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Honors Thesis

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Improvements made with Stem Cell Therapy

Medicine has greatly improved over the last several decades due to research and advances in technology. The quality of life for humans and animals has greatly increased due to the ability of doctors to treat more diseases and injuries than previously possible. A relatively new area in medicine that is still being researched is the use of stem cells to stimulate growth in certain areas of the body. Given the chance, this method of regenerative medicine could make a significant difference in how certain injuries are treated. A specific injury that is using stem cells is the superficial digital flexor tendon (SDFT) in horses. The superficial digital flexor tendon connects to the flexor muscles at the carpus region and runs to the area between the proximal and medial phalanx (Marfe et al., 2012). It is the most plantar structure in the metacarpus and metatarsal bone areas, sitting just behind the deep digital flexor tendon. Both the superficial and deep digital flexor tendons comprise the tendon sheath near the metacarpo-phalangeal joint and the proximal sesamoid bones (Marfe et al., 2012).

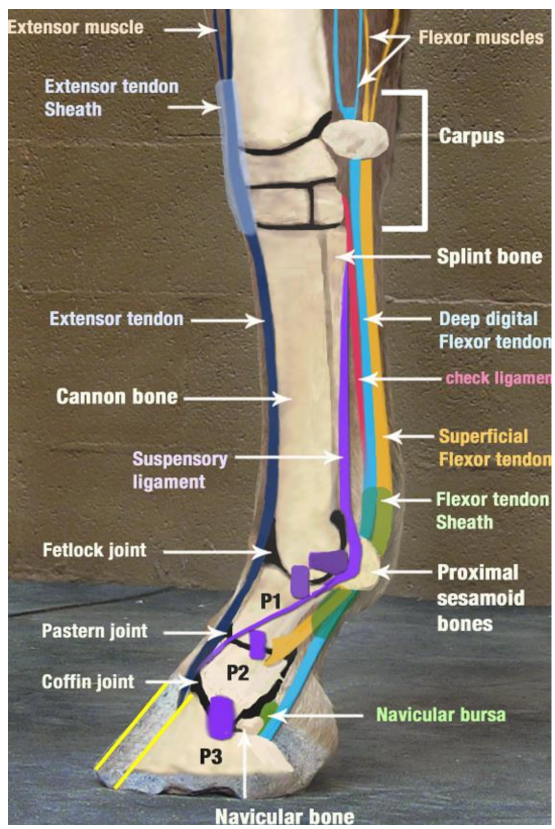

Fig 1. Image of the superficial digital flexor tendon in the equine leg. (Thal, 2016).

The collagen fibers in a tendon run parallel to each other, so the SDFT injury disrupts this arrangement and forms a lesion in the tendon fibers. The lesion will heal naturally by fibrosis, but the collagen fibers are smaller and have less crosslinks in the tendon, which affects the functionality of the tendon (Guercio et al., 2015). Tendons are a type 1 collagen, which is one of the most common connective tissues. The collagen fibrils are composed of three polypeptide chains, which are then arranged in a triple helix called tropocollagen (Depalle et al., 2015). The tropocollagen helices are stabilized by enzymatic crosslinks between amino acids in the polypeptide chains. Enzymatic reactions occur to form covalent crosslinks; two crosslinks are divalent, and another reaction will create trivalent crosslinks (Depalle et al., 2015). The stabilization and strength of the tropocollagen is directly proportional to the density of crosslinks in the tissue, which prevent the sliding of the fibrils (Sherman et al., 2015). If the fibrils slide too much, they can rupture the crosslinks and ultimately break the collagen. Therefore, a lack in crosslinks can decrease the amount of stress that a tendon is able to withstand (Depalle et al., 2015). A SDFT lesion that heals with less crosslinks in the fibers leave the tendon weaker than it started, which can be detrimental in an athletic horse that puts a lot of stress on their body.

