Predominant Energy-storing Tendons in Humans, Dogs, and Horses and Challenges With Healing

Energy-storing tendons are used to generate motion efficiently through stretch and recoil and therefore undergo extreme loading conditions with high stresses and strains during exercise. For this reason, energy-storing tendons are particularly prone to overload-induced injury. The most critical energy-storing and commonly injured tendon in humans is the Achilles, or calcaneal, tendon with which the famous phrase “Achilles’ heel” is associated, meaning one’s weak spot. The Achilles tendon serves to attach the plantaris, gastrocnemius, and soleus muscles to the calcaneus (heel bone), is surrounded by a bursa on each side, and enables flexion of the foot. The common calcaneal tendon (CCT) in dogs is very similar in structure and function to the Achilles tendon and has been used as both a naturally occurring and induced injury model for human Achilles tendon injury.

In contrast, the most critical energy-storing and most commonly injured tendon in horses is the forelimb superficial digital flexor tendon (SDFT). While also utilized as a model for Achilles tendon injury in humans because of its energy-storing capability, the forelimb SDFT in horses has markedly different anatomy. The forelimb SDFT attaches the SDFT muscle that originates from the humerus to the second phalanx, causing flexion of the metacarpophalangeal and proximal interphalangeal joints. It courses down the palmar aspect of the carpus within the synovial structure of the carpal canal, where it is joined with the accessory (superior/proximal check) ligament of the SDFT from the distal radius, and then continues down the palmar aspect of the metacarpus outside of any synovial structure until the level of the metacarpophalangeal joint. On the palmar aspect of the metacarpophalangeal joint, the SDFT forms a ring around the deep digital flexor tendon incorporated within the synovial structure of the digital flexor tendon sheath before ultimately splitting and inserting onto the medial and lateral aspects of the second phalanx. These differences in anatomy including the long length of the equine SDFT and split insertion sites not associated with...
bursae are likely to account for the differences in SDFT injury types and healing potential compared to the Achilles tendon and CCT, as described below.

In all species, tendon healing is challenging and associated with high failure and reinjury rates due to the inferior repair tissue that develops following injury. This repair tissue is more scar-like than normal tendon, with a higher content of collagen type III than the desired collagen type I and with a disorganized tendon fiber pattern. As such, tendon scar tissue is less elastic and has reduced biomechanical strength compared to normal tendon, which predisposes the tendon to reinjury. In the case of midbody or midsubstance tendon injuries, reinjury is particularly common at the interface or junction of the normal tendon tissue and the scar. In the case of tendon ruptures and insertional tendon injuries, tendon healing can be further complicated by tendon mineralization and adhesions, both of which can result in loss of tendon gliding properties, decreased range of motion, and pain. Adhesions are also a common complication of tendon injuries within synovial structures, as it is concurrent synovial inflammation in the form of tenosynovitis or bursitis. For all these reasons, novel therapies to improve tendon healing and reduce failure and reinjury rates are needed. The purpose of this manuscript is to review the evidence available for the use of mesenchymal stem cells (MSCs) to improve tendon healing in the horse and how we might apply what we have learned from the horse to other veterinary species and humans.

SDFT Core Lesions in Horses

While there are several types of SDFT injuries that can occur in horses, by far the most common type is the forelimb core lesion. These lesions occur most frequently in the extrasynovial midbody of the SDFT in the proximal to midmetacarpal region distal to the carpal canal and proximal to the digital flexor tendon sheath. SDFT ruptures and insertional disease have been described but are rare. Forelimb SDFT core lesions are overrepresented in horses working and performing at high speeds, such as racehorses and eventers, although they can occur in horses of any discipline. Just as a core is the central part of a fruit and a core value or belief is central to someone’s existence or character, a tendon core lesion is central to the tendon’s structure. While SDFT core lesions can be eccentric and of different shapes, they generally involve a substantial cross-sectional area of the SDFT with a remaining rim of normal tendon around them and result in clinical lameness. Visualized with ultrasonography, the lesion itself is hypoechoic in nature, especially in the acute stage, and the cross-sectional area of the entire SDFT is often markedly enlarged due to swelling (Figure 1). This SDFT swelling is grossly visible on the palmar aspect of the horse’s limb and is often referred to as a “bowed” tendon because of its appearance.

