in the sample syringe, with 3mls ACD-A prepared in a separation container provided by the Harvest SmartPReP kit. A sterile venipuncture sample was transferred to the separation
container for centrifugation. After processing, the platelet-poor plasma (PPP) was retrieved and discarded. The processed PRP concentrate (3mls) was collected from the chamber unit, and immediately administered under ultrasound guidance in a sterile manner.

The Actigraph accelerometer (Actigraph GT1M, ActiGraph LLC, Pensacola, FL, USA) was attached to the dorsal aspect of each dog’s harness starting the day of treatment. The device was worn for the full 6 week period. The ActiGraph is a small (3.8 x 3.7 x 1.8 cm), light weight (27 g) accelerometer is digitized at a rate of 30 Hz and integrated over a user-specified interval (epoch). At the end of each epoch, the summed value or “activity count” is recorded, which is used to estimate physical activity over time. Accelerometer records identified periods of when the patient was sleeping (sedentary), light-moderate intensity physical activity (PA), and time in vigorous intensity PA (23).

**Statistical Analysis**

Pressure mat data were analyzed using the statistical programming environment ‘R’. Data sets from pressure mat studies were tested for normality with the Shapiro-Wilks test. Data were first analyzed using mixed linear models to assess the effects of treatment (affected versus unaffected limb), time post-treatment, and the interaction (if any) between treatment and time.

Following initial analysis using mixed linear models, repeated measures ANOVA followed by Tukey’s Honest Significant Difference Test was used to assess between-time point changes for Peak Vertical Force. Stride Length Difference was inconsistent with a normal distribution, thus a nonparametric method (Friedman Test followed by Wilcoxon Paired Ranks test with Bonferroni correction) was used assess the effects of time on this parameter. Log transformation was used to achieve a normal distribution of data for Stride Length and Stance
Time in the forelimbs. Data for lameness score, accelerometry counts, goniometry measurements and CBPI were analyzed using statistical software (Prism 5.0, GraphPad Software, Inc., La Jolla, Ca) to test for normality with the D’Agostino & Pearson omnibus normality test. Data were then analyzed with a repeated measures ANOVA followed by Dunnett’s Multiple Comparison post-test to compare time points to pretreatment data. For all statistical analyses, a calculated value of P<0.05 was set as the threshold for statistical significance.

3.4. Results

Ten dogs were enrolled that fitted the inclusion criteria, however one dog lacked a six week follow-up and was excluded from the study. Breeds included the Border Collie (n=3), Labrador (n=1), Siberian Husky (n=1), Great Pyrenees (n=1), Mastiff (n=1), German Shepherd (n=1) and Chow mix (n=1). Dogs ranged from a bodyweight of 18.5kg to 53.2kg (mean 31.8kg), with age ranged from 5 to 11years (mean 6.9years). Sex distribution included female spayed (5), male neutered (4) with a body condition score range from 4/9 to 6/9 (Purina Body Condition Score).

The most common clinical presentation was intermittent hindlimb lameness ranging from one to more than 2 years. All dogs presented due to acute worsening of hindlimb lameness over the last 2-3 months (n=9), specifically refusing to jump (n=1) and lameness persisting for longer periods of time after exercise or play (n=2). Previous treatments included glucosamine/chondroitin (n=4), omega 3 fatty acids (n=2); elk velvet antler (n=1); gabapentin
(n=1); non-steroidal anti-inflammatory (n=4), and rest (n=2). Lameness was reported to attribute to right (n=6) and left (n=3) hindlimb.

Grading of pelvic radiographs was performed by a board-certified radiologist blinded to each image. Grading of hip conformations were based on 9 anatomic areas of the hip as dictated by the Orthopedic Foundation for Animals (OFA) phenotypic grading categories: craniolateral acetabular rim, cranial acetabular margin, femoral head, fovea capitus, acetabular notch, caudal acetabular rim, dorsal acetabular margin, junction of femoral head and neck, and trochanteric fossa. Pre-treatment radiographs were considered to be normal: fair (n=1) moderate (n=3), severe (n=5). Post-treatment radiographs at 6 weeks evaluation showed the same classification except for one dog that progressed from moderate to severe.