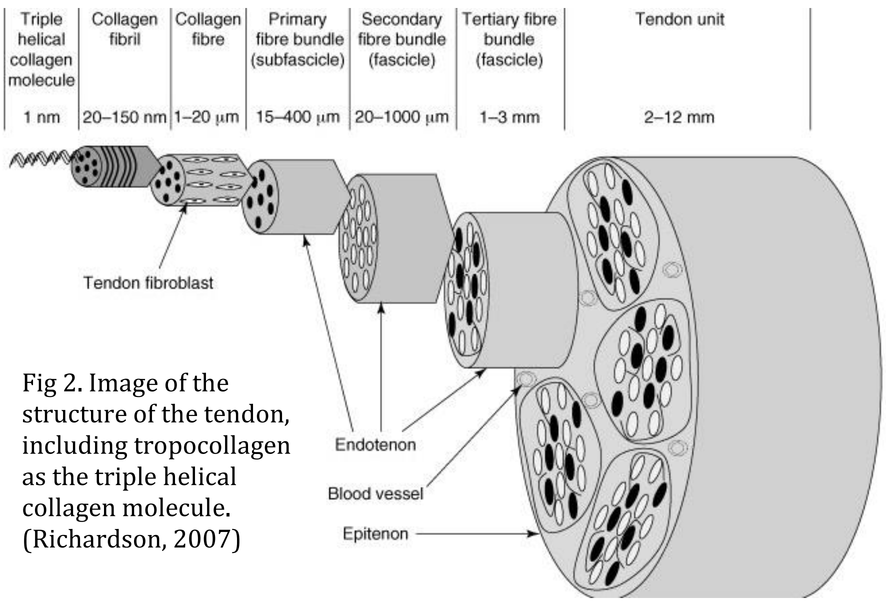

Fig 2. Image of the structure of the tendon, including tropocollagen as the triple helical collagen molecule. (Richardson, 2007)

The scar tissue that forms also decreases the functionality of the tendon by being less elastic, which causes tissues around the lesion to compensate and perform above their normal standards (Wood, 2006). The compensation by other tissues will increase the risk for re-injury and a decline in performance. In addition, the rate of healing is impeded by the lack of blood flow in the tendons, decreased mitotic division, and the absence of cells able to specialize into new tissues (Guercio et al., 2015). The repair process includes an accumulation of local cells to the lesion to synthesize new tissue, but this ability is limited by the mature tendon's lack of precursor cells that are able to differentiate (Godwin et al., 2011). The strength of the collagen fibers is also affected by the hydration of the collagen. When water is present in collagen, bonds will form between the collagen and water and allow more movement in the fibrils (Sherman et al., 2015). In a dehydrated tendon, bonds will instead form directly between fibers and limit the flexible sliding of the collagen. Without the vasculature that provides water to the body, the tendons are also at a greater risk for forming less water bonds and stiffening the tendon (Sherman et al., 2015). The shortage of growth factors in the tendon limits the repair process and the amount of regeneration that can occur on its own.

The SDFT injury typically results from a strain on the tendon that causes hyperextension of the metacarpophalangeal joint during activity, and is directly related to force on the limb (Godwin et al., 2011). The force on the limb increases with speed, which is also why SDFT injuries are especially prevalent in horses that are engaged in heavy training and competition. It is also thought that degeneration of the extracellular matrix contributes to SDFT injuries (Godwin et al., 2011). SDFT injuries are one of the most common causes of lameness horses at an estimated 30% of all injuries, have long rehabilitation periods, and have a high reoccurrence (Iacono et al., 2015). The frequency of this injury makes it a significant injury that cannot always be completely healed with conventional therapies. Conventional methods of rehabilitation have been successful in some horses, but other horses have had to end their athletic careers due to relapse or inability to fully recover from their injury (Marfe et al., 2012). Conventional methods of therapy include rest, cold therapy, surgery, anti-inflammatory drugs, steroid joint injections, and more (Wood, 2006). Of the 20-60 percent of horses that are able to return to their previous levels of athletic ability after conventional therapy, about 80% of those horses are susceptible to re-injury.

