PET ORTHOBIOLOGICS AND REGENERATIVE MEDICINE

KANSAS CITY CANINE ORTHOPEDICS

Orthobiological treatments include a number of different products such as “stem cells”, stromal vascular fraction, platelet-rich plasma, and others that are purported to promote biological activity, reduce inflammation, and tissue healing. Consequently, use of biological therapies are common methods of treatment in the field of “Regenerative Medicine”, the practice of trying to facilitate tissue healing or regeneration. Dr. Sam Franklin has extensive experience with orthobiologics and Pet Orthobiologics, having performed and published numerous research studies, book chapters, and presented nationally and internationally on use of platelet-rich plasma, cultured stem cells, stromal vascular fraction, autologous protein solution, and bone marrow aspirate concentrate.

Orthobiologics

Platelet-Rich Plasma

Platelet-rich plasma, or PRP for short, is the most thoroughly investigated and likely the most commonly used orthobiologic treatment in canine medicine. Platelet-rich plasma is made by drawing blood from the patient and then centrifuging the blood to separate the blood into its separate components including the red blood cell fraction, the platelet-poor plasma fraction, and the platelet-rich plasma fraction. The platelet-rich plasma fraction is, as its name denotes, rich in platelets. Platelets play an important role in clotting of blood, but they also release anabolic growth factors that consistently result in enhanced growth, expansion, and multiplication of different cell types in the laboratory, including cartilage, bone and tendon cells. Accordingly, numerous studies in dogs have shown that injecting PRP into abnormal joints in dogs can improve dogs’ comfort and function. There are fewer studies evaluating the injection of PRP into injured or abnormal tendons but there is the suspicion that injecting PRP into injured tendons may help control inflammation and improve tendon healing.

Autologous Protein Solution (APS)

Autologous protein solution is produced by centrifugation of the patient’s own blood, collection of plasma (akin to platelet-rich plasma), mixing of this plasma with activating and dessicating beads, and then a second centrifugation. There are two studies evaluating the efficacy of APS in dogs with naturally-occurring disease. The first study compared efficacy of APS to saline in a group of dogs with either elbow or stifle pathologic change. Based upon force plate data showed significant improvement at 12 weeks post injection; dogs treated with saline did not show improvement. More recently, the Dr. Franklin performed a small pilot study in dogs with bilateral hip osteoarthritis in which one hip was treated with saline and the other with APS. At study end one month later the limb treated with APS had significantly greater weight bearing. Therefore, these two studies are consistent in showing superiority of APS to saline in improving weight bearing with intra-articular injection. However, the studies are limited in the number of dogs completing them (19 and 5 respectively), the duration of follow-up, and the comparison group was saline in both studies. Hence, this is why Dr. Franklin is now doing a follow-up study comparing APS to a corticosteroid and hyaluronan and following dogs for 6 months.

Stromal Vascular Fraction

Stromal vascular fraction (SVF) is commonly, but inaccurately, referred to as “stem cell therapy” and is one of the first orthobiologic treatments to gain substantial attention and use in veterinary medicine. Production of stromal vascular fraction involves anesthetizing the patient, surgically removing a relatively small amount of fat, and then processing the fat by chopping it up, digesting it with collagenase to liquify the fat, and then centrifuging the liquid product to separate the fat into several different layers with different composition. The stromal vascular fraction layer contains numerous different cell types including, white blood cells, red blood cells, and a very small (<10%) percentage of fat-derived stem cells. There is no culturing of the SVF in a laboratory. This processing can be done patient side or the fat can be shipped to a commercial laboratory for processing and then shipped back to the hospital/clinic for administration to the patient. There are several studies that document to some extent the efficacy of treating osteoarthritis in dogs using SVF. In the opinion of the doctors at Kansas City Canine Orthopedics, that evidence for SVF is not as convincing as that for PRP for example. Consequently, we tend to use PRP more commonly for application into joints of dogs with osteoarthritis. With that said, Kansas City Canine Orthopedics does have experience using SVF, has done research on and published on use of SVF, and has the ability to procure and use SVF if we think it is the right option for your dog.

