

Novel Regenerative Therapy Options for Intervertebral Disc Disease

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Abstract

Clinical aspects of using Adipose Derived Stem Cells to treat Intervertebral Disc Disease are discussed. A two-pronged therapeutic plan is described which involves treating disc degeneration and damaged spinal cord with stem cells. Stem cell treatment offers prophylactic options for discs that have not yet ruptured and therapy, both alone and combined with standard surgical techniques. Injection of stem cells into the nucleus pulposus of the degenerating disc causes a rapid regeneration of the matrix and rehydration of the disc. This injection is facilitated using C-Arm Radiology. In addition, the effective outcomes of hemilaminectomy surgery are doubled by utilizing Adipose Derived Stem Cells injections into the spinal parenchyma during the surgery procedure⁽¹⁾. Successful outcome is based on access to MRI and C-arm radiography, surgical technique, in-house processing of stem cells, and rehabilitation.

Introduction

Intervertebral disc disease (IVDD) is a serious disease of the vertebral column affecting the spinal cord, causing pain and neurologic damage. IVDD can affect all dogs but is predominantly a disease of dogs with chondrodystrophy. Chondrodystrophic pets are literally those with “bad cartilage growth,” including but not limited to the Dachshund, Corgi, Cocker Spaniel, Shih Tzu, Basset Hound, and Beagle. In the United States, there are approximately 300,000 dachshunds. Over 25% of dachshunds will suffer from IVDD during their lifetime, resulting in 75,000 pets requiring some form of treatment for IVDD.

Intervertebral Disc Disease Anatomy and Pathophysiology

The vertebral column supports the neck and back and is composed of bony vertebral bodies connected by cushioning intervertebral discs. The vertebral canal is the hollow space formed by the vertebral bodies occupied by the spinal cord. The spinal cord is the nerve bundle responsible for all nerve impulses to and from the body.

The normal intervertebral disc (IVD) cushion is shaped like a jelly donut. The interior “jelly” is made of a rich extracellular matrix that forms the central gelatinous nucleus pulposus, which contains cartilage-like cells. This “jelly” is contained by the rest of the “donut” or annulus fibrosus, made of tougher fibroblast-like cells. This cushioning jelly donut structure is connected to the adjacent bone of the vertebral bodies by cartilaginous endplates, Figure 1.

The “jelly” consists of collagen and proteoglycans which are large molecules capable of attracting and retaining water. Functionally, the collagen provides shape and tensile strength, while proteoglycans confer tissue viscoelasticity, stiffness, and resistance to compression, through their interaction with water. A proper balance between matrix synthesis and degradation is responsible for the maintenance of the integrity of the IVD and hence the mechanical behavior of the IVD itself. This is related to the activity of cellular signaling molecules such as cytokines, growth factors, enzymes, and enzyme inhibitors

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that act to modulate the action of the cells (and stem cells) that make the collagen and proteoglycans. Degeneration of the structure of the annulus fibrosus and the nucleus pulposus occur early in life in chondrodystrophic breeds resulting in shape and molecular changes in the IVD. Progressive degeneration results in loss of water from the “jelly” in the disc and eventual expulsion of the dehydrated “jelly” from the disc resulting in compression of the spinal nerves and spinal cord itself. Intervertebral Disc Disease (IVDD) occurs when the cartilage disc deforms or ruptures causing pressure and or damage to the spinal cord, Figure 2.

