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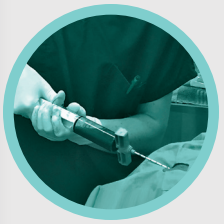

REGENERATIVE MEDICINE FOR SOFT TISSUE INJURY & OSTEOARTHRITIS

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Each year, the **NAVC Institute** gathers the top specialists in select areas of veterinary medicine to provide hands-on, one-on-one continuing education to the Institute attendees.

Practical Techniques from the NAVC Institute—brought to you by the **NAVC** and ***Today's Veterinary Practice***—provides the opportunity for you to experience the excellent education provided at the Institute within the pages of this journal.

This article reviews information from the session, **Canine Sports Medicine & Rehabilitation**, presented at the NAVC Institute 2015. The NAVC Institute 2017 takes place in Orlando, Florida, May 21 to 26; visit **navc.com/institute** for further information.

Regenerative medicine therapy has become increasingly popular in both human and veterinary medicine for treatment of multiple disease processes, and recent studies have demonstrated its efficacy in managing numerous orthopedic conditions in humans, dogs, and horses, including osteoarthritis and soft tissue injuries.[1-13]

While some tissues can heal to their original or near-original strength and stiffness, other tissues, such as cartilage, heal poorly. Regenerative medicine has been used to stimulate healing in areas that have not responded to more traditional approaches, helping injured tissues heal to their original or near-original condition.

Regenerative medicine is often used as an adjunct to surgical, medical, and/or rehabilitation therapy in a multimodal approach to treat a condition or injury. As with any other treatment modality, it is important to obtain a definitive diagnosis and tailor an appropriate treatment plan for the patient.

PLATELET-RICH PLASMA THERAPY

Platelet-rich plasma (PRP) is an autogenous fluid concentrate composed primarily of platelets and growth factors. Recent studies indicate that PRP mediates healing by supplying growth factors, cytokines, chemokines, and other bioactive compounds.[14,15]

PRP is used in both humans and animals to aid healing in numerous tissues. Recent studies have shown PRP to be efficacious in managing many different orthopedic conditions, including osteoarthritis and soft tissue injuries.[1,2,16-36] One recent study in dogs with partially transected cranial cruciate ligaments and meniscal release demonstrated improved range of motion, decreased pain, and improved limb function for up to 6 months—after treatment with 5 intra-articular injections of leukoreduced PRP—compared with the control group.[37]

Role in Tissue Healing

Platelets play roles in both hemostasis and wound healing, and PRP has been used as a regenerative medicine therapy to aid in tissue healing.

Platelets contain alpha granules that release growth factors to stimulate other cells of the body to migrate to the area of trauma, facilitating tissue healing. The growth factors—including platelet-derived, vascular endothelial, basic fibroblastic, and epidermal growth factors and transforming growth factor beta1 and beta2—contained within the platelets are important for tissue healing.[1,2,14-16]

Many growth factors act individually or synergistically to enhance cellular migration and proliferation, angiogenesis, and matrix deposition, which promotes tendon and wound

FIGURE 1. Blood collected for platelet-rich plasma (PRP) processing using an 18-gauge butterfly needle and syringe. Most systems require 10 to 60 mL of blood for PRP processing.

FIGURE 2. Both centrifugation and filtration systems are available for PRP processing. This centrifugation PRP system used for processing produces a leukocyte- and erythrocyte-poor PRP sample.

healing, aids in cartilage health, and counteracts the cartilage breakdown associated with osteoarthritis.[1-3,5,6,15-20,25-28,31,34,36] Platelets also recruit, stimulate, and provide a scaffold for stem cells.[32,36,38-45]

Components of PRP

Multiple formulations of PRP have been developed and studied. Previous studies in humans suggest that the ideal PRP product should lead to a 4- to 7-fold increase in platelets.[1,2,14,15,17]