Cultured Autologous Stem Cells

Cultured stem cells are obtained and produced by anesthetizing the dog and acquiring either fat or aspirating bone marrow from the center of a bone. The sample is then shipped to a laboratory and cells from the fat or bone marrow are then extracted and cultured for a period of weeks. During the process of cell culture, the population of cells becomes more homogeneous and the number of cells increases as the cells divide and multiply. The cells are subsequently harvested, packaged, shipped back to the hospital, and administered to the patient. One of the potential advantages of cultured stem cell therapy is that a far greater number of cells are administered to the patient as a result of the culture-associated multiplication of the cells. Despite this conceptual advantage, there are few studies directly comparing cultured cell therapy to less involved and less expensive alternatives such as PRP. However, as with PRP and stromal vascular fraction, Dr. Franklin has both clinical and research experience with cultured stem cell therapy and can provide this service for patients for whom we believe this would be beneficial.

Autologous Conditioned Serum/Interleukin Receptor Antagonist Protein

Autologous Conditioned Serum (ACS) is often also referred to as Interleukin Receptor Antagonist Protein (IRAP). ACS is prepared by acquiring blood from the dog, placing it in a container filled with propriety borosilicate beads, and then incubating the blood at body temperature for about 24 hours. During this period of incubation the white blood cells are supposed to be activated by the borosilicate beads to release interleukin receptor antagonist protein, or IRAP. This protein blocks the effects of interleukin 1β, the most influential cytokine that results in joint deterioration in patients with osteoarthritis.

After incubation of the blood the serum portion, which is rich in interleukin receptor antagonist protein, is harvested and then injected into the osteoarthritic joint. This treatment has been used commonly in horses for years, and has been used and studies in humans, but is only now gaining use and popularity in canine medicine. To date, there is not a single research study documenting the efficacy of ACS in dogs, although studies are ongoing. The doctors at Kansas City Canine Orthopedics now have the ability to provide this therapy, but acknowledge that experience with it is more limited. We are eagerly awaiting publication of evidence that either supports or refutes its use in dogs.

Bone Marrow Aspirate Concentrate

Bone marrow aspirate concentrate, or BMAC, is prepared by sedating the dog and aspirating a blood from the bone marrow of either the femur, humerus, or ilium. The bone marrow aspirate is then processed in a centrifuge, similar to how PRP is concentrated using a centrifuge, to try and concentrate the stem cells that live in the bone marrow into a small volume of blood. This concentrated portion of blood that contains bone-marrow derived stem cells is then immediately injected back into the patient. BMAC is not commonly used in veterinary medicine although a couple studies have evaluated its use with injection into dog knees and for augmenting bony healing in dogs. In human orthopedics, BMAC has been injected into joints to treat cartilage injury, as an adjunct to rotator cuff repair, and to augment bone healing.

Applications in Canine and Feline Orthopedics

There are several applications for biologics in canine orthopedics. The most common is the injection of an orthobiologic into an arthritic joint to try and ease pain and dysfunction attributable to osteoarthritis or joint deterioration. There is relatively good evidence for such application in dogs. However, it is important to note that such treatments are not shown to ‘re-grow’ the cartilage in joints with substantial cartilage wear and osteoarthritis. The goal of such treatment is to minimize inflammation and pain and improve mobility and function. As stated above, this has been shown. However, while these goals are often achieved, the benefits of the injection wear off after several months and the injections are often repeated every few months for those patients that benefit but that later show recurrent lameness when the effects wane.

Another common application is the ultrasound-guided injection of biologics into injured tendons such as the supraspinatus tendon near the shoulder, the iliopsoas tendon near the hip, or the Achilles tendon. Laboratory studies show that when tendon cells are treated with PRP the tendon cells proliferate and multiply rapidly, suggesting that PRP could be effective for treating tendon injury. Evidence proving that such treatment (i.e. injection of PRP through the skin and into the tendon) is effective in dogs with tendon injury is more limited. However, the ease, safety, relatively low cost, and some evidence of benefit make these appealing options for chronic tendinopathy cases that typically have a slow, prolonged recovery. Similarly to percutaneous injection of PRP into injured tendons, activated PRP gels can be placed in and around ruptured tendons at the time of surgical repair. Studies done in dogs have shown that intra-operative placement of activated PRP does enhance the healing response of such tendons. Consequently, placement of PRP at the time of Achilles tendon repair for example, is occasionally performed to enhance tendon healing.