Platelet count of PRP samples ranged from 1050 x 1000/ul to 2204x1000/ul (mean ± SD 1727 ± 429 x1000/ul) was significantly higher than platelet count of peripheral blood (246 ± 56 x1000/ul) (P < 0.0001). This represented a mean of 7 fold increase in platelet count in the PRP sample. White blood cell count of PRP sample ranged from 7670/ul to 34960/ul (mean 24034 ± 8070/ul) was significantly higher than peripheral blood (mean 7196/ul ± 1237/ul) (P < 0.0006). This represented a mean of 3.3 fold increase in WBC count in the PRP sample. In contrast, PCV of PRP sample (mean 17% ± 7%) was significantly lower than peripheral blood (mean 44% ± 2%) (P < 0.0001).

There was no significant difference in pain severity at pre-treatment (median 24.0, interquartile [25th and 75th percentile] range, 8.5 to 36.0) to 2 week post-treatment follow-up (median 16.0, interquartile range, 4.8 to 28), or 6 week post-treatment follow-up (median 15.1, interquartile range, 7.3 to 31).
Pain interference score did not show significant difference at pre-treatment (median 18, interquartile range 13.3 to 30) to 2 week post-treatment follow-up (median 19, interquartile range 14.3 to 38), or 6 week post-treatment follow-up (median 15.8, interquartile range 9 to 38). Lameness score and surgeon evaluation score showed no significant improvement at any time point.

Peak Vertical Force (%BWT) differs over time (p=0.0001) and between affected and unaffected limbs (p<0.05), however the effect of treatment and time do no interact (Figure 3). Vertical Impulse differs significantly between limbs (Affected vs Unaffected, p=0.00217) and as a result of time (p=0.0008), however there is no significant interaction between time and treatment as for PVF (Figure 4). Stride length difference (%) shows no significant effect of time (Kruskal Wallis Rank Sum Test, p=0.8497). Stride length shows no difference between limbs, or over time (Friedman Test, p=0.7 for limbs, 0.07 for time).

Stance time changed significantly with time (p=0.0024), with no significant difference between affected and unaffected limbs (p=0.301). Both affected and unaffected are significantly different to week 0 at week 2 (p=0.0025), however there is no difference between weeks 0 and 6 (p=0.5003) or week 2 and 6 (p=0.05003). Between times tested with Wilcoxon matched pairs test.

The mean hip extension angle was 122° (range 90° to 150°) at pre-treatment, 137° (range 105° to 160°) at 2 weeks and 135° (range 125° to 147°) at 6 weeks post-treatment. The mean hip flexion angle was 60° at all time points.
Accelerometry measurements showed that activity counts differed significantly during sedentary to light-moderate and vigorous activity, with no significant difference between activity level and different time points.

3.5. Discussion

The results of this study did not show any significant difference in gait or function over 6 weeks following a single intra-articular administration of autologous PRP for coxofemoral OA. The authors therefore reject the null hypothesis that a single intra-articular injection of autologous PRP can have a significant improvement in dogs with coxofemoral OA. This is in contrast to Fahie et al.’s study that found significant improvement in PVF, lameness scores at 12 weeks post-treatment following single intra-articular injection of an autologous platelet concentration for osteoarthritis (5). One reason for this difference is that the coxofemoral joint was not included in that study. Coxofemoral osteoarthritis frequently affects bilaterally, hence improvement may not be seen as readily. The patients in this study had clinical signs of lameness attributable to one coxofemoral joint, however, radiographic evidence of degenerative joint disease were present bilaterally. In addition, it has been previously reported that radiologic variables does not correlate well with owner or veterinarian pain scores on assessment of canine hip dysplasia (24). However, several comparisons can be made leading to the difference in results. One is the use of different commercial devices in PRP harvest. Fahie et al.’s study used a platelet filtration system compared to the platelet centrifugation system used in this study. The platelet yield was a 3 fold increase to peripheral baseline compared to a 7 times increase in this study. White blood cell count was 1.8 fold increase compared to peripheral baseline in Fahie et
al’s study in contrast to the 3.3 fold increase in WBC in this study (5). The ideal level of platelets and white blood cell ratio in a PRP sample remains controversial in determining its effect on inflammation, healing and efficacy of treatment.