With the location of the SDFT injury and the factors involved, the long length of rehabilitation and rate of re-injury are common. Since it is such a prevalent injury in athletic horses, methods have been developed to aid in the healing process. One of the newer therapies still being explored is the use of stem cell therapy. In this specific injury, mesenchymal stem cells (MSCs) are being used to increase efficiency of regeneration (Guercio et al., 2015). These stem cells can be extracted from bone marrow and adipose tissue and then administered in areas with tissue damage to stimulate the healing of the lesion in the tendon. The stem cells are multipotent, which means that they can differentiate by dividing and developing into specialized adult cells (Iacono et al., 2015). The ability of these cells makes it possible for them to heal the injury by essentially making new tissue that is comparable to healthy tissue (Guercio et al., 2015). The stem cells minimize the development of scar tissue by providing healthy tissue, whose permanent tissue regeneration has provided improved results compared to conventional methods.

One case study was performed to test the intralesional injection of stem cells in horses that have ligament and tendon injuries. Although only one third of the 98 horses tested had the superficial digital flexor tendon injury, the other two thirds that were suspensory ligament lesions will still show a correlation to the stem cell therapy upon connective tissue (Burk and Brehm, 2011). All of the horses were injected with bone marrow stem cells and were tested with follow up exams for more than 12 months. Information for only 58 horses could be found for follow up exams, so data was analyzed based on the horses and information available. For the treatment to be considered a success, the horse needed to return to their original use or previous training ability without injury. After 12 months, there was an 84.5% success rate for horses returning to the previous abilities, with zero significance in success rate differences between the SDFT and suspensory ligament (Burk and Brehm, 2011). Horses that had their injury previously did not show as improved return rates, with only 50% returning compared to the average 85% from one time injuries. In addition, this study found that the age of the horse could influence the success rate of the treatment. Horses under the age of 12 years old had an 88.9% return, while older horses had a decreased return of 75% (Burk and Brehm, 2011). The discipline of the horse was also found to be significant. Horses that participated in competitions were only reinjured with a 10% rate, but more intensive flat and trotting racehorses had higher reinjury rates of 30 to 66% (Burk and Brehm, 2011). Overall, the study showed that not only was the stem cell therapy successful and had higher success rates, but that there were differences in the recovery due to the background of the horse.

Some stem cell studies have shown slight differences in the cultures of two types of stem cells, but not necessarily the healing ability of the stem cells. In a study with racehorses that had suffered a superficial flexor tendon injury, they researched stem cells from equine adipose tissue (fat tissue) at the base of the tail and the other from bone marrow in the iliac crest. The horses qualified for the study by undergoing an ultrasound that confirmed their injury lesion was a grade 2 and less than 15 days old (Iacono et al., 2015). The stem cell samples taken from each horse were cultured, and osteogenesis, chondrogenesis, and adipogenesis differentiation were all confirmed in staining procedures. Five racehorses were treated with the blood marrow stem cells, and the other five horses were treated with the adipose tissue stem cells. The animals were put into a standard recovery program, and the injury site was checked to record the progress of the lesion. By day 30 after implantation, the tendon tissue had nearly been completely reformed and the fibers resembled healthy tendon alignment. After 12 months the lesion was completely healed and the fibers were aligned as a healthy tendon would be (Iacono et al., 2015). Although all the horses returned to racing, the equine adipose tissue stem cells showed better adipogenic and chondrogenic differentiaton, as well as higher cell growth (Iacono et al., 2015). For this reason, adipose tissue stem cells could be considered advantageous to use in regards to cell banking and culturing the cells, but both types of stem cells were effective in returning the horses to their athletic career.

