Regenerative medicine therapies have become significant tools for treatment of joint, soft tissue, and a variety of other conditions in animals and humans. Regenerative medicine aims to restore form and function of injured tissues using the body’s own resources such as cells, fluids (ie, plasma and serum), and their resulting anti-inflammatory and prohealing cytokines. Other terms used to describe these regenerative treatments include biologic therapy and orthobiologic therapy. Theses therapies achieve anti-inflammatory and healing effects without the use of corticosteroid therapy. This response is an advantage when treating young animals or human patients, and in animals with metabolic or hormonal issues such as equine pituitary pars intermedia dysfunction. Also, these therapies may have beneficial effects when traditional IA treatments such as corticosteroids and/or hyaluronan are no longer effective at reducing joint inflammation and pain. Examples of hemoderivative regenerative therapies to be discussed include platelet-rich plasma, autologous conditioned serum, autologous protein solution, and α-2 macroglobulin. Regenerative therapies are used to facilitate healing and restoration of function in acute and chronic injuries. The response of soft tissue injuries to these regenerative therapies is often more rapid with higher quality healing than for previously used methods of therapy. For example, a study of experimentally induced superficial flexor tendon injury in horses found that intralesional injection of platelet-rich plasma (PRP) resulted in a stronger and more elastic tendon after healing compared to saline (0.9% NaCl) solution-treated controls. In a survey of board-certified specialists (American College of Veterinary Surgeons and American College of Veterinary Sports Medicine and Rehabilitation), PRP was the most commonly used biologic for treatment of soft tissue injuries. Autologous conditioned serum (ACS), a serum derivative that concentrates interleukin-1 receptor antagonist protein (IL-1Ra) and other anti-inflammatory cytokines, has also been evaluated for treatment of equine superficial digital flexor tendon (SDFT) injury. This paper will describe several of the most common regenerative therapies that originate from autologous whole blood and convey their indications, mechanism of action, and methods of application.

General Indications

- Soft tissue injuries.
- Synovitis.
- Osteoarthritis.
- Muscle injury.
- Wounds.
- Ophthalmic injuries.
In naturally occurring SDFT injury, treatment with ACS was compared to controls, which were either injected with saline solution or had no treatment. ACS-treated tendons had reduced lameness and swelling and increased collagen type I expression compared to controls.8

An in vitro study9 reported ACS sourced from dogs with osteoarthritis had anti-inflammatory and regenerative activity. The authors concluded that the hemoderivatives from dogs with arthritis contained the bioactive factors that would be appropriately used for osteoarthritis management.9

Platelet-rich plasma and other whole blood derivatives have application for soft tissue injuries (eg, tendonitis and desmitis) and joint disorders such as osteoarthritis, cartilage injury, and synovitis. These therapies result in beneficial joint effects without the use of corticosteroid therapy. This response is an advantage when treating young animals and animals with metabolic or hormonal issues such as equine pituitary pars intermedia dysfunction. Also, these therapies may have beneficial effects when traditional IA treatments such as corticosteroids and/or hyaluronan are no longer effective at reducing joint inflammation and pain. These effects of regenerative therapies will be described in greater detail in this paper. Examples of hemoderivative regenerative therapies include PRP, ACS, autologous protein solution (APS), and α-2 macroglobulin (α2M).

Platelet-rich Plasma

• PRP is an autologous biologic that has a multitude of beneficial actions: it is anti-inflammatory, provides pain relief, and promotes healing.
  - Indications include treatment of soft tissue injuries, joint inflammation, wounds, and corneal injuries.
  - Processing is uncomplicated and may be accomplished at the point of care.
  - PRP is a general term for whole blood that is processed in a way to concentrate platelets.1 Processing of whole blood obtained with anticoagulant (eg, anticoagulant citrate dextrose solution A [ACD-A]) results in platelet concentrations that differ on the basis of the donor animal’s parameters such as health status and the processing technique used. Generally, a platelet concentration that is 3 to 4 times or greater than that in whole blood is considered PRP.1
  - PRP is an autologous biologic; hence, obtaining and processing it is not complicated, and it is safe when administered to the animal from which the whole blood was obtained.1 It has many applications in musculoskeletal conditions including treatment of tendinitis, desmitis, synovitis, and cartilage injury. PRP is also used for treatment of wounds and corneal disorders.12–15
  - Platelets play a primary role in the healing response to injury by activating the clotting process, resulting in the release of bioactive cytokines and growth factors, thereby enhancing angiogenesis and growth of new tissue.1,10,11,13 Platelet-rich plasma enhances these responses when injected in the site of injury.1,10,11,15