The study participants were treated unilaterally based on clinical presentation and gait analyses. The low sample size, short-term follow-up and possibility of bilateral disease can be further confounding factors. Ideally, arthroscopy is the gold standard to determine the extent of OA in joints, and this was not performed for any patient. Although previous studies assessing treatment outcomes of the hip joint utilize PVF and VI, we have further included StT and SL to investigate if other variables may be of influence (25-27). Kinematic studies involving dogs with hip dysplasia found angular acceleration was greater in the mid-to end of the stance phase (extension), and deceleration greater in the mid to end of the swing phase (flexion) (28). Correlation of this kinematic information may correspond to kinetic gait analyses parameters such as StT and SL, however, significant changes of these measurements were not detected in this study. Bennett et al found that SL was increased, PVF was decreased and StT did not change in dogs with hip dysplasia (29). The reliability of goniometry measurements was validated to be a reliable and objective method of determining joint range of motion (30, 31). Hip extension measurements in this study were less than that reported for normal hip range of motion in German Shepherd Dogs and Labrador Retrievers using goniometry measurements (extension 155-162°) and slightly higher for flexion angles (flexion 44°-50°). The decreased range of motion in hips extension is consistent with previous studies of dogs with hip dysplasia having a decreased range of motion and reduced hip joint extension (31, 32). Breed differences could explain the increased hip flexion angles in this study.
The accelerometer has been reported as a valid and reliable device for objective measurement of habitual physical activity and at home activity monitoring in dogs (32, 33). It has been used to effectively discriminate standardized activity levels, hence this validates the use of accelerometry as an objective measurement of quantifying activity post-treatment. An increase in vigorous or light-moderate activity may mean increased comfort, compared to an increase in sedentary activity which may relate to discomfort on movement. Unfortunately, pre-treatment data was not collected, hence the available information indicates only that there was no significant difference in any activity level at 2 week and 6 week post-treatment. Our results correspond to a previously reported study that found dogs were sedentary most of the time (34).

The CBPI is an established reliable measure of owner’s assessments of severity and impact of pain on quality of life (18, 35). Pain severity and pain interference score improved over the study period, and the overall quality of life also improved except for one dog that went from ‘excellent’ to ‘very good’. This may have been due to progression of the disease process either unilaterally or bilaterally, although the owner noted improved exercise tolerance since treatment.

The components of the PRP samples were similar to manufacturer’s guidelines of 8.2 ± 2.3 times platelet concentration above baseline, with a WBC concentration of 4.0 ± 1.2 times above baseline. Depending on the literature, platelet concentration for a PRP sample should be 200% (36) or 2 to 5 fold increase compared to baseline peripheral platelet count (37-40). These growth factors include TGF-β that stimulates chondrogenesis and extracellular matrix synthesis (41); IGF-1 that induce chondrogenic differentiation of MSCs and protect synovial membrane from chronic inflammation (42), and BMP-7 induces hyaline cartilage-like regeneration of full-thickness osteochondral defects in a dog model (43); and BMP-2 stimulates matrix synthesis,
enhance cartilage matrix turnover, increase collagen type II and aggrecan expression (44). The use of intra-articular PRP for OA in 40 human patients with hip OA showed 57.5% had reduced pain with 40% excellent response sustained to 6 months (8). Other studies comparing the use of PRP versus a viscoelastic substance such as hyaluronic acid, showed that PRP had improved clinical performance and reduction in pain in human patients with knee OA (14, 45).

Autologous platelet concentrate were also injected into horses with OA and found significantly improved lameness and joint effusion, with most marked improvement at 2 months post-treatment and persisted for 8 months. The treatment protocol in this study used a single injection and follow-up period of only 6 weeks, a series of PRP injections (such as 3 injections 2 weeks apart), or longer follow-up evaluations may have provided further information on the effectiveness of PRP on hip OA in dogs.