Treatment of SDFT Core Lesions in Horses With MSCs

Treatment of SDFT core lesions in horses has evolved substantially over the past 2 decades, particularly regarding biologic therapies such as stem cells, rehabilitation treatment modalities, and controlled rehabilitation protocols. The purpose of the current manuscript is to focus on the use of MSCs for the treatment of SDFT core lesions, and as such, other treatments will not be discussed. Compared to tendon tears and ruptures, tendon core lesions offer the unique and seemingly ideal opportunity to deposit MSCs in a confined location, particularly prior to the formation of any granulation or scar tissue. While it is known that MSCs don’t persist within the core lesion long-term, they do survive and remain within the lesion in large numbers for at least 10 days, with viability declining over the following 30 to 60 days. Equine clinicians have been taking advantage of this opportunity for many years, performing ultrasound-guided intralesional injections of MSCs. In many parts of the world, these MSC injections are considered the standard of care for core lesions and are also covered by insurance policies for performance horses. As shown (Figure 2),

![Figure 1](image-url)
the needle is advanced through the rim of the normal tendon and into the hypoechoic core lesion. During and following injection of the MSC suspension, the MSC suspension and any associated hyperechoic air bubbles can be seen within the core lesion. The horse should be adequately sedated with the limb locally anesthetized, and a small-gauge needle (typically size 20 to 23 gauge) should be utilized to avoid any substantial iatrogenic damage to the normal tendon, through which the needle must pass. Even with small-gauge needles, needle tracks may remain visible for several weeks on recheck examinations. While there has been some concern initially regarding needle size and MSC viability following injection, it has been shown that MSCs maintain their viability, even through needles as small as 30 gauge as long as they are injected only through that needle and not aspirated repeatedly or quickly for resuspension. MSCs should be resuspended very gently in the vial in which they are transported, by gentle agitation of that vial, either by inversion, as long as sterility of the cap has not been compromised, or flicking the bottom of the vial where the cell pellet is. The MSCs should then be aspirated once with a large-gauge needle (20 gauge or larger) prior to switching to the needle size of choice for injection. The timing of the first treatment, optimal dose or number of stem cells per treatment, optimal MSC vehicle for suspension, and optimal number of treatments have yet to be determined.

Despite the above statement, there are many things we have learned not to do over the past 20 years of clinical MSC use in the horse. It is important that fetal bovine serum (FBS) be removed from the MSC culture media at a minimum of 48 to 72 hours prior to cell preparation to avoid an immune reaction to the FBS. In many cases, FBS is replaced by autologous serum from the patient and the MSCs are injected into the core lesion suspended in the same autologous serum, although platelet lysate and other autologous biologics have also been used. In addition, the MSC suspension is not combined with antibiotics or steroids, as both can cause MSC death. Regardless of the type of vehicle used, it is important to realize that most SDFT core lesions can hold only a relatively small injection volume of < 1 mL, unless the core lesion is long in length proximal to distal. It is critical to stop injecting the core lesion as soon as back pressure is felt upon injection, as forcing further volume of MSC suspension (or any other substance for that matter) can cause splitting of the tendon fibers and enlargement of the lesion, which may also result in increased lameness. How many MSC treatments the patient receives is largely guided by core lesion size and clinician’s preference, with consideration of needle placement through normal tendon and ability to inject a reasonable volume of MSC suspension into the lesion as just discussed. If the lesion is substantially smaller or filled in with repair tissue on recheck ultrasound examination, further intratessional injection is typically avoided to prevent injury to the normal rim of tendon and so as not to force injection volume into the lesion. An excellent alternative at this stage, if needed, is regional limb perfusion of MSCs using either the cephalic vein or the median artery. A schematic overview of the treatment decision-making process and general rehabilitation protocol for a typical SDFT core lesion is presented (Figure 3).

The optimal timing of the first MSC treatment remains a particularly intriguing question in terms of MSC interaction with the injury environment and MSC response to inflammation. While it was originally believed that inflammatory cytokines could cause MSC death or limit the desired MSC response, more recent evidence suggests that MSC exposure to inflammatory cytokines is beneficial through stimulation or priming of MSCs to secrete factors important for healing. This concept of MSC stimulation or priming is commonly referred to as MSC licensing and is addressed in the companion Currents in One Health by Koch and Schnabel, AJVR, October 2023. In a recent study, inert ultrafiltration probes were placed into surgically induced SDFT core lesions in horses to determine the temporal cytokine profile present in the core lesions following injury. In this study, it was found that the predominant proinflammatory cytokines were IL-1β and IL-6 and that both