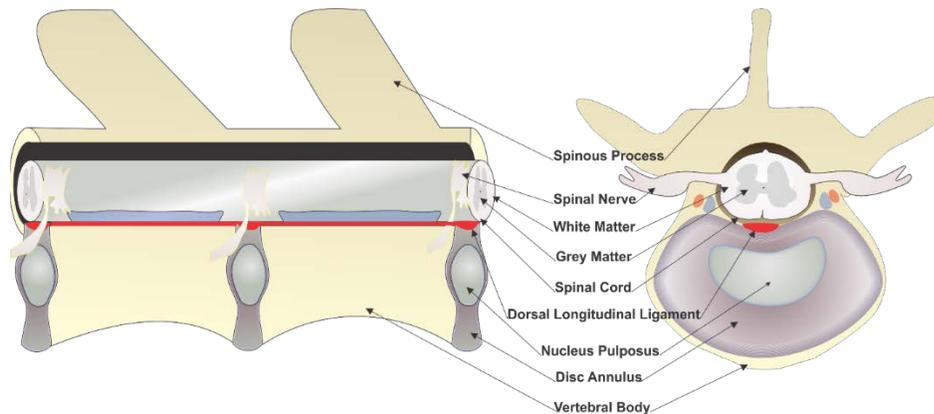


Figure 1: Normal Anatomy of Intervertebral Disc and Spinal Canal.

As the intervertebral disc degenerates we see dehydration and tears of the disc annulus and nucleus pulposus. At a molecular level, we see decreased cell viability and proteoglycan synthesis and reduced diffusion of nutrient and waste products. This leads to accumulation of dead cellular debris, degraded matrix macromolecules, and an increased degrading enzymatic activity along with a modification of the collagen distribution. ⁽²⁾ It is at this level that stem cells function to regenerate healthy matrix and reform the normal shape of the IVD.

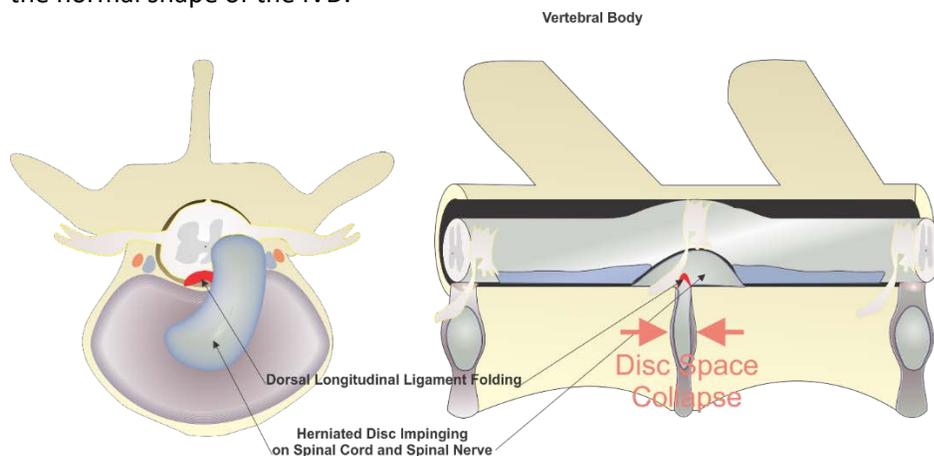


Figure 2: Intervertebral Disc Disease: Spinal nerve root and spinal cord compression caused by extruded nucleus pulposus. The disc space is narrower, causing folding and dorsal impingement of the spinal cord by the dorsal longitudinal ligament.

Spinal cord damage from IVDD results from outward to inward compression of the spinal cord. The spinal cord neurons are organized with the less important neurons on the outside and the more important ones on the inside. The outer neurons conduct proprioceptive messages that tell the location and position of the limbs to the brain. The innermost tissues of the spinal cord contain nerve tracts

responsible for relaying information from the brain to the muscles. The grey matter of the spinal cord contains nerve cell bodies responsible for spinal reflexes and for coordination. Initial damage to the spinal nerves results in pain as a primary sign, as compression becomes more severe, loss of proprioception is evident, followed by loss of coordination, followed by loss of motor control, then finally loss of deep pain perception. Localization of damage to one side of the spinal cord is evident by comparing the degree of damage and loss of nerve function on one side versus the other. Figure 3.