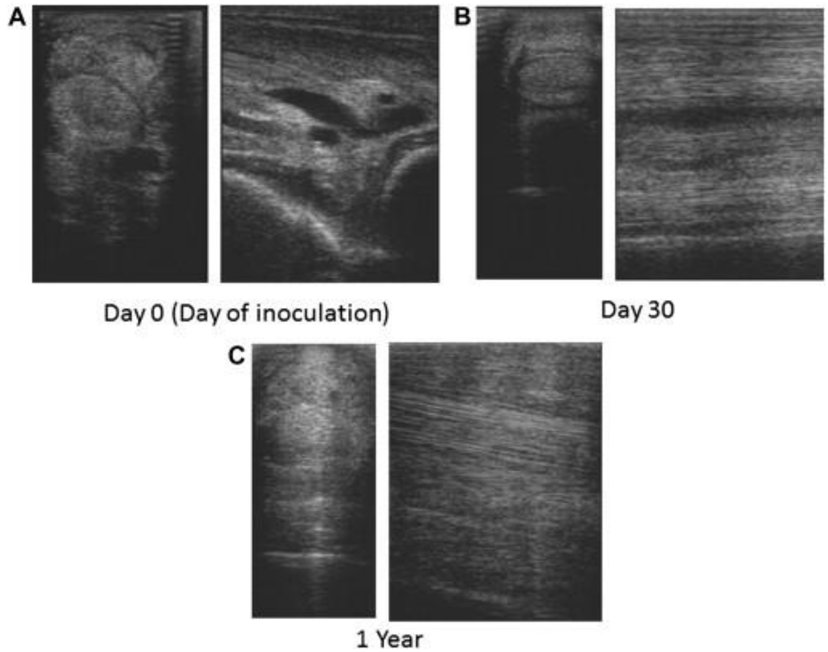

Fig 3. Longitudinal and transversal ultrasound images of an injured tendon (A) treated with bone marrow stem cells at 30 days (B) and 1 year (C). (Iacono et al., 2015)

Adipose tissue and bone marrow stem cells are typically the two stem cells most used and researched in this type of injury. Both are multipotent and allow the stem cells to differentiate to fat, bone, and cartilage cells (Wood, 2006). However, there are advantages to each type of stem cell. Adipose mesenchymal stem cells are arguably more accessible than bone marrow stem cells, especially in equine. Adipose stem cells will usually be taken from the gluteal region (Guercio et al., 2015), whereas a bone marrow stem cell sample is more invasive and requires a small procedure, such as a sample from the sternebrae or iliac crest. Stem cells from adipose tissue have showed a higher adipogenic specialization due to their ability to form more lipid vacuoles, and a higher chondrogenic differentiation shown by more glycosaminoglycans (Iacono et al., 2015). Due to the accessibility and differentiation of the adipose tissue stem cells, it may be more beneficial to use adipose stem cells in comparison to bone marrow stem cells, although there shows no difference in results.

In one study, 113 horses from Flat racing or National Hunt racing were injured and implanted at the injury site with cultured bone marrow stem cells. The stem cells were taken from the sternebrae and cultured to attain a higher concentration of stem cells in the sample (Godwin et al., 2011). The stem cells were injected into the lesion site, and a 42 week rehabilitation program started immediately following treatment. Although the recovery program was not regulated, 111 horses returned to racing and only 31 horses were reinjured on the same limb within three years of the treatment. With a 98.2 percent return rate to racing and a 27.4 percent re-injury rate, the stem

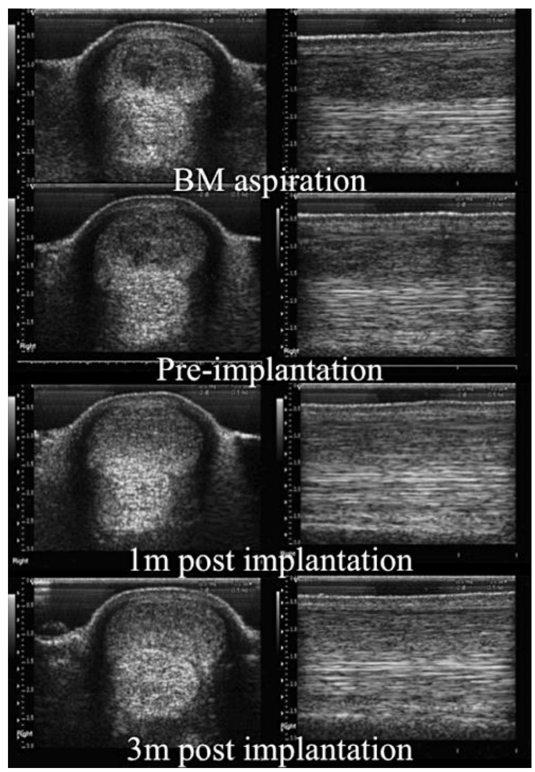

Fig 4. Ultrasound of a tendon treated with bone marrow stem cells. Godwin et al., 2011).