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**Figure 2**—The same superficial digital flexor tendon (SDFT) core lesion as shown in Figure 1, panel B, now slightly off angle to allow for ultrasound-guided intratessional injection of mesenchymal stem cells (MSCs). Image with hyper-echoic needle in place within the outlined SDFT core lesion preinjection (A) and postinjection with MSCs and hyperechoic air bubbles filling the outlined SDFT core lesion (B). DDFT = Deep digital flexor tendon.
peaked within 48 hours following injury. When bone marrow–derived MSCs were then stimulated in vitro with the same concentrations of IL-1β or IL-6 as measured from the SDFT core lesions, it was found that IL-1β induced upregulation of many genes beneficial to tendon healing, as identified by RNA sequencing. Additionally, this resulted in increased protein expression of IL-6, vascular endothelial growth factor, and prostaglandin E2, all of which are key players in tendon healing. These findings suggest that treatment of a tendon lesion with MSCs early in the acute phase of injury could be advantageous for endogenous in vivo licensing of the cells. This acute phase is rather short, however, and may not be practical or feasible in the clinical setting.

Currently, a challenge in MSC treatment of equine tendon injuries is the timing and availability of MSCs, as the greatest preclinical and clinical evidence to date is with the use of autologous bone marrow–derived MSCs. Timing of the first MSC treatment is therefore dependent on when the horse is diagnosed and how quickly bone marrow can be harvested for autologous MSC culture, expansion, and injection preparation, typically requiring 2 to 3 weeks. There are many reasons why allogeneic bone marrow–derived MSC use would be desirable, such as to avoid bone marrow harvest, have high-quality screened cells ready for “off-the-shelf” use, and allow for earlier treatment. Numerous in vitro and in vivo studies in the horse, however, have demonstrated cellular and humoral immune responses to major histocompatibility complex (MHC) mismatched bone marrow–derived MSCs and MSC cytotoxicity in response to antibody production. As MHC haplotyping and matching is not

**Figure 3**—Schematic overview of the treatment decision-making process and general rehabilitation protocol for a typical equine superficial digital flexor tendon (SDFT) core lesion. Created with BioRender.com. PRP = platelet-rich plasma. RLP = regional limb perfusion.
practical in the clinical setting, efforts are underway to control bone marrow–derived MSC immunogenicity through methods such as culture with TGF-β2, which has been shown to downregulate MHC expression on the surface of MSCs and prevent MSC cytotoxicity.16,37 Interestingly, TGF-β2 has also recently been shown to enhance expression of bone marrow–derived MSC paracrine factors with known associations to tendon healing.38

**Clinical Evidence for the Treatment of Tendon Core Lesions With MSCs in the Horse**

To date, 1 small and 2 large retrospective clinical studies of naturally occurring forelimb SDFT core lesions have been performed with long-term follow-up, and all have supported the use of autologous bone marrow–derived MSCs.39,19,40 In all 3 studies, naïve (nonlicensed) MSCs were used as a single treatment. In the first small clinical case-controlled series, 11 flat racehorses treated with autologous bone marrow–derived MSCs were compared to 15 control racehorses from the same practice treated with traditional methods that were not described.39 Both MSC-treated and control horses underwent the same rehabilitation protocol. The number of MSCs used ranged widely from 600,000 to 31.2 million cells, and days to injection ranged from 18 to 35 days. Nine of the 11 (82%) MSC-treated horses returned to racing between 9 and 12 months and were racing without reinjury at 2 years post–MSC treatment. In contrast, all 15 of the control horses experienced a reinjury event within 1 year, with a median reinjury time of 7 months. Interestingly, the horse that received the lowest number of MSCs was 1 of the 2 that did not heal well or return to racing.

In a later large clinical case series, 105 National Hunt horses (racing with jumping) treated with autologous MSCs and controlled rehabilitation were compared to historical controls from the literature on the treatment of naturally occurring SDFT core lesions.19 Control horses were treated with other non-MSC methods including controlled exercise and medical treatment with hyaluronan or polysulfated glycosaminoglycans, intralesional injection of IGF-1 injection, and firing or desmotomy of the accessory (superior/proximal check) ligament of the SDFT. A small number of flat Thoroughbred racehorses were also included, but the numbers were too small to compare statistically or draw any conclusions. The number of MSCs used in this study also ranged widely from a few million MSCs to over 50 million MSCs, as did duration between injury and implantation from around 20 days to over 180 days. A significant reduction in reinjury rate was found for National Hunt horses treated with MSCs (25.7%) compared to National Hunt horses treated with traditional therapies (56% in one historic study and 53% in another).19 No differences in the percentage of National Hunt horses treated with MSCs compared to historical controls were found in terms of return to racing and completing 3 and 5 races. When MSC-treated horses that did not reinjure were compared to MSC treated horses that did reinjure, reinjured horses on average received fewer MSCs, were older, and had an increased duration between injury and implantation, although these differences were not found to be significant.