Limitations of this study included the presence of bilateral disease detected radiographically, however, clinical presentation was lameness confined to one hip. Radiographs of other joints of the affected hindlimb were also not assessed although clinical examination did not reveal abnormalities. Arthroscopy and joint fluid analysis (such as synovial joint markers) of the hip joint would have provided further information of the progression or improvement of the disease process pre- and post-treatment. A strength of this study is the use of client-owned dogs in the study. However, this can also be limitation, as naturally occurring OA in dogs can be associated with confounding factors: such as concurrent medications, variations in home environment, and client compliance.

The use of intra-articular PRP has the potential to be a palliative alternative to restore metabolic balance and joint homeostasis in degenerative joint disease, whilst avoiding long-term adverse effects of NSAIDs, inconsistencies of nutraceuticals and salvage procedures such as total
hip replacements and femoral head and neck ostectomies. However, given the lack of significant changes post PRP treatment, further research with larger case numbers, longer follow-up, and comparison to other treatment methods such as intra-articular hyaluronan injections or mesenchymal stem cell therapy is recommended to evaluate the efficacy of PRP as a treatment option for OA in dogs.

3.6. References


Chapter IV

4.1. Conclusion

The concept of autologous PRP has led to a transition from the traditional approach focusing on ‘repair’ to the emerging approach of ‘regeneration’. Effective therapies for the treatment of tendinopathies and chondropathies have been under extensive research in the last decade. The progression of ‘biotherapeutics’ to regenerate progressive loss of cartilage have turned its focus to platelet-rich plasma, autologous conditioned serum, and mesenchymal stem cell therapy (MSC). Systemic reviews on the use of PRP in tendon and joint models showed several potential advantages of faster recovery, reduction in recurrence and improved pain and functional scores. Despite its use in orthopedic and sports-related injuries, the clinical efficacy of PRP therapy and varying mechanisms of action have yet to be established.

There are inherent issues surrounding the application and outcome of PRP to individuals. Furthermore, PRP can produce different effects according to the particular disease phase, for example, acute versus chronic disease states. There is currently a milieu of multi-center trials analyzing the use of regenerative medicine in human and veterinary clinical studies. The immunomodulatory effects of bone-marrow MSCs, adipose-derived MSCs, use of specific cytokine or growth factor as scaffolds/matrices, interleukin-1 receptor antagonist protein (IRAP) as alone or in combination with PRP have shown positive results in patient comfort and increase in functional ability as an intra-articular injection in OA affected joints. Although much research lies ahead, standardization of preparation, administration and application of PRP procedures are essential to determine the existing potential for PRP in the treatment musculoskeletal diseases in the dog.
4.2. Figures

Figure 1. Peak vertical force (percentage body weight) in dogs with unilaterally affected ST. Results are shown for the affected (red) and unaffected (blue) limb at pre-treatment, 2 weeks, 6 weeks, and final (>16 weeks) follow-up evaluation. Values are presented as mean ± standard deviation (grey bars).

Figure 2. Vertical impulse (percentage body weight*seconds) in dogs with unilaterally affected ST. Results are shown for the affected and unaffected limb at pre-treatment, 2 weeks, 6 weeks, and final (>16 weeks) follow-up evaluation. Values are presented as mean ± standard deviation (grey bars).
Figure 3. Peak vertical force (percentage body weight) in dogs with coxofemoral OA. Results are shown for the affected (red) and unaffected (blue) limb at pre-treatment, 2 weeks and 6 weeks follow-up evaluation. Values are presented as mean ± standard deviation (grey bars).

Figure 4. Vertical impulse (percentage body weight*seconds) in dogs with coxofemoral OA. Results are shown for the affected and unaffected limb at pre-treatment, 2 weeks and 6 weeks follow-up evaluation. Values are presented as mean ± standard deviation (grey bars).
### 4.3. Tables