The beneficial regenerative biologic within platelets originate in the α granules.1,11,13 Platelet-dense granules secrete proteins that modify the inflammatory response and promote clotting.11 Activation of the concentrated platelets releases cytokines that enhance healing. Several of the growth factors released and their actions in enhancing healing include the following11:
  - Transforming growth factor β (TGF-β1): promotes proliferation of undifferentiated mesenchymal stem cells and growth of epithelial and vascular endothelium; regulates collagen production; and inhibits macrophage and lymphocyte proliferation.
  - Platelet-derived growth factor (PDGF): enhances mesenchymal cells and osteoblasts mitogenesis; stimulates chemotaxis of fibroblasts, glial, smooth muscle cells, and neutrophils; and regulates collagen synthesis and secretion.
  - Vascular endothelial growth factor: increases angiogenesis and mitogenesis of endothelial cells.
  - Insulin-like growth factor-1: augments chemotaxis of fibroblasts and enhances protein synthesis and bone formation.

Rather than using individual cytokines to enhance tendon healing,16 the combination of cytokines released with platelet activation results in enhanced healing similar to what is accomplished by the body.1,17

The cumulative effect of these cytokines is to attract appropriate cells to the injury site such as fibroblasts and stem cells and increase cell proliferation, angiogenesis, and collagen production.18,19 In joints, PRP has anti-inflammatory, antibacterial effects.20,21 Fibrin-rich PRP contains the cytokines and growth factors of PRP, plus the fibrin creates a scaffold for wound healing and bone healing.12,22,23

PRP application has been found to reduce infection and improve wound healing.24 An example of PRP’s effect on healing of infected wounds was reported in dogs.25 Treatment of full-thickness skin wounds in dogs infected with methicillin-resistant Staphylococcus aureus was compared in 2 groups. One group was administered autologous PRP SC weekly, and the second group was administered topical clindamycin twice daily. The cases were followed for 21 days. The PRP group had more advanced healing when compared to the clindamycin group due to reduced methicillin-resistant Staphylococcus aureus levels in the wound.25

PRP Processing

Differences in autologous whole blood processing techniques result in different types of PRP. These different types are classified on the basis of postprocessing components. The components differ in platelet concentration, leukocyte concentration, and fibrin content. A universal classification for PRP is yet to be agreed upon. A working classification of PRP is as follows26:
  - Pure PRP (P-PRP): no leukocytes and a low-density fibrin network. Indicated for IA administration.
• Leukocyte and platelet-rich PRP: platelets and leukocytes in high concentrations and a low-density fibrin network. Indicated for treatment of soft tissue injuries.
• Pure platelet-rich fibrin: no leukocytes and a high-density fibrin network. Indicated for treatment of wounds and corneal injuries.
• Leukocyte- and platelet-rich fibrin: increased concentration of leukocytes and a high-density fibrin network. Indicated for treatment of wounds and corneal injuries.

Optimal leukocyte levels in PRP for treatment of tendon and ligament injuries remain controversial. The leukocyte population measurable in PRP is composed of neutrophils, lymphocytes, and monocytes. Neutrophils provide the initial inflammatory response necessary to initiate the healing response of an injury. However, continued inflammation during healing of tendon injuries, which may be associated with high leukocyte levels, results in excessive scar tissue and poor quality of repair.