In the most recent large clinical case-controlled series, a total of 213 Thoroughbred flat racehorses with naturally occurring SDFT core lesions were all prescribed the same 12-month controlled exercise rehabilitation program.40 Sixty-six of these horses received a single treatment of intralesional autologous bone marrow–derived MSCs (10 million cells) approximately 3 weeks after injury diagnosis, while 17 received a single treatment of intralesional allogeneic adipose-derived MSCs (21 million cells) approximately 7 days after injury diagnosis. All horses were followed up for a minimum of 2 years after return to racing. Compared to the control horses with a controlled exercise rehabilitation program alone, only the horses treated with autologous bone marrow–derived MSCs had both increased odds of returning to racing (OR, 3.19; 95% CI, 1.55 to 6.81) and increased odds of completing 5 or more races postinjury (OR, 2.64; 95% CI, 1.32 to 5.33).

In summary, these 3 clinical case series support the use of autologous bone marrow–derived MSCs combined with a controlled rehabilitation protocol for the treatment of SDFT core lesions in horses. Findings of increased return to performance and reduced reinjury rates compared to control horses treated with controlled rehabilitation alone are suggestive of improved tendon healing consistent with preclinical studies that have utilized histology to demonstrate improved tendon architecture with greater ratios of type I to type III collagen and improved tendon organization.

**Achilles Tendon Injuries in Humans and Dogs**

When reflecting on what we now know about the use of MSCs for treatment of SDFT core lesions in horses, several key questions about Achilles tendon injuries in humans and dogs become apparent and may account for the lack of MSC evidence in these species to date.41 Achilles tendon injuries in both species are commonly chronic and degenerative, so would they have the same inflammatory environment as an acute SDFT core lesion in the horse? If not, perhaps in vitro MSC licensing would be even more critical to improve treatment outcomes, as the MSCs would not otherwise be receiving the optimal endogenous signals from the injury environment. Achilles tendon injuries in both species can be either noninsertional or insertional in nature and often involve partial or complete ruptures or avulsions, respectively (Figure 4).1–6,12,42,43 This scenario is much different from the contained SDFT core lesion in the horse that is an ideal location to deliver and retain cells, allowing MSCs time to interact with the injured tissue environment and secrete paracrine factors. When injected around the region of the rupture or
avulsion, it is unknown how long the MSCs remain near the site of injury and how much contact they have with the injured tendon environment. In such a scenario, should the MSCs be delivered in a gel or on a scaffold to help with retention? Diffusion of MSCs or MSC-secreted factors out of the calcaneal bursa to reach the injured tendon is also an interesting concept that warrants further exploration. Lastly, when considering insertional tendinopathies, should MSCs be licensed to target production of type II and type X collagen, which are present in tendon specifically at that insertion site? Further studies in this area are warranted, as MSC licensing might represent a novel approach for location and injury-specific treatment of tendon injuries.

Conclusions

We as clinicians and scientists in both veterinary and human medicine are just beginning to understand how MSCs exert their effects on tissue healing and how to best stimulate and capitalize on MSC effector functions. We must consider the type and stage of tendon injury we are treating, the type of environment to which the MSCs may be exposed, and how to best deliver MSCs to the injury site. The positive outcomes documented in horses for the treatment of naturally occurring SDFT core lesions very well may be because lesions are confined and surrounded by normal tendon and with a relatively simple architecture compared to tendon insertion sites. Therefore, strategies to improve MSC function such as MSC licensing may increase their translational potential to treat tendon injuries in other locations and species. Comparing and contrasting the differences between SDFT core lesions in horses and Achilles tendon injuries in humans and dogs is a useful exercise to contemplate ways we can collaborate to improve MSC treatment outcomes. In particular, the concept of MSC licensing has the potential to substantially change how we utilize MSCs to treat tendon injuries.

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