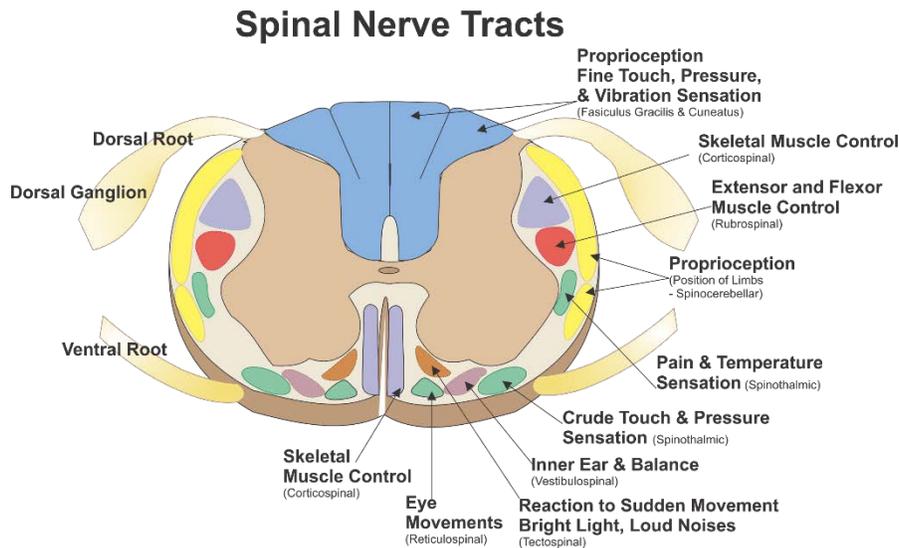


Figure 3: Spinal Nerve Tracts: Nerve bundles to and from the brain are organized into tracts. These tracts are compressed during IVDD resulting in loss of their function. Depending on the location and severity of the compression, different functions are lost allowing prognostic decisions to be made.

Central nervous system regeneration is highly limited after injury. Spinal cord injury (SCI) leads to cell death, particularly in neurons, oligodendrocytes, astrocytes, and precursor cells. Any cavities and cysts resulting from this cell death and loss may interrupt healing of axonal tracts. SCI culminates in glial scarring, a multifactorial process involving reactive astrocytes, glial progenitors, microglia, macrophages, fibroblasts, and Schwann cells⁽³⁾. Such scars are often oriented across the neuron path and contain transmembrane molecular inhibitors of axon growth, and appear impenetrable. Lack of regenerative capacities of adult spinal cords results from neuron growth inhibitors of myelin proteins, glial scar formation and a poorly defined extracellular matrix-derived factor⁽³⁾. Stem cells regenerate the spinal cord by differentiating into astrocytes and oligodendrocytes, as well as neuronal cells. Neurons derived from engrafted stem cells may relay signals from disrupted fibers in the host, including local circuit interneurons or ascending fibers that are present in the dorsal column and grey matter. This neuronal transdifferentiation (changing from one cell type to another based on chemical and molecular by-products) process that occurs with adipose derived stem cells results from the interactions of cells, cytokines provided by these cells, growth factors, and intercellular signals. Adipose derived stem cells have been shown to secrete multiple angiogenic (blood vessel growth) and anti-apoptotic (anti cell death) cytokines that support tissue regeneration and minimize tissue damage. Engrafted stem cells and the chemical factors released by the spinal cord injury play important roles in the proliferation, migration and differentiation of existing spinal cord stem cells. The adipose derived stem cells that survive produce large amounts of fibroblast growth factor and vascular endothelial growth factor in the host spinal cord. These cytokines have been shown to promote neurogenesis.

Adipose Tissue Derived Mesenchymal Stem Cell Therapy

Tissue engineering and regenerative medicine techniques offer long-term tissue repairing options with the possibility to restore intervertebral disc function. Stem cells have the potential to repopulate and regenerate the IVD. There are several potential sources of stem cells, including adipose tissue, bone marrow, and other tissues such as embryonic and fetal stem cells. Embryonic and fetal stem cells are pluripotent cells with a potential to differentiate into any body tissue. These cells are highly flexible since they can differentiate into any type of tissue, but both the procurement of embryonic stem cells and research are limited, given the related ethical issues. Embryonic cells are not used clinically in the treatment of pets. Figure 4.

