Musculoskeletal disorders and open wounds are common in veterinary medicine.

Advances in Stem Cell Therapy: Application to Veterinary Medicine

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**Article Overview**

- In veterinary medicine, there are options available for both small and large animal practitioners to utilize autologous adipose-derived stem/stromal cells (AD-SCs) to promote healing for injuries and degenerative joint disease.¹
- By provision of a living bioscaffolding to encourage stem cell adherence–proliferation, the additional cell availability can be further enhanced with addition of high-density platelet-rich plasma (HDPRP).
- The potential of stem/stromal cells, coupled with important inflammatory promotion (HDPRP), is recognized as safe and efficacious in both open wound surgical care and guided placement.
- The ability to prepare a site for skin graft by placement of AD-SCs in recalcitrant full-thickness wounds speeds the healing and recuperation of small and large defects in animals.
- AD-SCs are of significant value in musculoskeletal tissue injury or disease because there is gradual depletion of native stem/stromal cells in chronic injury or degenerative states.
- Multiple studies support the effectiveness of AD-SCs for use in connective tissue and joint repair, among other potential uses.
- Controlled veterinary clinical trials are continuing, which will provide statistical documentation of the safety and efficacy of AD-SCs, as well as comparisons of different protocols for administration.
- Utilization of AD-SCs, with or without HDPRP concentrates, have proven very effective in several thousand injections in preclinical and clinical use by both human and veterinary physicians in the U.S. and elsewhere.

**Reference**

Osteoarthritis afflicts 10 to 12 million dogs in the United States and is the most common cause of chronic pain in dogs. Tendon and ligament injuries are common in performance horses and potentially more threatening than a fracture to the horse’s athletic ability.

Clinical evidence is growing that autologous adipose-derived stem/stromal cells (AD-SCs) can dramatically improve healing of injuries and decrease degenerative processes.

**USE IN VETERINARY MEDICINE**

**Horses**
Veterinarians have used autologous adipose-derived mesenchymal stem cells to treat tendon and ligament injuries and joint disease in horses on a commercial basis since 2003.

This procedure involves:
- Extraction of a fat sample from the animal
- Sample sent to a laboratory for stem/stromal cell processing
- Processed sample returned to treating veterinarian for direct placement or injection into the injured tissue or joint.

Successful outcomes from this treatment have been shown in horses treated from 2003 to 2008, with:
- 77% returning to prior level of performance
- 94% stable 1 year or more after treatment for acute and chronic suspensory ligament injuries
- 57% with joint injuries returning to prior level of performance.

No systemic adverse events were reported and less than 0.5% had local tissue reactions.

**Companion Animals**
In 2007, this technology was made available to small animal veterinarians for dogs and cats, with more than 1000 cases of connective tissue and joint repair reported through 2009, and a greater than 80% success rate in blinded, placebo-controlled canine clinical trials.

Recently in-office stem cell procedure kits have become available, allowing for treatment at the clinic or on the farm, depending on the species of animal receiving therapy.

**STEM CELL CHARACTERISTICS**
There are 2 kinds of stem cells:
- Embryonic (prenatal) stem cells
- Adult (postnatal) stem cells

Although most lay people recognize the term embryonic stem cells, the important potentials of adult stem cells have been recognized in the veterinary and human medical literature since 1963, when Becker, et al, reported on the regenerative nature of bone marrow.

**Embryonic Stem Cells**
Embryonic stem cells are, in theory, able to transform into any type of tissue; they are totipotent when an egg is fertilized; then after several divisions are pluripotent.

Although less of an issue in veterinary than human medicine, there are religious, political, and ethical issues that limit the use of fetal stem cells.

**Adult Stem Cells**
Postnatal adult stem cells:
- Retain regenerative or reparative capabilities as undifferentiated cells
- Maintain homeostasis in all tissues
• Are capable of repair or regeneration in a tissue or organ system.

These multipotent cells are locally activated to proliferate and differentiate into some, or all, of the major specialized cell types of tissue when required for maintenance or repair. They facilitate tissue maintenance, regeneration, growth, and wound healing throughout life with the capability of differentiation to a wide variety of types of adult cells, such as muscle, bone, cartilage, tendon-ligament, and adipose tissues. 14

Adult stem cells are found in all tissues in the body 17 in varying quantities, with major reservoirs in adipose tissue 16 and, to a lesser extent, bone marrow. 7 Both bone marrow and adipose tissue are derived from embryonic mesodermal tissues and contain a microvascular network, including extracellular matrix and extensive perivascular stroma, which is credited for clinical promise in regenerative medicine applications. 18