However, platelet concentration is not the only important component of a PRP product. Inclusion or exclusion of mononuclear cells, neutrophils, and red blood cells not only defines an autologous platelet product but also affects the clinical efficacy of the product and the inflammatory responses after PRP injection.[15,16,19,25-28,46-50] In general, red blood cells and neutrophils should be reduced because they have an inflammatory effect, while the effect of mononuclear cells remains largely unknown.[42,46,47,51-54]

Recent studies compared key parameters of the PRP product from the commonly used commercial canine PRP systems in healthy, adult canines and found variations in product composition.[50,55,56]

Performing PRP Therapy

PRP therapy is a minimally invasive procedure that typically can be performed on an outpatient basis. It is often performed as a series of 1 to 3 injections, with 2 weeks between each injection. If PRP is being used to manage moderate to severe osteoarthritis, in my experience, about 50% of dogs require more than 1 injection for significant improvement.

To perform PRP therapy:

- Approximately 30 to 60 mL of blood is obtained using an 18-gauge needle or butterfly needle, processed, and prepared for injection (**Figures 1 and 2**).
- Once the PRP is processed, the area to be treated is clipped and aseptically prepared.
- Sedation or general anesthesia may be required for injection, depending on the location of the injection.

For osteoarthritis, PRP joint injections are usually performed without sedation; however, some joints, such as the hip, require sedation and may also require advanced imaging (fluoroscopy) for guidance. If one is not familiar with joint injections, it is wise to sedate patients until comfort with the procedure is obtained.

PRP has been used for tendon and ligament injuries, and is most commonly used for low grade strains or sprains. For soft tissue injuries, ultrasonography guidance is used to ensure accuracy of the injection because PRP is most

effective when administered directly into the lesion. If musculoskeletal ultrasound is not available for obtaining a definitive diagnosis or guidance for treatment administration, referral should be considered. Ultrasound-guided injections also require sedation.

Pain Management & Rehabilitation

The most common side effect is discomfort associated with the injection, which can be managed with pain medications, if needed, and typically resolves within 12 to 24 hours of the injection. However, nonsteroidal anti-inflammatory medication and steroids need to be avoided 2 weeks *before* and *after* PRP therapy because they have been shown to alter platelet function.[57]

A dedicated rehabilitation therapy program is often recommended in conjunction with PRP therapy to achieve and maintain the fullest musculoskeletal potential and performance level. Since the effects of certain modalities on PRP have not been well documented, therapeutic ultrasound, electrostimulation, and hydrotherapy are not recommended during the 4 weeks *following* PRP therapy.

STEM CELL THERAPY

Stem cells are the body's progenitor cells, from which all other cells are derived. Recent studies have shown that stems cells can regenerate and heal injured tissue, decrease inflammation, stimulate new blood supply to support healing, activate resident stem cells, create a scaffold for healing tissue, protect cells from death, and break down scar tissue.[9,10,58-61]

Mechanisms of Stem Cells

The mechanisms by which stem cells initiate healing within the body are complex. Mesenchymal stem cells (MSCs) become immunosuppressive after activation by soluble factors; then secrete factors that inhibit T-lymphocyte activation and proliferation.[9,10,58-61] They:

- Use their diverse plasticity to help numerous types of injured tissues regenerate and heal
- Decrease proinflammatory, while increasing anti-inflammatory, mediators
- Secrete bioactive levels of cytokines and growth factors that support angiogenesis, tissue remodeling, differentiation, antiapoptotic events, and neovascularization.[9,10,58-61]

Orthopedic Treatment Indications

Recent studies have demonstrated the efficacy of stem cell therapy for canine osteoarthritis.[62-64] While many factors play a role in the decision to choose stem cell therapy for a patient, in our experience, patients with severe to end-stage osteoarthritis typically respond better to a combination of stem cell and PRP therapy rather than PRP therapy alone.