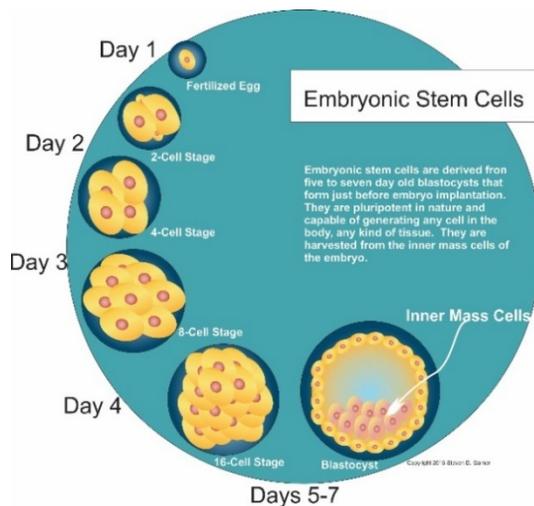


Figure 4: Embryonic Stem Cells: These cells are harvested from developing embryos at the Blastocyst stage, killing the embryo in the process. These cells have huge, unlimited potential but are difficult to control and have ethical issues associated with them. Therefore, we do not use these cells in a clinical setting.

stem cells for clinical use, given the ease of the procedure to retrieve adipose tissue. This procedure is minimally invasive and large numbers of cells are obtained. Treatment of IVDD can occur with an injection of autologous adipose stem cells (ASCs). The adipose derived cells can be mixed with Platelet Rich Plasma to add growth factors and increase potency. Hyaluronic acid can also be used to provide a support matrix for the stem cells⁽⁵⁾.

Adipose derived stem cells injected into collapsed, degenerated discs act to regenerate the disc. The stem cells produce a collagen, proteoglycan, and macromolecule matrix that is indistinguishable from a normal disc. This chemical structure attracts water,

Adult stem cells can be used to treat intervertebral disc disease. Many studies have shown that IVDD can be treated or repaired with the injection of Mesenchymal Stem Cells (MSCs)^{(3) (2) (4)}, multipotent cells able to differentiate into tissues of mesenchymal origin, including bone, cartilage, fat, muscle, and fibrous tissues, depending on the biological environment.

These cells can be obtained from multiple adult tissues, including bone marrow, trabecular bone, articular cartilage, muscle, and adipose tissue, which represent a variant of the adult stem cell, Figure 5.

While a variety of cell sources in the field of stem cell research have been proposed for clinical application, Autologous Adipose Stem Cells (ASCs) as a cell source in bone and cartilage repair have gained significant attention. In fact, adipose tissue is considered a suitable source of

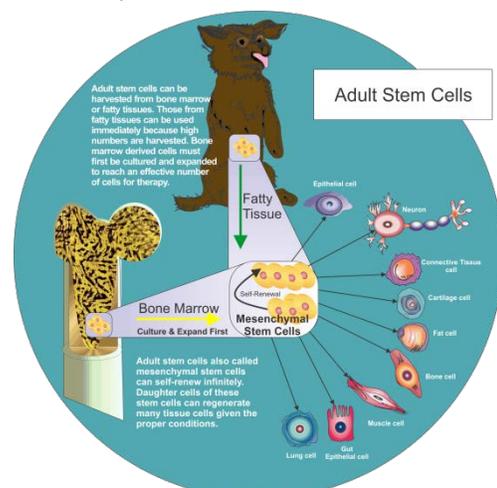


Figure 5: Mesenchymal Stem Cells. These adult stem cells may come from bone marrow or from fatty tissue. Bone marrow derived cells require culture and expansion prior to clinical use. Adipose derived mesenchymal stem cells are ready to use immediately. All stem cells may transmute into other tissue cells as depicted.

causing the collapsed disc to re-inflate and expand the disc space between the vertebrae. This flattens out the previously folded ligaments on the floor of the spinal canal, thereby reducing pressure on the spinal cord. The extruded disc material pressing against the spinal cord now has more space and less pressure on it. The anti-inflammatory effects of the ASCs and stromal vascular fraction of cells reduces damaging effects of the previously extruded disc material on the spinal cord. Disc regeneration is rapid, occurring within two weeks of treatment and continuing for at least six months after therapy. Disc regeneration can also be given to patients before complete degeneration and collapse of the disc space to prevent disease associated with compression of the spinal cord.