Adipose-Derived Adult Stem Cells

Adipose stroma contains large numbers of undifferentiated stem/stromal cells capable of producing all tissue types derived from the mesodermal layer. 19,20 These AD-SCs have the potential to differentiate to tenoligamentous, skeletal muscle, smooth muscle, and cardiac muscle. 21,22 AD-SCs also have the potential to differentiate into tissue derived from ectodermal and endodermal origins, such as organ tissue, nerves, and skin, suggesting that they have pluripotent and multipotent capabilities (see Figure 1). 23-32

Recent studies have determined the safety and efficacy of implanted/administered AD-SCs in various animal models as well as human clinical trials. AD-SCs also meet certain criteria described for the ideal stem cell for regenerative medicinal applications: 33,34

- Found in abundant quantities
- Harvested with a minimally invasive procedure
- Can be differentiated along multiple cell lineage pathways in a regulatable and reproducible manner
- Can be safely and effectively transplanted.

As a result, adipose tissue has become an important resource for research and patient care applications in human and veterinary medicine.

HIGH-DENSITY PLATELET-RICH PLASMA CONCENTRATES

Enhanced healing capability is possible when platelet concentrations are increased within injured or damaged tissue. 35 Platelet-rich plasma has been used successfully as a treatment modality in both veterinary (equine) and human medicine.

High-density platelet-rich plasma (HDPRP) is defined as plasma with platelet concentrations > 4 times the levels found in circulating blood. It is isolated and concentrated from a peripheral venous blood sample by bidirectional centrifugation. 36

Various portable commercial centrifugation units exist for in-office use; however, there are only a few that

NAMING ADIPOSE-DERIVED STEM/STROMAL CELLS

There has been some variation and question regarding the correct terminology for stem/stromal adipose cells.

At first, mesenchymal stem cells (discovered to have an active role in connective tissue repair in the early 1990s) were thought to be the most important contributor to tissue regeneration; however, it became evident that within the adipose tissue complex is a key extracellular matrix, which included mature adipocytes and adipocytic precursors (known as progenitor cells). In addition, there was a variety of additional nucleated, undifferentiated, multipotent, and pluripotent cells, including pericytes and endothelial cells, which are all thought to play important roles in mesenchymal/stromal-derived tissue regeneration (Figure 2).

Therefore the term adipose-derived stem/stromal cells (AD-SCs), rather than simply “mesenchymal stem cell,” is used to describe this population of cells.

Reference

are capable of consistently concentrating platelets to high-density therapeutic levels, including the Harvest Technologies Smart PReP2 centrifugation system (harvesttech.com), which has been cleared by the FDA.

The Inflammatory Cascade
Platelets contain a significant number of growth factors, key signal proteins, chemokines, cytokines, and other proinflammatory bioactive factors that initiate and regulate most basic aspects of the inflammatory cascade, resulting in natural wound healing (Table).77

- Elevated platelet concentrations are known to stimulate proliferation and differentiation, recruitment, and migration of mesenchymal and stromal repair cells to an injury site.78
- Circulating platelets, when activated, begin a degranulation process that secretes a variety of important growth factors and cytokines/chemokines.79
- Activated platelets also secrete stromal cell-derived factor 1 alpha (SDF-1α), which supports primary adhesion and migration of mesenchymal stem/stromal cells.80

Platelet’s Role in Wound Healing
In many human stem/stromal cell protocols and some veterinary stem cell protocols, HDPRP is combined with AD-SC for placement into specific injury sites. There are also many examples of utilization of HDPRP, with and without stem/stromal cell additives, that have proven effective in acute and chronic full-thickness wound defects. Stimulation of the inflammatory cascade, coupled with the addition of AD-SC, remarkably shortens the healing process by promoting tissue regeneration (see Figure 3).81

RESEARCH
Multiple studies, both in the human and veterinary literature, have clearly demonstrated the ability of AD-SCs to actively participate in tissue homeostasis, regeneration, and open wound healing.82-65 AD-SCs also differentiate into and repair musculoskeletal connective tissue, including:
- Ligament
- Tendon
- Cartilage

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**Figure 3.** Use of high-density platelet-rich plasma in equine open wound defect: (A) Pre-operative avulsion, 4-degree tissue loss, bone surface exposed, wound 8 days old; (B) postoperative wound at 48 hours with 1 sharp debridement and thrombin-activated HDPRP topical concentrate; (C) postoperative wound on day 8 with 2 debridements plus second activated HDPRP topical concentrate; (D) split-thickness skin graft taken from left chest, suture fixed, and meshed, with activated HDPRP under graft and platelet-poor plasma as fibrin gel on surface.
mesenchymal and stromal vascular stem-like cells exist in adipose compared to bone marrow.56-79 For further information on ongoing stem cell research, go to todaysveterinarypractice.com.

STEM CELL HARVESTING & ADMINISTRATION

The ability of AD-SCs to support and serve as a cell reservoir for connective tissue and joint repair is the basic theory for their use in joint regenerative medicine.

