The use of stem cell therapeutics has become one of the largest areas of scientific research around the globe. It was first introduced into equine practice in 1995 with the injection of bone marrow derived cells into tendon injuries. The use of stem cell in canine bone marrow transplants date back as early as the 1960's. Since, it has found great interest in its use in small animals and humans for many different medical conditions, in particular osteoarthritis. It is estimated that 10 to 12 million dogs in the US are affected with osteoarthritis, and it is the most common cause of chronic pain. Stem cells are unspecialized cells capable of self-renewal and differentiation into a specialized cell, and perhaps any type of tissue in the body that is needed. Another term for regenerative medicine is Prolotherapy. Prolotherapy is a regenerative injection treatment used to stimulate the healing mechanism to repair damaged or injured areas in the spine and joints. This term may be inclusive of both stem cell therapy and platelet rich plasma. Both treatment modalities can be used in conjunction to serve as a transport to the intended treatment area.

Regenerative medicine therapies should only be implemented or recommended following a thorough examination with a Veterinarian. Diagnostic imaging and further treatment options should be discussed prior to starting prolotherapy as it is known to be contraindicated in the presence of cancer, infection, coagulopathies, NSAID's and steroids.

The patients are monitored closely before, during and after initiating treatment for any adverse effects. By routinely tracking our patients closely with examinations, surveys and all otherwise client communications; we are able to adjust their therapies as needed and determine the patients progress.

Cell Differential	Technique	Indications/Comments
Mesenchymal Stem Cells (MSC's)		
• Primarily found in abundance in adipose tissue. Can also be found in bone marrow, dental pulp (marrow) and placenta. • Multipotent stem cells derived by culturing various biological tissues such as adipose and bone marrow. • Contains osteoblasts, myocytes, adipocytes, chondrocytes	• MSC's are found in excess in **cultured** adipose tissue and bone marrow. • Sample size is system dependent ranging from 5g-60g+ minimum. • Culturing, cloning and storage is available through cGMP certified labs. • Processing In-house per manufacturer	• Induces antiapoptosis (VEGF, TGF-B, bFGF) • Angiogenesis IGF-1, IL-6, VEGF) • Chemoattractants induce leucocyte migration to injured tissue. **See Below**
Bone Marrow Derived Mesenchymal Stem Cells (BM-MSC's)		
• BM-MSC's form superior cartilage compared to AD-MSC's • Contains osteoblasts, myocytes, chondrocytes • Abundant in multipotent cells • Pluripotent cells present with the ability to regenerate as other cell lineage.	• Collected from long bones such as the iliac crest, femur or humerus. • Should be administered through a micron filter to prevent a systemic infection due to the higher count of neutrophils and debris that may be present in the sample IF processed in-house. • Culturing, cloning and storage is available through cGMP certified labs. • Have limited plasticity. • General anesthesia needed to collect sample. • BM-MSC's have a slower PDT than AD-MSC's. • Processing In-house per manufacturer • 600,000 to 2 million stem cells per 1g of BM can be expected with SVF processing based on system .	• Can be used for bone marrow transplantation following chemotherapy to regenerate the destroyed bone marrow. • Used in conjunction with HSC's and PRP to recruit cells where needed. • Tissue Regeneration • Lineage priming • Heterogeneity • Osteoarthritis • Degenerative Joint Disease • Spondylosis • Sprains/Strains • Ligament Ruptures • Gastrointestinal diseases (IBD, Chrohn's, etc.) • Autoimmune Disorders such as Lupus and MS • Diabetes • Cataracts • Self-renewing. May harvest samples multiple times as/if warranted.