A lower level of leukocytes in the administered PRP reduced expression of catabolic cytokine IL-1β, thereby diminishing inflammation. PRP processed with low leukocyte levels and optimal platelet concentrations resulted in release of higher levels of anabolic growth factors PDGF-ββ and TGF-β. In an equine tendon explant study, reduced-leukocyte PRP resulted in lower expression of inflammatory cytokines compared to high-leukocyte PRP. However, phagocytic macrophages, which originate from blood monocytes, are necessary for the initiation of healing. Using a rat ligament injury model, macrophages were found to be critical for the healing response. Inhibition of macrophages in early ligament healing in this model led to reduced granulation tissue formation but also in reduced ligament strength.

Low-leukocyte PRP is preferred for IA use, but this conclusion also remains controversial. A meta-analysis of PRP response for treatment of human osteoarthritis of the knee joint concluded that leukocyte-poor PRP, a single-spin technique, and platelet concentration < 5 times over baseline were factors associated with the “very good responders group.” Contrary to the previous reference cited, a study using leukocyte-rich PRP found positive effects in human knee osteoarthritis. Improved functional outcomes in the study were time to standing, quality of life, pain scores, and balance parameters; however, gait and range of motion were not improved over pre-PRP treatment measurements.

There are many commercially available kits for processing PRP. Some of the kits include provisions for obtaining leukocyte-rich or leukocyte-poor PRP. There are reports that document PRP results following processing with various kits in canine and equine species. High variability in PRP platelet, leukocyte, and erythrocyte concentration is common across species and kits. NSAIDs should be withheld prior to obtaining blood for PRP processing, but the specific withdrawal times have not been definitively determined for animals or humans. Whole blood from human subjects who had recently received NSAIDs were found to have impaired platelet aggregation compared to blood processed from subjects that had not taken NSAIDs for at least 2 weeks. The authors concluded that the measured reduction in platelet aggregation may result in less effective biologic responses associated with PRP. Other drugs have also been identified as interfering with the positive effects of PRP, but further research is required to identify the clinical effects relevant in veterinary medicine.

The final PRP characteristics are dependent on animal (donor) and processing factors. Donor factors include platelet concentration, presence of illness or inflammation, and presence of extraneous medications such as NSAIDs. Processing factors include anticoagulant choice, centrifugation protocol, and method of platelet activation.

The most common method of PRP processing is centrifugation. Centrifugation results in separation of the blood components: platelets, leukocytes, erythrocytes, and plasma (Figure 1).

Figure 1—Equine whole blood with anticoagulant following centrifugation with resulting separation of cells and plasma. A—Platelet-poor plasma. B—Platelet-rich, leukocyte-poor plasma. C—Buffy coat (leukocytes and platelets). D—Erythrocytes. Courtesy of Sue Murad.
have been described. A filtration technique that separates platelets from whole blood has been validated for use in horses and dogs. When choosing a processing system, determine that it has been validated for the intended species, it is compatible with the location of use, and the system manufacturer/distributor provides support as needed. For example, a system that is effectively used in a clinic setting may not be appropriate for processing PRP on a farm.

**Protocol**

- Select the appropriate processing system for the point-of-care site (eg, hospital or farm) and the type of PRP needed for the target tissue (eg, leukocyte and platelet-rich PRP or P-PRP).
- Withhold NSAID administration before PRP processing (see above).
- Use aseptic technique for whole blood withdrawal and administration of PRP.
- Ultrasound control helps assure PRP injection in target site.
- Quantity of PRP injected depends on targeted joint space or tissue characteristics.
- Reexamine in 1 to 4 weeks to determine whether additional PRP injection or other treatment modalities should also be used to facilitate healing.