ASCs injected into damaged spinal cord, aid in healing by reducing the glial scar formation and promoting neurogenesis. Studies have shown that in dogs with no deep pain perception prior to surgical decompression with hemilaminectomy that received a single injection of ASCs into the spinal cord had better clinical outcomes than dogs with surgery alone. ⁽¹⁾ Improved clinical signs after transplantation of ASCs are primarily attributed to a modified inflammatory environment and increased survival of endogenous nerve cells. Moreover, transplanted ASCs reduce regulatory signal molecules related to glial scar formation and can partially differentiate into neural cells.

Procedures and Methods

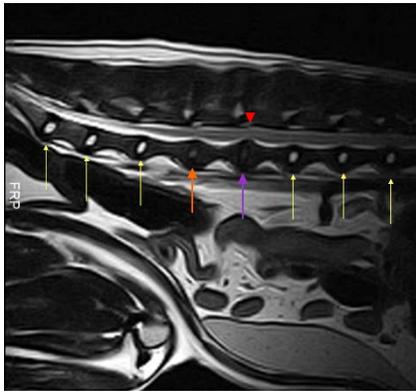


Figure 6: Sagittal T2 MRI image. Yellow arrow discs are hyperintense indicating high water content typical for normal disc. Orange arrow shows reduced intensity indicating less water and degeneration. Purple arrow shows degeneration and collapse with narrowing of the disc space as well as compression of the spinal cord (red arrow) due to dorsal longitudinal ligament. This pet was a 3-year-old shih tzu with back pain only. Injection of stem cells into all lumbar discs resulted in complete resolution of signs.

Clinical cases of IVDD present at different stages of the disease and so require customizing of therapy. Some pets present with mild behavioral abnormalities such as reluctance to jump, climb or descend stairs, or painful or stiff back or neck. Some pets have mild to moderate proprioception deficits. Other pets have ataxia, crossing of the limbs and reluctance or inability to stand while the most severe pets have no pain perception and are tetraparetic or quadriplegic. Almost all pets are candidates for stem cell therapy as a prevention or treatment with or without surgical decompression. However, the best decisions are made after complete neurologic

examination and imaging. MRI imaging is the preferred modality because not only can compression of the spine be perceived but also the amount of water within the disc is an indication of disc health and can be seen on T2 weighted MRI images ⁽⁵⁾, Figure 6 & Figure 9. CT

images have proven adequate when MRI is not available, and CT scans outperform myelogram studies when evaluating IVDD ⁽⁶⁾.

Autologous Adipose Derived Stem Cells (ADCs) are obtained from the patient by harvesting the fat filled falciform ligament through a 2cm midline abdominal incision anterior to the umbilicus. The fat is transferred to a sterile container and processed in a biological safety hood, Figure 7. The fat is minced, washed with PBS and mixed with collagenase type I then incubated, agitated, and centrifuged at 37°C to

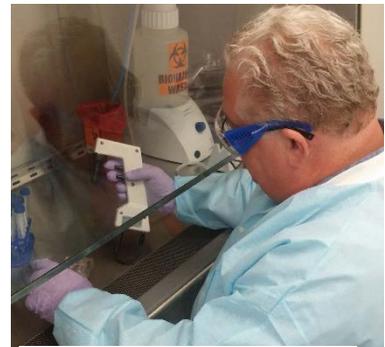


Figure 7: Biologic Safety Cabinet: Laminar flow prevents bacterial contamination when dealing with stem cell processing.

separate fat from the Stromal Vascular Fraction (SVF). The SVF contains mesenchymal stem cells, preadipocytes, endothelial progenitor cell, T cells, B cells, and mast cells, as well as adipose tissue macrophages. The SVF is filtered through a 100 µm pore filter then filtered again through a 60 µm pore filter. The cells are counted and a ratio of live cells to dead cells is used to calculate live cell doses.