Orthobiologics, and PRP, BMAC, and cultured stem cells, have also been evaluated for injection into knees of dogs with cranial cruciate ligament disease and "partial rupture" of their CCL. To date there is effectively no evidence that orthobiologics significantly decrease the likelihood that dogs with CCL disease will progress to complete ligament rupture. More specifically, one study compared the use of PRP to dogs that received no treatment and failed to find any decrease in the likelihood of complete CCL rupture with PRP. Studies using cultured stem cells for such purpose are ongoing and have not yet substantiated such use of cultured stem cells. One case series exists that describes treating these dogs with PRP + cultured stem cells or PRP + bone marrow aspirate concentrate. The study attempted to claim that such treatments were efficacious. However, such study never compared these treatments to dogs that didn't receive such treatments and there were a substantial percentage of patients that progressed to have complete ACL rupture and were treated with surgery. As a result, at Kansas City Canine Orthopedics we will be willing to discuss and provide such treatments for dogs with CCL disease, but we will warn all such owners that progression to complete ligament rupture is very possible if not probable. Accordingly, because performing TPLO in dogs with ACL disease and partial ligament rupture has been shown to preserve the remaining CCL in some dogs, TPLO surgery tends to be our recommendation for dogs that are symptomatic (i.e. limping, having difficulty rising, having decreased quality of life) for ACL disease.

PRP and BMAC have also been used and studied for augmenting bone healing in dogs. Although one study showed improved bone healing in dogs using PRP, other studies have not shown benefit. Dr. Sam Franklin performed an extensive, involved study funded by the AO Foundation to assess whether PRP placed in the osteotomy site at the time of TPLO would hasten healing of the TPLO. The study effectively showed that TPLOs, with or without PRP, healed equally effectively and in an average of just 10 weeks. Therefore, we do not tend to use PRP to hasten bone healing in dogs treated by TPLO.

Regulation of Orthobiologics

The Center for Veterinary Medicine at the Food and Drug Administration regulates all "cell-based products" for animal use and the FDA issued "Guidance for Industry: Cell-Based Products for Animal Use" in June 2015. One of the major purposes of this document is to provide guidelines to companies on what is needed to achieve FDA approval for their cell-based therapy. Such guidelines are most stringent and most relevant to cell-based therapies that come from species other than dogs (xenogenic stem cells from pigs for example), cell-based therapies that come from dogs other than the patient itself (i.e. allogeneic), or autologous products that have undergone substantial manipulation outside the body (culture expansion of stem cells in a laboratory that will then be administered back to the patient in the future for example). There is far less concern or scrutiny by the FDA over autologous (made from one's self) products that are minimally manipulated, such as PRP and BMAC. With that stated, the following are true. Many orthobiologics continue to be used in veterinary medicine with few if any adverse events reported including use of PRP, BMAC, IRAP, cultured autologous stem cells, and stromal vascular fraction. However, none of these treatments are FDA approved at this time.

Pre-Injection PRP Instructions

Virtually all patients are sedated, not anesthetized, for their PRP injections. Please refrain from feeding your dog breakfast the morning of their injection. Patients will go home the same day as their injection. Please note that due to sedation, patients may be slightly groggy for a few hours afterward.

Patients will have hair clipped under the neck for blood collection, on their forearm or foreleg for catheter placement, and at the location of the injection (eg over the joint of interest).

Post-Injection PRP Instructions

Your dog may resume eating and drinking as soon as your dog seems adequately awake to eat and drink. Most dogs are uncomfortable following joint injections for 24-48 hours. Please make sure you have a pain medication available, such as a canine specific non-steroid anti-inflammatory, to give to your dog for 48 hours afterwards. If you do not have a pain medication please ask our staff at the time you are checking out.

If your dog remains more uncomfortable for 96 hours following injection please contact a veterinarian. This would raise concern for the possibility of an infection in the joint. The chance of this complication is very low, far less than 1%, but is not something that we want to leave untreated if it occurs.