PRP is injected into the injury site or joint using aseptic technique with the animal under sedation (if needed). The site may also need to be prepared for local and/or regional analgesia. Ultrasound control is usually necessary for assuring the appropriate placement of the injection. Volume of injection depends on the size of the lesion or joint. In a soft tissue lesion, the PRP is infiltrated within the injury site without causing excessive distension of the tissue. Within a joint, the quantity administered depends on the normal joint volume, with the intent to fill the joint without excessive distension. A single dose of antimicrobial medication is often administered parenterally at the time of PRP treatment. The site of injection is bandaged if the anatomy allows for it. Follow-up examinations should be made at 1 week and 4 weeks after a single treatment to determine whether any swelling or undue discomfort is present. For soft tissue injuries, ultrasonography may be conducted between 4 and 8 weeks after PRP administration to determine tissue response. If progression of healing is not evident by 8 weeks after the first PRP injection, a second injection may be indicated. For animals with osteoarthritis treated with PRP, improvement of joint pain in dogs has been reported as soon as 15 days and within 3 months. In horses, improvement of pain may require 6 to 12 weeks.

When PRP is administered for soft tissue injuries, platelet activation can be enhanced by conducting extracorporeal shock wave therapy immediately following the injection. The extracorporeal shock wave therapy treatment parameters for platelet activation remain empirical. Based on an in vitro study that mimicked conditions of equine soft tissue, 300 impulses at 0.12 to 0.28 mJ/mm² energy resulted in significant increases in TGF-β₁ and PDGF-ββ compared to controls.

In most soft tissue cases, a single injection of PRP is made and results in an effective response. In a placebo-controlled clinical trial in horses with SDFT injury, a single injection of PRP administered up to 8 weeks after injury was found to result in earlier reduction of lameness and advanced organization of repair tissue evident on ultrasonography compared to saline-injected controls. Improvement in lameness and tissue organization and a higher level of performance out to 12 months following treatment was reported in the PRP-treated group.

In a report of equine suspensory injury in 9 racing Standardbreds in which a single intralesional injection of PRP and a controlled exercise program were used for treatment, the authors concluded that the protocol was effective in returning horses to racing.

Other research also identifies prolonged effects of a single PRP treatment. Significantly greater neovascularization was identified 23 weeks after a single PRP injection within a surgically created equine SDFT lesion compared to controls. A report identified increased strength, elasticity, and tissue organization in an experimental equine SDFT injury treated with a single PRP injection compared to controls out to 23 weeks post-treatment as well.

Treatment intervals for PRP used within joints have been reported in dogs and horses. Intra-articular treatment of osteoarthritis associated with cranial cruciate ligament rupture was reported. PRP was administered in the affected joint 4 times at 30-day intervals. Lameness improved most substantially as measured by peak vertical force, vertical impulse, and angular range of motion. In a study of PRP treatment of bilateral canine hip osteoarthritis, 20 treatment group dogs were administered PRP twice at 14-day intervals and compared to 20 dogs that were administered 2 injections of saline solution at 14-day intervals and were followed for 180 days. Outcome measures included 5 different visual analog scales for assessment of pain and function. Administration of 2 IA platelet injections significantly improved pain and function compared to control treatment.

In dogs with naturally occurring stifle osteoarthritis due to cranial cruciate ligament injury, a single 2.5-mL injection of P-PRP was found to improve measured gait values for as long as 12 weeks following administration.

Response of PRP treatment in 12 horses with naturally occurring osteoarthritis was reported. A single dose of PRP obtained via a commercially available filtration technique was administered following objective gait analysis and response to IA analgesia. Response to PRP was variable, with 6 horses having improved gait analysis at 6 weeks and 6 horses having improvement of gait at 12 weeks following administration.

**Autologous conditioned serum and autologous protein solution**

Indications for these 2 hemoderivatives are as stated earlier. In horses, these products are most commonly used to treat joint injury and inflamma-
tion. Clinical reports of ACS and APS use in dogs are limited.

ACS- and APS-type biological products require further processing to concentrate the beneficial anti-inflammatory cytokines. ACS processing requires contact of whole blood with glass beads and incubation. APS processing is a 2-step process completed without incubation.