A recent study in dogs with elbow osteoarthritis caused by spontaneous fragmented coronoid process demonstrated that those that underwent arthroscopic fragment removal and a proximal ulnar ostectomy and received stromal vascular fraction (SVF; see **Adipose-Derived Stem Cells**, page 56) or allogeneic stem cells had a more favorable outcome than those treated with surgery alone.[62]

In another recent study, dogs with hip osteoarthritis that received a single intra-articular injection of adipose-derived cultured stem cells had a better outcome than control patients and those that received plasma rich in growth factors (PRGF).[12]

Similarly, other recent studies also demonstrated superiority in osteoarthritic dogs treated with adipose-derived cultured stem cells over control patients and those treated with PRGF on controlled blinded force platform analysis.[13,63] A recent study in a dog with a gastrocnemius strain concluded that stem cell therapy with a custom, progressive, dynamic orthosis may be a viable, minimally invasive treatment option.[64]

Sources of Stem Cells

Stem cells that can be obtained from the patient's own body are called *autologous adult-derived MSCs*. The most common places from which to harvest adult-derived MSCs are the patient's bone marrow or adipose tissue (**Figures 3 and 4**, page 56). Both bone marrow-derived and adipose-derived stem cells can differentiate into cartilage, bone, tendons, and ligaments. To date, no evidence supports superiority of one over the other in terms of viability or efficacy of the derived stem cells. However, adipose tissue may be a preferred source in dogs (see **Adipose-Derived Stem Cells**).

Stem Cell Procedure

Once the sample is obtained, it is processed and prepared for injection. Both bone marrow-derived stem cells and adipose-derived stem cells can be processed onsite or shipped to a university or private company for processing, culturing, and banking for future use.[65]

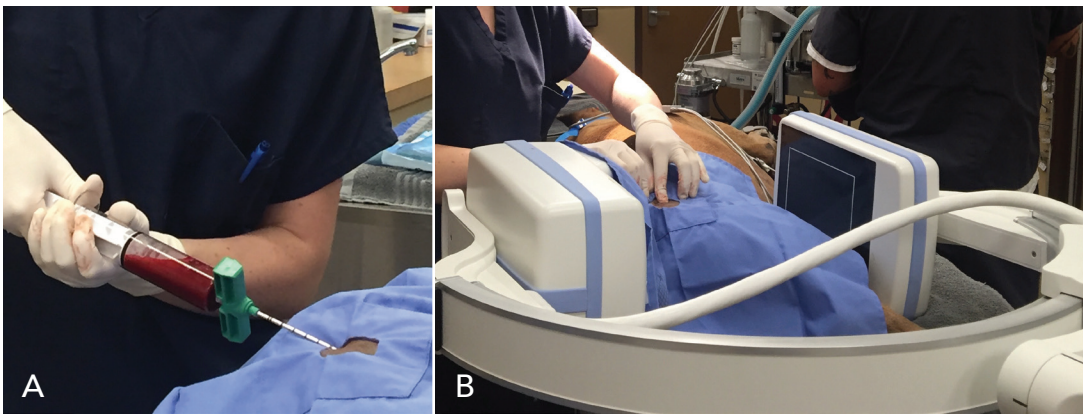

FIGURE 3. Bone marrow collection (**A**) from the proximal femur with fluoroscopic guidance (**B**) for processing of bone marrow aspirate concentrate (BMAC).

As with other forms of regenerative medicine, stem cell therapy is a minimally invasive procedure that typically can be performed on an outpatient basis with or without sedation, depending on the location of the injection. In addition, because recent studies have shown that PRP recruits and stimulates stem cells, PRP is often combined with stem cells before injection to both activate and act as a scaffold for the stem cells.[43,66-71]

Adipose-Derived Stem Cells

Almost all veterinary research has focused on adult stem cells, specifically MSCs, derived from bone marrow (BM-MSCs) or adipose tissue. Adipose tissue may be a preferred source in dogs for several reasons, including ease of access, low morbidity and pain associated with collection, and high-yielding MSC count (especially falciform) (**Figure 4**).