Autologous AD-SC therapy involves:
1. Harvesting fat from the available sites
2. Isolating the stem and regenerative cells
3. Administering the cells back to the patient.

In veterinary practice, these cells are typically found in the adipose deposits near the tail (horses) and from the ventral fat pad (dogs). In these cases, fat is either lipoaspirated80 or, if taken surgically, en bloc, morselized to permit separation of the stem–stromal elements within the adipose complex. After preparation, this tissue, combined with HDPRP, can then be injected during open surgical intervention or by guided injection.

It is important to understand that undifferentiated stem/stromal cells must adhere to other cells (cell-to-cell contact) or to extracellular matrix/perivascular tissues in order to proliferate effectively. Within an injured or degenerative site, the stem/stromal cell fate is controlled by a complex set of physical (cell-to-cell) and chemical signals (paracrine-autocrine effects) dictated by the cellular and chemical microenvironment (niche).81

Further, these cells are believed to proliferate and differentiate from signaling based on the environment in which they are placed. Therefore, if AD-SCs are placed within and adherent to damaged connective tissue, uncommitted progenitor and stem/stromal elements within the AD-SC graft are activated and differentiate toward the specific connective tissue lineages for growth and repair.

CONCLUSION

Stem cell therapy in regenerative veterinary medicine is a viable option for the equine as well as the small animal veterinarian, offering a safe and clinically effective tool for the clinician to assist in his/her treatment of the animal with difficult wounds or unresolved musculoskeletal or joint pain.

AD-SC = adipose-derived stem/stromal cell;
HDPRP = high-density platelet-rich plasma

Go to TodaysVeterinaryPractice.com for further information on how adipose-derived stem cells were originally introduced for use in medicine as well as details regarding current research taking place.
DISCOVERY OF MESENCHYMAL STEM CELLS

In the early 1990s, adult mesenchymal stem cells were discovered to have an active role in connective tissue repair. These cells were first labeled as *mesenchymal stem cells* (MSCs) because of the ability to differentiate to lineages of mesenchymal tissue, and were recognized to be an essential component of the tissue repair process.

An interesting observation made about MSCs was the ability to “home in” and help repair areas of tissue injury using chemotactic mechanisms. While bone marrow has historically been used as a source of MSCs, adipose-derived stromal elements were shown to have nearly identical fibroblast-like morphology and colonization, immunophenotypic capabilities, successful rate of isolation, and differentiation capabilities. The healing potentials of the very heterogenic, adipose-derived stem/stromal cells (AD-SCs) were demonstrated in early clinical use for cranial defect and chronic fistula repair, without side effects.

MSCs, along with other cells within the adipose stroma (together making up AD-SCs), react to cellular and chemical signals, and have been shown to differentiate and assist in healing for a wide variety of cellular types. This includes cartilage repair, angiogenesis in osteoarthritis, tendon defects, ligament tissue, intervertebral disk repair, ischemic heart tissue, graft-versus-host disease, and osteogenesis imperfecta.

Of particular interest in musculoskeletal medicine is the observation in degenerative diseases, such as advanced osteoarthritis, that an individual’s local adult stem cell frequency and potency may be depleted, with reduced proliferative capacity and ability to differentiate. It has been suggested that addition of these missing stem cell elements might help these conditions. Studies have demonstrated such improvement with adult stem cell therapy by successful regeneration of osteoarthritic damage and articular cartilage defects.

AD-SC USE IN HUMAN COSMETIC SURGERY

Cosmetic-plastic surgeons have studied, and safely and successfully utilized, autologous fat grafting for structural augmentation via transplantation of lipoaspirants for many years. In the past decade, better understanding of the cellular mechanisms responsible for successful soft tissue augmentation has been better defined, focusing on the plentiful undifferentiated stem/stromal elements rather than the survival of mature adipocytes.

During the 1990s, further understanding and enhancements by cosmetic plastic surgeons to improve the “take” of fat grafts led to the effective addition of high-density, platelet-rich plasma (HDPRP) concentrates to further enhance the success of autologous fat grafts (AFG).

Several publications within the human cosmetic-plastic surgical literature have reported significant contributions to successful adipose tissue transplantation when these autologous grafts were blended with highly concentrated platelet elements (PRPs). Recognition of the significant clinical contribution to structural fat grafting when transplanted with the multitude of platelet-derived growth factors, cytokines, and chemokines, became a valuable aid in retaining improved structural augmentation.