Both hemoderivatives have increased interleukin-1 receptor antagonist protein (IL-1Ra) compared to whole blood in equine51,52 and canine studies.53 IL-1 inflammatory activity in joints is inhibited by IL-1Ra by binding at the IL-1 receptors.54,55 Increased concentration of IL-1 is found in joints with synovial and chondrocyte inflammation, and its presence upregulates enzymes that further degrade the joint.52 IL-1Ra also inhibits other inflammatory mediators such as cyclooxygenase-2 and metalloproteinases.56

Additional APS and ACS cytokines include IL-4, tumor necrosis factor-α (TNF-α), IL-10, and IL-6.7

APS is reported to include significantly greater concentrations of blood-derived growth factors and cytokines such as IL-1RA, insulin-like growth factor-1, TGF-β, IL-10, and growth factors such as epidermal growth factor and PDGF, compared with PRP alone.51,57

**Autologous conditioned serum**

It has been reported that incubation of human whole blood with glass beads stimulates the production of IL-4, IL-10, and IL-1Ra as well as fibroblastic growth factor-1, hepatocyte growth factor, and TGF-β1, resulting in higher concentrations of those beneficial cytokines in ACS.58 The increase in these anti-inflammatory mediators does not appear to be accompanied by an increase in the proinflammatory cytokines IL-1β or TNF-α. Because IL-1Ra expression is as much as 140-fold greater than other anti-inflammatory proteins in ACS after such stimulation of whole blood, IL-1Ra is considered one of the major mediators responsible for clinical improvement in patients with osteoarthritis and muscle injuries.58,59

ACS cytokines obtained by processing equine blood have been described and include IL-1Ra and TNF-α.60 The concentration of the anti-inflammatory cytokine IL-10 was found to increase in incubated whole blood even when not incubated with glass beads.60 ACS IL-1Ra levels in processed canine blood are similar to the levels obtained in human and equine blood.61

The first significant study of ACS in horses was published in 2007.62 Using a model of surgically induced osteoarthritis, horses were injected with ACS 4 times at weekly intervals. Horses with experimental osteoarthritis treated with ACS had significant improvement in lameness and significantly decreased synovial membrane hyperplasia, compared with placebo-treated joints. ACS-treated joints also appeared to have less gross cartilage fibrillation and synovial membrane hemorrhage. Synovial fluid concentration of IL-1Ra was increased following treatment with ACS compared to controls.62

ACS obtained from dogs with naturally occurring osteoarthritis was evaluated in vitro.9 The canine ACS in this study included significantly increased concentrations of IL-1RA, vascular endothelial growth factor, and TGF-β. In chondrocyte cell cultures, anti-inflammatory effects were identified, as were proliferation of fibroblasts and osteocytes, and extracellular matrix gene expression was elevated.9

**ACS processing and protocol**

ACS is produced from aseptically obtained whole blood, which is incubated for 24 hours in a proprietary tube that contains medical-grade glass beads (IRAP II; Arthrex Inc; and Orthokine vet iap; Dechra). The beads increase the surface area of blood contact during incubation, which results in increased cytokine production by activated monocytes.63 The incubated tube is centrifuged, and the ACS serum is aspirated. The serum may be used immediately or frozen for future use. The ACS is injected into the affected joint(s) 3 to 6 times at 7- to 10-day intervals.56,62

**Autologous protein solution**

APS is processed autologous whole blood that contains IL-1RA, white blood cells, and platelets.51,53 APS may be processed stall-side and is administered as a single IA injection.51,53,54 Both ACS and APS have similar symptom-modifying effects in joints, and both have not been shown to have disease-modifying effects.64 APS is often used because of the point-of-care same-day processing and the need for only 1 injection of APS for effective treatment.51,53,64 However, in a survey of board-certified equine specialists, ACS was used more commonly than APS for treatment of joint inflammation.9

A group of 40 horses with naturally occurring osteoarthritis were randomly selected for treatment or control groups.51 Twenty horses received a single 5-mL injection of APS, and 20 horses were injected with 5 mL of sterile saline. All horses were exercised on a treadmill and evaluated for lameness grade, joint circumference, kinetic gait analysis, and range of motion for 14 days. Clients assessed and reported lameness before treatment and at 12 and 52 weeks following treatment. The APS group had significant improvements in lameness, range of motion, and gait symmetry by day 14. Owners of horses that were treated with APS reported improved lameness at 12 and 52 weeks over controls. Concentrations of IL-1Ra in APS were an average of 5.8 times the level found in whole blood.