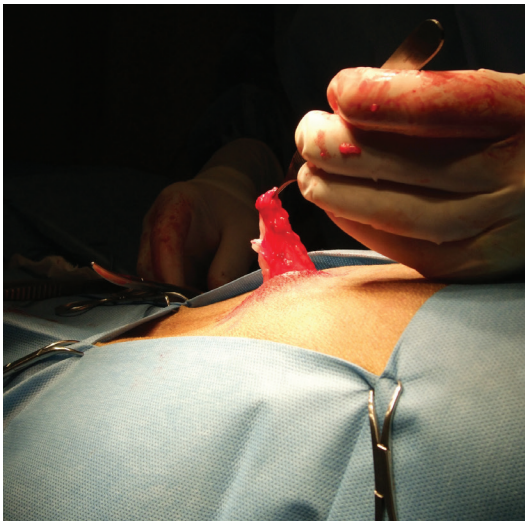

FIGURE 4. Collection of adipose tissue from the falciform ligament for processing of stromal vascular fraction (SVF).

The cells isolated from the adipose tissue include not only the MSCs but also endothelial progenitor cells, pericytes, immune cells, fibroblasts, and other growth factor-secreting bioactive cells. The use of this combination of stem cells and other regenerative cells is known as SVF therapy, and this mixture can be injected directly into the injured tissue or joint or can be administered by IV route. However, recent studies have shown that stem cells given by IV do not actually reach joints or injured tissues[72]; thus, for orthopedic applications, we currently do not recommend administering stem cells IV.

Alternatively, stem cells can be isolated from adipose tissue, cultured, and expanded. This technique, which yields a more homogenous population with a larger quantity of cells for injection, is known as *adipose-derived cultured progenitor cell*, or *ADPC*, *therapy*. To date, no studies show superiority of adipose-derived SVF versus culture-expanded adipose-derived MSCs for treatment of canine orthopedic conditions.

Bone Marrow-Derived Stem Cells

BM-MSCs are most commonly used in equine regenerative medicine but can also be used in dogs. There are 2 primary techniques for canine BM-MSC therapy: bone marrow aspirate concentrate (BMAC) and cultured-expanded.

Only 2% to 4% of the mononuclear cell population of bone marrow is considered an MSC. The BMAC technique evolved such that the nucleated cellular portion of tissue aspirates obtained from bone marrow was concentrated and then applied to the injured tissue. This therapy is appealing for several reasons:

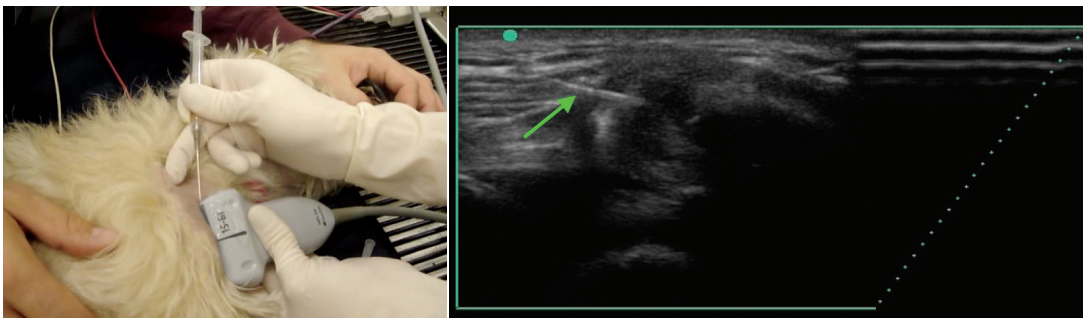

FIGURE 5. Ultrasonography-guided injection of BMAC and PRP into the supraspinatus tendon. The **green arrow** points to the needle being inserted into the supraspinatus tendon.

- The cells can be processed quickly for faster therapeutic application. Processing takes only 1 to 2 hours if it can be performed in-house by using a commercially available kit, which allows the practitioner to initiate therapy 3 to 4 weeks earlier than can be done with culture-expanded cells.
- These cells are not manipulated in culture to the extent that culture-expanded cells are, meaning that they do not undergo adherence, expansion, or trypsinization through multiple passages, which can alter cellular phenotype.
- This cellular therapy also delivers portions of the bone marrow cell pool that could potentially participate in tissue regeneration.