It is believed that these effects are largely a result of provision of a undifferentiated cell population and HDPRP’s ability to improve active angiogenesis, stimulation and promotion of undifferentiated cell adherence, proliferation, and differentiation activities of precursor cells in the grafts, reflecting the niche in which they are received (Figure).
RESEARCH AT THIS POINT IN TIME
When adipose-derived stem cells are placed within osteoarthritic degenerated cartilage, chondrogenic differentiation is believed to be encouraged.11-14 In the 1990s, Young, et al, showed repair of an Achilles tendon tear when placed in a collagen matrix, then placed in a tendon defect.15

In 2003, Murphy, et al, reported significant improvement in medial meniscus and cartilage regeneration with autologous stem cell therapy in an animal model. Not only was there evidence of marked regeneration of meniscal tissue, but the usual progressive destruction of articular cartilage, osteophytic remodeling, and subchondral sclerosis commonly seen in osteoarthritic disease was reduced in MSC-treated joints compared with controls.16

In 2008, Centeno, et al, reported significant knee cartilage growth and symptom improvement in a human case report using culture-expanded autologous MSCs from bone marrow.17

In 2010, Little, et al, demonstrated the successful differentiation of human AD-SCs to ligamentous tissue following placement in a simulated ligament matrix composed of native ligamentous material combined with collagen fibrin gel. Cells placed in this manner showed changes in gene expression consistent with ligament growth and expression of a ligament phenotype.18

In 2011, Albano and Alexander successfully reported an autologous fat graft as a mesenchymal stem source and living bioscaffold (autologous regenerative matrix) to repair a persistent patellar tendon tear.19 Growth factors and chemical elements, such as present in HDP, are believed to provide favorable influences within the microenvironment to enhance adherence, proliferation, differentiation and migration of cells towards this end.20

CONTINUING RESEARCH
There are more than 43 ongoing U.S. human controlled clinical trials with approximately half of them still recruiting participants.21 Studies include the use of AD-SCs for degenerative arthritis. In that trial, AD-SCs will be culture expanded, then administered into a cartilage tissue lesion via orthopedic surgery.22

Another trial pending (Scarpone and Alexander, sponsored by Trinity Health Systems) explores the use of PRPs alone versus AD-SC alone versus PRPs and AD-SCs together. A similar planned veterinary study will also explore PRP with and without AD-SCs.

Other ongoing human studies include AD-SCs for the treatment of diabetes, rectovaginal and perianal fistulas, peripheral vascular disease, ischemic heart disease, coronary arteriosclerosis, hemifacial atrophy, liver cirrhosis, breast reconstruction after breast cancer, anti-aging, polycystic ovary syndrome, metabolic syndrome X, fecal incontinence, graft versus host disease, chronic critical limb ischemia in diabetic patients, lipodystrophy, Crohn’s Disease, spinal cord injury, Buerger’s disease, and neurologic diseases such as amyotrophic lateral sclerosis.23

The use of stem cell on equine tendinitis and articular cartilage regeneration has been reported from research centers including Cornell University School of Veterinary Medicine. In 2010, Fortier, et al, compared concentrated bone marrow aspirate compared with microfracture for full-thickness cartilage repair in the equine model. At 8 months there was superior and statistically significant improvement in repair tissue in the bone marrow concentrate group compared with the microfracture group, with MRI evidence of increased filling of the defects and improved integration of repair tissue into surrounding normal cartilage, greater type-II collagen content, and improved orientation of the collagen.24

In another study at Cornell, the histologic evaluation of stem cell treated tendons versus controls revealed a significant improvement in tendon fiber architecture, reductions in vascularity and inflammatory cell infiltrate, and improvements in tendon fiber density and alignment in AD-SC treated tendons. The study concluded that the use of adipose stem cell therapy in horses with tendonitis appears warranted.25

Small animal studies include 2 double-blinded, placebo-controlled studies in canine osteoarthritis, 1 for the hip and the other for the elbow. In both cases a small amount of fat was extracted from the animal, placed in a temperature-controlled (28°C) transport box, and shipped overnight to Vet-Stem laboratory for processing and return of concentrated stem/stromal cells.

Veterinary evaluation incorporated history, physical examination, and lameness determination. Eighteen dogs completed the 90 day study. Statistically significant improvement in lameness, pain, and range of motion was noted in the stem-cell-treated group compared with a blinded, saline-injected control group, with significant improvement over time from baseline. Three of the owners were considering euthanasia prior to the study because of their animals’ pain and functional disability. At the time of writing the publication those animals were reported to be living relatively pain free.26
References


42. Autologous adipose tissue derived mesenchymal stem cells transplantation in patient with degenerative arthritis (Identifier: NCT01300598).

43. Searching results in clinicaltrials.gov: http://clinicaltrials.gov/ct2/result s?term=adipose+adult+stem+cells&err=3&itl=type=&cond=&int r=&outc=0&states=1&state3=&cntry3=&locn=&gndr=0&rcv_s=&rcv_e=&lup_s=&lup_e


References


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