<table>
<thead>
<tr>
<th>Device</th>
<th>Blood Vol (ml)</th>
<th>PRP Vol (ml)</th>
<th>Prep Time</th>
<th>Platelet yield</th>
<th>Activator (+/-)</th>
<th>WBC (+/-)</th>
</tr>
</thead>
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<td>SmartPReP®</td>
<td>20-120</td>
<td>3-20</td>
<td>15min</td>
<td>Up to 9x</td>
<td>/-thrombin</td>
<td>+</td>
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<tr>
<td>GPS III</td>
<td>60</td>
<td>10</td>
<td>12min</td>
<td>Up to 8x</td>
<td>Thrombin</td>
<td>+</td>
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<tr>
<td>Magellan®</td>
<td>30-60</td>
<td>6</td>
<td>15min</td>
<td>Up to 10x</td>
<td>CaCl₂</td>
<td>+</td>
</tr>
<tr>
<td>PCCS</td>
<td>50</td>
<td>8-12</td>
<td>20min</td>
<td>Up to 7x</td>
<td>Thrombin + CaCl₂</td>
<td>NS</td>
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<tr>
<td>Symphony II®</td>
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<td>5-10</td>
<td>15min</td>
<td>Up to 6x</td>
<td>Thrombin + CaCl₂</td>
<td>NS</td>
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<td>Cascade®</td>
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<td>4-9</td>
<td>20min</td>
<td>1-1.5x</td>
<td>CaCl₂</td>
<td>-</td>
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<td>-</td>
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<td>2-3x</td>
<td>CaCl₂</td>
<td>-</td>
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<td>Angel®</td>
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<td>+/-</td>
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<td>15min</td>
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<td>Genesis CS®</td>
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<td>E-PET</td>
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<td>1-18</td>
<td>15min</td>
<td>Up to 7x</td>
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Table 1. Different commercial PRP systems available.

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<th>L-PRP</th>
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<tr>
<td>Pure-PRP</td>
<td>Leukocyte-Rich PRP</td>
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<tr>
<td>• Autologous Conditioned Plasma (ACP) Arthrex</td>
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</tr>
<tr>
<td>• Preparation Rich in Growth Factor (PRGF) Biotechnology Institute</td>
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<tr>
<td>• Gravitational Platelet Separation (GPS) Biomet</td>
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<tr>
<td>• Symphony II DePuy</td>
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<td>• SmartPReP 2 Harvest Technologies</td>
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<tr>
<td>• Magellan Arteriocyte Medical Systems</td>
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<table>
<thead>
<tr>
<th>P-PRF</th>
<th>L-PRF</th>
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<tbody>
<tr>
<td>Pure Platelet Rich Fibrin</td>
<td></td>
</tr>
<tr>
<td>• Cascade® Musculoskeletal transplantation foundation</td>
<td></td>
</tr>
<tr>
<td>• PRGF Scaffold Biotechnology Institute</td>
<td></td>
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<tr>
<td>• Fibrinet Aesthetic Factors</td>
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<table>
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<th>L-PRF</th>
<th>Pure Leukocyte + Platelet Rich Fibrin</th>
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Table 2. Types of PRP
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<tr>
<th>Sex</th>
<th>Age yrs</th>
<th>Wgt kgs</th>
<th>Duration Of Signs</th>
<th>Side Affected</th>
<th>Lameness Grade - Pre</th>
<th>Lameness Grade - Final</th>
<th>U/S Lesion Size – Pre</th>
<th>U/S Lesion Size – Final</th>
<th>Outcome</th>
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<td>NM</td>
<td>2</td>
<td>80.5</td>
<td>7mths</td>
<td>Right</td>
<td>2/4</td>
<td>0/4 (6wks)</td>
<td>1.26 cm²</td>
<td>2 cm²</td>
<td>Returned to normal function, competing</td>
</tr>
<tr>
<td>NM</td>
<td>8</td>
<td>13.5</td>
<td>10mths</td>
<td>Left</td>
<td>1/4</td>
<td>1/4 (12mths)</td>
<td>1.6 cm²</td>
<td>2 cm²</td>
<td>Returned to normal function</td>
</tr>
<tr>
<td>SF</td>
<td>4</td>
<td>36.8</td>
<td>8mths</td>
<td>Bilateral</td>
<td>2/4</td>
<td>0/4 (12wks)</td>
<td>R: 1.36 cm²</td>
<td>L: 1.52 cm²</td>
<td>Improving, able to jump, normal activity</td>
</tr>
<tr>
<td>SF</td>
<td>3</td>
<td>31.8</td>
<td>12mths</td>
<td>Left</td>
<td>3/4</td>
<td>0/4 (6wks)</td>
<td>1.12 cm²</td>
<td>1.26 cm²</td>
<td>Improving</td>
</tr>
<tr>
<td>IM</td>
<td>8</td>
<td>32</td>
<td>5yrs</td>
<td>Right</td>
<td>3/4</td>
<td>2/4 (12mths)</td>
<td>1.3 cm²</td>
<td>1.3 cm²</td>
<td>Returned to competing until lumbosacral instability</td>
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<tr>
<td>IM</td>
<td>12</td>
<td>39.9</td>
<td>5mths</td>
<td>Left</td>
<td>3/4</td>
<td>2/4 (16wks)</td>
<td>2.27 cm²</td>
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<td>1/4 (6wks)</td>
<td>R: 1.52 cm²</td>
<td>L: 0.95 cm²</td>
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<tr>
<td>SF</td>
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<td>34</td>
<td>5mths</td>
<td>Bilateral</td>
<td>2/4</td>
<td>1/4 (6wks)</td>
<td>R: 1.2 cm²</td>
<td>L: 1.36 cm²</td>
<td>Returned to normal function</td>
</tr>
<tr>
<td>IF</td>
<td>5</td>
<td>18.7</td>
<td>1yr</td>
<td>Bilateral</td>
<td>3/4</td>
<td>0/4 (26wks)</td>
<td>R: 0.9 cm²</td>
<td>L: 1 cm²</td>
<td>Returned to normal function</td>
</tr>
</tbody>
</table>