		• Will differentiate into a variety of mesodermal lineages
Culturing MSC's will prevent the cells from passing through the blood-brain barrier due to their engorged diameter.		
Adipose Derived Mesenchymal Stem Cells (AD-MSC's or AMSC's)		
• Contains osteoblasts, myocytes, adipocytes, chondrocytes • Abundant in multipotent, pluripotent and unipotent cells • Produces 100x the amount of MSC's than BM-MSC's**.** o Not a self-renewing harvest site.	• Gonads will yield the highest number of cells. • Falciform ligament is the most commonly used site for harvesting adipose tissue • The scapula, flank and inguinal canal may also be used as a donor site • Culturing, cloning and storage is available through cGMP certified labs. • Adipose tissue has a particularly high concentration of regenerative cells due to the high density of micro vascularization of this tissue • These regenerative cells can be isolated, concentrated and administered at the point-of-use in a single procedure within the OR • Adipose tissue is not as essential as bone marrow • Adipose tissue can be harvested by a minimally invasive procedure under local anesthesia • **5-20 million stem cells per 1g of adipose can be expected with SVF processing.**	• Liver disease • Corneal lesions • Articular lesions • Cutaneous Lesions • Osteoarthritis • Degenerative Joint Disease • Spondylosis • Sprains/Strains • Ligament Ruptures • Gastrointestinal diseases (IBD, Chrohn's, etc.) • Other Autoimmune Disorders such as Lupus and MS • Diabetes • Cataracts
Hematopoietic Stem Cells (HSC's)		
• Constantly repopulating all types of blood cells.	• Have limited plasticity. • Collected from long bones such as the iliac crest, femur or humerus.	• Skin Laxity • Stimulation of hair growth • Acute myeloblastic leukemia.

• Bone marrow or blood derived. HSC's are more prominent in PRP, Serum therapy and IRAP • Multipotent stem cells derived by culturing bone marrow and whole blood. • Contains a higher number of WBC's and RBC's than MSC's. • *Not recommended by experts for IV or epidural use without filtration due to high counts of WBC's and RBC's. Peri-spinal injections are acceptable with caution. • Abundant in omnipotent, multipotent pluripotent and unipotent cells • Used to treat a wide variety of hematological disorders. • Found in cord blood, bone marrow and peripheral blood.	• Mechanically processed to separate from any MSC's present in the sample • Should be administered through a micron filter to prevent a systemic infection due to the higher count of neutrophils and debris that may present in the sample IF processed in-house. • Culturing, cloning and storage is available through cGMP certified labs. • Found sparingly in PRP and PPP. • General anesthesia needed to collect sample. • These regenerative cells can be isolated, concentrated and administered at the point-of-use in a single procedure within the OR • 600,000 to 2 million stem cells per 1g of BM can be expected with SVF processing based on system.	• Chronic myeloid leukemia. • Non-Hodgkin's lymphoma • Tay-Sachs disease. • Osteoarthritis. • Sport injuries – ligament ruptures. • Fractures. Can be complimented with the use of granulocyte-macrophage-colony stimulating factors to allow the HSC's present in the blood for recruitment. Similar to BM-MSC's in function, but are far less elastic.
Platelet Rich Plasma (PRP)		
• Commonly referred to as autologous conditioned plasma. • Is a whole blood product that contains a high concentration of platelets. • Contains various different cytokines and growth factors to treat a variety of deep and soft tissue injuries. • Abundant in pluripotent and unipotent cells • Scarce in multipotent cells	• Derived of whole blood. • Can be processed in-house or at a cGMP certified lab. • Processing varies depending on the system used. • All in-house systems require whole blood is collected under aseptic technicique; 10-60 mls needed. • Closed-system is highly encouraged to not contaminate the sample	• Ligament injuries • Sprains and Strains • Burn wounds • Cataracts • Open wound • Degenerate Joint Disease (DJD) • Stomatitis • Keratoconjunctivitis Sicca (KCS) • Bone Graft Healing (Osteotomies, Fracture Repairs

• Concentrated platelets in plasma • Platelets release growth factors IGF-I, PDGF, VEGF	during processing and prior to injection. • Platelet Poor Plasma (PPP) is considered the supernatant of PRP and the sample and is discarded during processing.	**The sample should be 3-4x the patients baseline platelet levels. A CBC may be ran on the final sample for quality control.**