Similar effects of APS in canine and human osteoarthritis have been reported.53,65 Dogs with stifle or hip osteoarthritis received a single injection of APS in the affected joint and were compared to non-treated dogs and followed for 12 weeks. APS-treated dogs had reduced pain scores and improved weight-bearing as measured by peak vertical force and vertical impulse compared to controls.53 Humans with mild to moderate osteoarthritis of the knee joint and treated with a single injection of APS had reduction of pain recorded for as long as 3 years.65

APS is primarily used for treatment of joint inflammation, but it has been evaluated in an equine collagenase-induced tendinitis model.66 Horses were...
studied with collagenase-induced tendinitis in both forelimb SDFTs. Comparisons were made over a 12-week period between the APS treated tendon and the control tendons that had been treated with saline. The findings included no significant difference in elastic modulus between groups. Evidence of improved healing was based on reduced gene expression of collagen type III in the APS compared to control tendons. The authors concluded that APS injection within an SDFT lesion resulted in some improvement in healing.66

APS processing and protocol

Whole blood with ACD-A anticoagulant is processed at the point of care (clinic or stall side) in 2 steps, which do not require incubation (ProStride). Aseptically obtained whole blood is placed in the APS separator device and centrifuged to form PRP. Plasma is then withdrawn and used or transferred to a proprietary separator device and centrifuged to form PRP. Platelet-poor plasma is removed, and the remaining PRP is placed in the concentrator device that contains polyacrylamide beads and is centrifuged again, resulting in APS. The APS is injected immediately after processing in the joint(s) to be treated. Volume of APS administered depends on joint size. The treatment protocol requires only a single injection of APS.51

α-2 Macroglobulin

α2M is a protein found in blood and synovial fluid that protects cartilage and reduces joint inflammation. α2M traps proteases and other inflammatory cytokines including IL-1β and facilitates clearance of the trapped proteins from the joint.67,68 In cultured human chondrocytes and in a rat osteoarthritis model, α2M was found to inhibit matrix metalloproteinase-13 induction by IL-1β.69 A concentrated serum of α2M was obtained from humans and pigs and evaluated in human chondrocyte culture exposed to IL-1 and a pig model of osteoarthritis. In chondrocyte culture, concentrated α2M serum was found to promote chondrocyte proliferation and reduce apoptosis and catabolic gene expression. The pig osteoarthritis model involved anterior cruciate ligament reconstruction. Comparisons were made between treatment of the affected joint with concentrated α2M serum or saline. The α2M group had reduced levels of inflammatory cytokines, improved gait, and less cartilage deterioration when compared to saline solution–treated joints.70 In a rat model of collagen-induced arthritis, administration of α2M resulted in an anti-inflammatory response and reduced cartilage and bone damage when compared to saline.71 There are no published reports that describe the clinical use of α2M in horses or dogs.

α2M can be processed using a commercial kit developed for use in horses (Alpha2EQ; Astaria Global). The manufacturer of the kit recommends the horse not have sedation for 3 days and no NSAIDs for 5 days prior to blood draw for processing. Whole blood with ACD-A anticoagulant is aseptically obtained and placed in tubes for centrifugation. The resulting plasma is then transferred to a proprietary filtration vial that is also centrifuged. The concentrated α2M is then withdrawn and used or transferred to syringes or vials and frozen for future use. The α2M protein captures inflammatory cytokines and proteases, thereby inactivating them.

Summary

The practitioner has many regenerative therapy options for treatment of arthritis, soft tissue injuries, and other disorders that make use of hemoderivatives, with more options being developed. None of the therapies presented in this report are definitively better than another. Each hemoderivative has advantages and disadvantages that should be understood prior to application. A key factor is to recognize the significant healing response of injured tissues and joints to these techniques and make use of them as part of routine, primary therapy.

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