Figure 8: C-Arm Fluoroscopic Radiology. Allows real-time precision needle placement into intervertebral discs.

In a separate process, blood from the patient is harvested for the creation of Platelet Rich Plasma (PRP)⁽⁷⁾. The stem cells are mixed with PRP to dilute them and stimulate them to release cytokines. The PRP is activated with autologous thrombin and mixed with the SVF in the proper proportions for administration of 100 µl volume containing 1 x 10⁷ live cells/ml.

Individual luer-lok syringes are prepared containing 100 µL stem cell mixture for each disc to be injected. This process takes about 2 hours. The pet is prepared

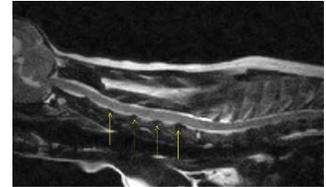


Figure 9: MRI Image: Degenerated discs causing pain and mild neurologic signs. This is the same 6 year old Yorkie in Figure 10 and Figure 13.

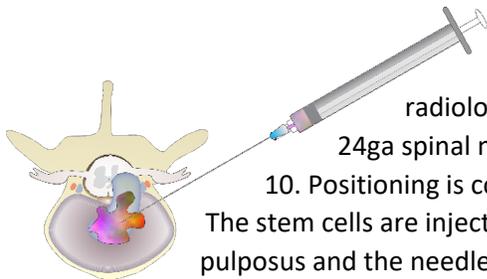


Figure 11: Nucleus Pulposus. Injection with Stem Cells.

for general anesthesia and the area of skin over the site is surgically prepared. A C-Arm radiology unit is used to properly guide 24ga spinal needles into the disc space. Figure 10. Positioning is confirmed at opposing 90° angles. The stem cells are injected into the center of the nucleus pulposus and the needle is then removed. Figure 11.



Figure 10: C-Arm Image. Needles placed into cervical spine for injection of stem cells into the disc.

For the animals who have significant neurologic disease, hemilaminectomy surgery is recommended with stem cell therapy as an adjunct to the surgery and to treat other discs that may be affected. The surgical technique for hemilaminectomy is the standard removal of the bony wall lateral to the spinal cord. Disc material is removed with small atraumatic instruments, Figure 12. The spinal cord is then injected with stem cells. A syringe is prepared with pure platelet rich plasma enriched adipose derived stem cells containing 150 µL volume with 1 x 10⁷ stem cells⁽¹⁾. These cells are injected using a 24GA spinal needle through the dura matter in three locations of 50µL each at the location of damage and above and below the area of damage.

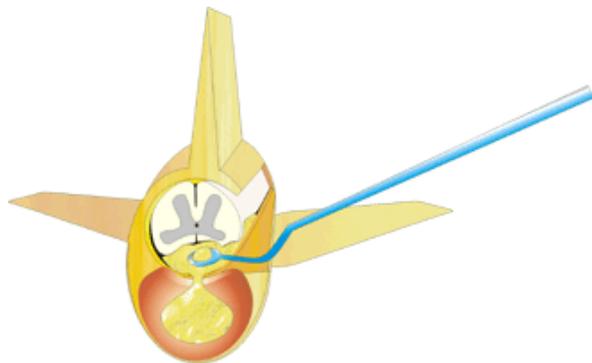


Figure 12: Hemilaminectomy. Removing disc material from under spinal cord.

In animals with spinal cord damage as indicated on physical examination or diagnostic imaging, but that are not receiving decompressive surgery, the stem cell and PRP mixture is injected sub-arachnoid much the same as if doing myelography using the cisterna magna or lower lumbar access areas. Total volume of stem cell mixture should be 150 μ L per kg and contain 1×10^7 cells per ml.