Table 6. Demographics, characteristics and outcome of 10 dogs diagnosed with supraspinatus tendinopathy. (NM = neutered male, SF = spayed female, IM = intact male, IF = intact female, yrs = years, wgt = weight, kgs = kilograms, mths = months, wks = weeks, Pre = pre-treatment, Final = >16 weeks final evaluation, U/S = ultrasound, R = right, L = left)

4.4 Appendix

Appendix A: Lameness Score

Please enter a lameness score below based on the following scale:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stands and walks normally</td>
<td>0</td>
</tr>
<tr>
<td>Stands normally, slight lameness when walking</td>
<td>1</td>
</tr>
<tr>
<td>Stands normally, obvious lameness when walking</td>
<td>2</td>
</tr>
<tr>
<td>Stands abnormally, slight to obvious lameness when walking</td>
<td>3</td>
</tr>
<tr>
<td>Non-weight bearing lameness</td>
<td>4</td>
</tr>
</tbody>
</table>

Lameness Score ____________________________
4.4 References


131. Loeser RF, Pacione CA, Chubinskaya S. The combination of insulin-like growth factor 1 and osteogenic protein 1 promotes increased survival of and matrix synthesis by normal and osteoarthritic human articular chondrocytes. Arthritis & Rheumatism 2003;48:2188-2196.


4.5. Vita

ACADEMIC HISTORY

Masters in Veterinary Science
Oregon State University, Corvallis, Oregon, USA, 2014

Graduate Diploma in Animal Chiropractic
Australian Veterinary Chiropractic Association
Royal Melbourne Institute of Technology University, Melbourne, Australia, 2007

Bachelor of Veterinary Science, Class I Honors
University of Sydney, Sydney, Australia, 2004

SCIENTIFIC PRESENTATIONS

Ultrasonographic Findings of Canine Supraspinatus Tendinopathies Following Intralesional Platelet-Rich Plasma Injection
Podium Speaker, Veterinary Orthopedic Society (VOS) Conference, Utah, USA, 2013

Femoral Fixation Technique Comparisons in Canine Cranial Cruciate Ligament Reconstruction
Podium Speaker, American College of Veterinary Surgeons (ACVS) Veterinary Symposium, Chicago, USA, 2012

PUBLICATIONS


ABSTRACTS


POSTER PRESENTATION

Pike N, Angus W, Ho LK. A comparison of aquatic fauna in two river systems in Sydney. Poster Presentation Session. The University of Sydney and CSIRO (Commonwealth Scientific and Industrial Research Organization) Student Research Scheme, Australia, 1998