Exercise: Your dog can resume exercise after a joint injection. In general, we advise that exercise be consistent with their pre-injection routine as soon as they are ready (for example, after 48 hours after the initial discomfort of the injection has passed). Dramatic increases in exercise beyond a typical daily routine should be avoided to avoid making the dog dramatically more sore and hindering our ability to determine whether the injections were beneficial.

Timeline for recognizing the benefit of injections: Dogs will rarely show demonstrable benefit in less than 2 weeks following a PRP joint injection thus we have to wait at least 2 weeks before drawing conclusions regarding benefit. Most dogs that will show benefit will do so by 6 weeks. Therefore, if a dog has not appeared to benefit by 6 weeks following injection, that dog may not respond at all. However, the optimal protocol is not yet clearly established (see next).

Repeat injections: There is no clearly defined (i.e. optimal) injection protocol with regard to the number of injections or frequency of injections. Dr. Franklin typically offers owners 2 protocols (though more are possible): 3 injections each spaced 1 month apart. This protocol offers the benefit of repeat delivery of the anabolic growth factors in the PRP but also allows a reasonable time frame in between for owners to try and gauge whether there has been some benefit of the injection(s). Note that Dr. Franklin does offer the use of frozen/thawed injections. He has done research showing the anabolic growth factor concentrations in such frozen aliquots of Angel PRP remain very high. However, we do not know if frozen injections are equally efficacious as fresh injections. The second protocol to choose from is one in which a single injection is performed, the response evaluated by the owner, and an injection is repeated when the benefit (if seen) wanes. Some doctors, veterinarians, and owners claim benefits for up to a year. The objective research suggests a maximal benefit of intra-articular injections in dogs to last up to 6 months.

Evidence / Resources

The following studies can be provided to owners upon request.

Franklin SP. A Pilot Clinical Study Assessing Treatment of Canine Hip Dysplasia using Autologous Protein Solution. Frontiers in Veterinary Science 2019; 6:243 doi.org/10.3389/fvets.2019.00243

Franklin SP, Birdwhistell KE. Assessment of Canine Autologous Conditioned Plasma™ Cellular and Transforming Growth Factor-β1 Content. Frontiers in Veterinary Science. 2018;11(5):105. doi: 10.3389/fvets.2018.00105

Ludwig HC, Brainard BM, Birdwhistell KE, **Franklin SP.** Use of a cyclooxygenase-2 inhibitor does not inhibit platelet activation or growth factor release from platelet-rich plasma. American Journal of Sports Medicine. 2017;45(14):3351-3357. doi: 10.1177/0363546517730578

Birdwhistell KE, Karumbaiah L, **Franklin SP.** Sustained release of transforming growth factor-β1 from platelet-rich chondroitin sulfate glycosaminoglycan gels. Journal of Knee Surgery. 2017. doi: 10.1055/s-0037-1603801.

Franklin SP, Burke E, Holmes SP. The effect of platelet-rich plasma on osseous healing in dogs undergoing high tibial osteotomy. PLoS One. 2017; May 16;12(5):e0177597. doi: 10.1371/journal.pone.0177597.

Franklin SP, Birdwhistell KE, Strelchik A, Garner BC, and Brainard B. Influence of Cellular Composition and Exogenous Activation on Growth Factor and Cytokine Concentrations in Canine Platelet-Rich Plasmas. Frontiers in Veterinary Science. April 5, 2017. https://doi.org/10.3389/fvets.2017.00040

Birdwhistell KE, Basinger RW, Hayes BR, Norton NA, Hurley DJ, **Franklin SP**. Validation of commercial ELISAS for quantifying anabolic growth factors and cytokines in canine ACD-A anticoagulated plasma. Journal of Veterinary Diagnostic Investigation. 2017 Mar;29(2):143-147 DOI: 10.1177/1040638717690186.

Franklin SP, Garner BC, and Cook JL. Characteristics of canine platelet-rich plasma prepared with five commercially available systems. American Journal of Veterinary Research, 2015; 76:822–827.

Franklin SP and Cook JL. Prospective trial of autologous conditioned plasma versus hyaluronan plus corticosteroid for elbow osteoarthritis in dogs. Canadian Veterinary Journal, 2013;54(9):881-884.