Alternatively, BM-MSCs can be isolated, cultured, and expanded. This yields a more homogenous population with a larger quantity of cells for injection. To date, no studies show superiority of BMAC over culture-expanded BM-MSCs in the treatment of canine orthopedic conditions. In addition, no studies have documented the superiority of BM-MSCs over adipose-derived stem cells or identified the number of stem cells needed for treating soft tissue injuries or osteoarthritis.

REHABILITATION AFTER THERAPY

A dedicated rehabilitation therapy program guided by trained and certified individuals in canine rehabilitation is often recommended for 12 weeks after regenerative medicine therapy, depending on the diagnosed condition. Rehabilitation therapy should be performed weekly in conjunction with an at-home exercise program.

During PRP/Stem Cell Therapy

Rehabilitation therapy helps speed healing by decreasing inflammation and swelling, building muscle mass, increasing range of motion, and improving overall comfort. Therapy sessions often include manual therapies, standard isometric exercises, and class IIIb low-level laser therapy, which is recommended because recent studies have shown that this laser therapy can stimulate stem cell differentiation, proliferation, and viability.[73]

Certain therapies are contraindicated within the first 8 weeks of regenerative medicine therapy because their effects on stem cells and PRP have not been fully studied; these therapies include class IV low-level laser therapy, therapeutic ultrasound, shockwave therapy, neuromuscular electrical stimulation/transcutaneous electrical neurostimulation, and nonsteroidal anti-inflammatory drugs.

After PRP/Stem Cell Therapy

Underwater treadmill therapy can usually be initiated 8 weeks after the start of rehabilitation therapy.

Once the tissue has healed, as confirmed via orthopedic examination, gait analysis, and diagnostic ultrasonography or needle arthroscopy,

Procedural Pearls

Injection of PRP or stem cells is a minimally invasive procedure that typically can be performed on an outpatient basis.

- Sedation or general anesthesia may be required, depending on the location of the injection; sedation is required for injections administered under ultrasound guidance.
- Joint injections are usually performed without sedation; however, some joints, such as the hip, require sedation and may also require advanced imaging (fluoroscopy) for guidance. If one is not familiar with joint injections, it is wise to sedate patients until comfort with the procedure is obtained.
- For soft tissue injuries, ultrasonography guidance ensures accuracy of the injection because both PRP and stem cells are most effective when administered directly into the site of injury (**Figure 5**).
- The most common side effect is mild discomfort associated with the injection, which typically resolves within 12 to 24 hours.

the rehabilitation program focuses on strengthening and conditioning.

After appropriate muscle mass has been attained, dogs are cleared for retraining and return to sport. On average, patients treated with regenerative medicine therapy typically return to competition or normal activity within 4 to 6 months of treatment.

IN SUMMARY

Regenerative medicine has been used to stimulate healing and help restore injured tissues to their original or near-original condition. Canine regenerative medicine therapy can be used to help treat medial shoulder syndrome, shoulder tendinopathies (eg, supraspinatus tendinopathy or biceps tendinopathy), iliopsoas strain, Achilles tendon injury, early partial cranial cruciate ligament tear, carpal and tarsal ligament injuries, and osteoarthritis.

It is important to obtain a definitive diagnosis and ensure that the patient is an appropriate candidate for regenerative medicine. It is equally important to incorporate a dedicated rehabilitation therapy plan into the recovery process to optimize results from regenerative medicine therapy.

ADPC = adipose-derived cultured progenitor cell; BMAC = bone marrow aspirate concentrate; BM-MSC = bone marrow–derived mesenchymal stem cell; MSC = mesenchymal stem cell; PRGF = plasma rich in growth factors; PRP = platelet-rich plasma; SVF = stromal vascular fraction

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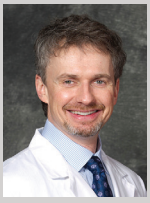

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