Intravenous administration of stem cells is optional but may be recommended if cells are not to be cryopreserved or cultured. The average sized pet will yield between 40 and 80 million live cells. Each disc requires about 1 million cells and intrathecal administration may use an additional 20 million cells. This may leave an additional 20 million cells that would be wasted if not given intravenously. The SVF has significant anti-inflammatory properties and there is evidence that intravenous stem cells will seek damaged tissue to regenerate. It has been our experience that pets receiving additional intravenous stem cells seem to recover faster with less pain.



Figure 13: Proprioceptive Training. Increased texture stimulates spinal communication to the brain.

Physical therapy is an important part of the post-operative recovery plan. The day after the procedure, the pet should be receiving spinal proprioceptive inputs in the form of spatial alterations using foam or inflatable “peanuts” to roll the pet back and forth. Limb placement and massage also stimulate ascending neurons. Figure 13. When neurons damaged by IVDD are stimulated they will release cytokines when the nerve impulses reach the damaged area. These cytokines influence the genetic neurogenesis of the stem cells enabling more accurate repair. Utilizing electrostimulation, massage, or passive range of motion exercises for paraparetic or

quadraparetic patients enables spinal tracts to receive information destined for the brain⁽⁸⁾. Mental stimulation using the pet’s name, treats and other encouragements creates impulses along the cerebrospinal tracts also liberating cellular chemical signals that communicate to the newly engrafted stem cells. The importance of these chemical messengers in the instruction of stem cell development cannot be overemphasized. Two to three times a day the pet’s spinal and centrally mediated reflexes need to be stimulated using different and alternating modalities. Strength training with water treadmill needs to be started as soon as possible, taking into consideration the need to protect a surgical wound from the water.

Discussion

Regenerative therapy modalities using Adipose Derived Stem Cells are clinically relevant. Stem cells from fatty tissue that does not require culture and expansion is revolutionary. Previously stem cells were derived from bone marrow which was then cultured to increase stem cell numbers over three to six weeks. The next generation of stem cell treatments involved sending fat samples to outside labs which processed the cells and sent them back with no way of the clinician knowing how many live cells were in the final cell sample. Stem cells that can be processed within a couple of hours and are autologous with little if any chance for adverse reactions represent a paradigm shift for all of medicine. Stem cell therapy for neurologic damage to the spinal cord resulting in loss of deep pain perception has been shown to double the success rate⁽⁹⁾. This doubling of pets that have complete return to function over surgery alone is a substantial reason that every pet with neurologic disease should have stem cells as an adjunct

to surgical therapy. The intervertebral disc nucleus pulposus matrix of molecules is largely devoid of cells with cells counts less than 6000 cells per mm². Very few cells are required to produce the matrix⁽⁴⁾. In fact, too many cells added to the nucleus pulposus matrix will cause death of the cells. The matrix attracts and holds water molecules resulting in a change in the structure of the disc. The disc expands, and this expansion reduces pressure on the spinal cord and restores normal function to the disc. This regeneration of the disc is also paradigm shifting, as many of our previous therapies were designed to destroy the disc by fenestration, laser ablation, etc. Today we can rebuild it within days.

This novel approach must be holistic, encompassing all aspects of diagnosis, treatment, post-operative care and rehabilitation. The following are considered essential skills or assets in this regard:

1. MRI is an essential tool to determine the health of the intervertebral disc. When surgery is anticipated, CT scans or Myelograms are great but they cannot tell you the amount of water in a disc. Disc water content is a key indicator for disc degeneration.
2. Surgery capability is still an essential tool for those pets with severe compressive disease.
3. Stem Cell Processing Laboratory with biological safety cabinet, cell counting, cell viability, cryopreservation and culture capacity. You must know how many live cells you are placing into the tissues. For example, too many cells in the nucleus pulposus will not live.
4. C-Arm Fluoroscopy for accurate placement of stem cells into the nucleus pulposus. This can be done without a C-Arm using anatomical guidelines, skill, touch and many many radiographs to confirm needle location but it is not feasible or advisable for most patients.
5. Rehabilitation is essential starting the day after the procedure if possible.

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