Post Injection Care

- Therapeutic inflammation is often noted 2-7 days afterwards.
- Can be managed with pain medication (Gabapentin or Opioids)
- Avoid steroids and NSAID's 2 weeks afterwards and 1 week before injection.
- Hypothermic therapy to the area 1-2 times daily for 5-10 minutes where the injection was given. This therapy can decrease platelet activation.
- The pets activity should be restricted to mild/moderate activity for 3-4 weeks after a SC injection as to not cause a soft tissue injury.
- Commit to rehabilitation programs for 8-12 weeks.
- Redness from the injection site is NOT a normal side effect of any injection.

***As of 2019, there are over 9,000 published papers on the efficacious use of regenerative medicine.**

Other Forms of Regenerative Medicine

- Interleukin-1 Receptor Antagonist Protein (IRAP)
- Gene and Genome Therapy
- Tissue Engineering
- Autologous Cancer Therapy
- Biomedicine.

When Is Regenerative Medicine Indicated?

- Clearly defined diseases.
- Non-surgical candidate – surgery first, if needed.
- Can be used in conjunction with surgery.
- Limited intra-articular osteophytes
- One or two joints maximum injected at a time to reduce the risk of sepsis IF sample is contaminated.
- No other major systemic diseases such as cancer

- No major spinal disease
- NSAID – non-responsive or lack of tolerance
- Owner has rational expectations

Key Words and Terms

Hematopoietic Stem Cells (HSC's)
Mesenchymal Stem Cells (MSC's)
Adipose Derived Mesenchymal Stem Cells (AD-MSC's or AMSC's)
Bone Marrow Derived Mesenchymal Stem Cells (BM-MSC's)
Multipotent Stem Cells
Multipotent Progenitor Cells
Stem Cell Therapy (SCT)
Platelet Poor Plasma (PRP)
Platelet Poor Plasma (PPP)
Satellite Stem Cells
Neural Stem Cells
Embryonic Stem Cells
Osteoblasts
Myocytes
Adipocytes
Chondrocytes
Cytokines
Chemokines
Acute myeloblastic leukemia.
Chronic myeloid leukemia.
Non-Hodgkin's lymphoma
Tay-Sachs disease.
Osteoarthritis (OA)
Degenerative Joint Disease
Spondylosis

Platelet-derived growth factor
Insulin growth factor
Platelet Factor 4
von Willebrand factor
Basic fibroblast growth factor
Compassionate Care Use
Totipotent (*Omnipotent*)
Pluripotent
Unipotent
Autologous (autochthonous).
Allogeneic
Xenogeneic
cGMP Certified Laboratories
Thrombin
Calcium Chloride
Apoptosis
Antiapoptosis
Angiogenesis
Prolotherapy
Chemoattractants
Stromal Vascular Fraction

Rules and Regulations
US FDA regulates regenerative therapies under TITLE 21 Federal Regulation 1271 • Category I – whole blood, BM, organ transplants • Category II – cell & tissue products not dependent on metabolic activity of living cells, require minimal manipulation • Category III – dependent on metabolic activity of living cells and include products that are cultured or more than minimally manipulated, not intended for homologous use, combined with a drug or device • Currently, the FDA approves the use of autologous regenerative medicine therapy.

- **Allogeneic and Xenogeneic biological products may be used for organ and tissue transplantation, including blood-derived products such as with albumin and whole blood, plasma and platelets for emergent use, only.**
- Compassionate care use must be approved with the FDA prior to initiating treatment.

Precautions and Contraindications

• Pet's diagnosed with cancer should NOT be treated outside of a controlled setting. Local injections of regenerative cells may be injected. It is discouraged to give stem cells by intravenous use if a cancer morphology has been detected.
• Not a compatible therapy with infection.
  • Infection with *Pseudomonas, Enterococcus or Klebsiella.*
• Coagulopathies should be taken into consideration.
  • Thrombocytopenia.
  • Septicemia.
• NSAID use can still be considered (2 days).
• Do not use in conjunction with Steroids. (7 days) OR Immunosuppressant's (14 days).
• Should not be used to replace emergent intervention procedures.
• Antibiotic and bacteriostatic drugs can inhibit the effects of both SCT and PRP.

Recommendations and Standards of Care
Common Soft Tissue and Orthopedic Use
Brachii tenosynovitis, Supraspinatus insertionopathy, Infraspinaturs Contracture, Teres Minor Myopathy, ossification, laxity, tendinopathy and instability, flexor carpi ulnaris tendinopathy/avulsion, digital flexor tendon elongation, medial and lateral collateral liagement rupture, abductor pollicis longus tenosynovitis, psoas, iliopsoas, popliteal muscle avulsion, gastrocnemius muscle avulsion, superficial digital flexor tendon luxation. Acute and chronic fractures, luxations, osteoarthritis, osteophytes, hip dysplasia, myopathy, neuropathy, ligament ruptures, patellar and digital extensor tendonitis and achilles tendon avulsion.
PRP Dosing*

• 3-4x above the patients platelet baseline is currently what is recommended by the worlds leading experts. • 1-2x is more than likely not to produce a noticeable effect on the patients condition • 5x + may lead to a systemic or local infection due to the high number of WBC's present in BD-MSC's, HSC's, PPP and PRP • **+ Platelet concentration** • **- RBC concentration** • **+ Monocyte concentration** • **- Neutrophil concentration**
Stem Cell Dosing*
• Widely dependent on degree of injury/disease   o <u>More is not always better.</u> • Research shows that roughly a minimum of 1 million cells may be needed to elicit an efficacious response for a majority of injuries and illness. • In-house systems support data ranging from 1-5 million stem cells with a SVF sample • Culturing cells with a cGMP certified facility will yield the highest cell count
Regenerative Cell Activation
It is widely speculated that our own body's cells must be activated by the use of thrombin and calcium chloride to release various growth factors from the present alpha granules.
A Hgb < 10 g/dL and A PLT <10^5/µL. is recommended for PRP

Common Injection Sites		
Anatomical Landmark	**Technique/Approach**	**Recommended Volume***
Hind Limb		
Stifle	• Flex stifle to 90-degree angle • Appreciate joint space medially or laterally to patella ligament o Insert 22g Needle	0.5-3cc
Hip	• Lateral recumbency • Abduct hip	0.5-3cc

	• Insert 22g Spinal Needle cranial to the greater trochanter positioned medially and slightly dorsal into the joint.	
Hock	• Lateral recumbency • Flex the hock and approach the joint space from the caudomedial aspect • 22 g needle recommended	0.5-1cc
Achilles Tendon	• Lateral recumbency • Flex the hock and approach the tendon sheath from the caudal aspect; injecting directly into any lesions, adhesions or surgical repair site. • 22 g needle recommended	0.5-2cc
Thoracic Limb		
Carpus	• Lateral recumbency • Dorsal approach of the carpal joint • Flex the carpus to expose joint • 22g needle recommended	0.5-1cc
Elbow	• Medial aspect • Flexed or straightened • Insert 22 g needle distal to the medial epicondyle of the humerus.	0.5-1.5cc
Shoulder	• Lateral recumbency • Abduct humerus • Lateral approach off acromion process, aim it slightly proximal. ○ Use 22g 1 ½ in needle.	0.5-3cc
Other		
Epidural	• Place the patient in sternal recumbency with hindlimbs flexed. • Locate the lumbosacral space caudal to L7. Redirect needle if CSF or blood is seen. • Use 22g Spinal Needle	0.5-1cc
Muscle/Tendon Injuries	• Ultrasound is highly recommended to appreciate the intended injection site.	0.5-6cc*

	• Patient may be placed in sternal dorsal, or lateral recumbency.	
Pododermatitis, Wound and Skin Grafting	Dermal needle/insulin needle recommended	***Dependent on treatment area.**
***Dosing and volume administration will be dependent on the patient size, the treatment plan and area.**		

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