cell therapy was determined to be more effective than medical management practices by nearly halving the re-injury rate (Godwin et al., 2011). The amount of time between injury and implantation was also studied and showed results that may be consistent with the healing process, but will need a larger sample size in order to be statistically significant. The longer the period of time between injury and implantation could directly affect the amount of scar tissue that forms, which would affect the effectiveness of the stem cell therapy (Godwin et al., 2011). Although the results were not statistically significant, the percentage of re-injury rates increased by 15% as the length of time increased by an average of 3 weeks (Godwin et al., 2011). Ultimately, bone marrow stem cell therapy shows much better return and lower re-injury rates than conventional therapy would in flat and National Hunt racehorses.

A different case study was done on 168 racehorses that had superficial digital flexor tendon injuries. These horses were injected with bone marrow MSCs that had been culture expanded to obtain a greater number of stem cells. Of the 168 horses tested, 78 percent of the horses had no injury for more than one year after treatment. In conventional therapy treatments, typically only 44 percent of the horses recover from their injuries for more than a year (King, 2015). In addition, the 13 percent re-injury rate of horses with the stem cell treatment far surpasses the 56 percent re-injury rate of horses with conventional therapies (King, 2015). This statistic also shows that the new tendon tissue from the stem cells is better fit for normal activity than the scar tissue that develops without stem cells. The stem cells not only have the ability to return the tissue to the same structure, function, and tensile strength after regeneration, but also have immunomodulatory paracrine effects (King, 2015). Therefore, the stem cell therapy shows great improvement over other methods that may try and mechanically repair the tendons because it more closely resembles the normal tissue.

Immunomodulatory paracrine effects include the stem cells ability to influence the immune system. Depending on the conditions of which the stem cells are exposed to, they can regenerate, sustain function, and help cell survival (Fontaine et al., 2016). The ability of the stem cells to do this is mainly due to the secretion of biological factors that have a paracrine effect. These factors include the transforming growth factor (TGF-B1), insulin-like growth factor, vascular endothelial growth factor (VEGF), and others (Fontaine et al., 2016). These factors promote reproduction, healing, and the development of new blood vessels, which have made stem cells a possible way to treat inflammatory and degenerative diseases (Wood, 2006). The stem cells affect the immune system when the secretion of the biological factors come in contact with dentritic cells, lymphatic T cells, monocytes and macrophages (Fontaine et al., 2016). This process is not completely understood, but clinical studies and other tests have been able to link stem cells with these effects on the immune system.

Another stem cell study was done on nine athletic horses that had sustained a superficial digital flexor tendon lesion in the foreleg. Each horse showed symptoms including pain, swelling, and edema along the tendon, and tendon lesions were confirmed by ultrasound. Mesenchymal stem cells from adipose tissue were used due to their accessability, isolation procedure, and ability to increase the concentration of MSC's in vitro (Guercio et al., 2015). In addition, platelet-rich plasma (PRP) from the jugular vein was injected with the stem cells to increase collagen type 1 production, multiplication, and vascularization of the injury site (Guercio et al., 2015). Since the tendon does not get as much blood flow, the PRP mimics blood and its growth factors. The combination of the PRPs and the MSCs used on each horse had the same concentration and was injected into the lesion site of each injury. The goal of this therapy was to increase the regenerative ability of the injury site and return the horses back to their previous level of athletic activity (Guercio et al., 2015).

For the next six months, the horses were subjected to physical therapy and increased conditioning until they returned to their careers. The lesion started at a grade 3 at the time of injury, and had already decreased to a grade two after one month. The grades by Reef are an indication of the fiber alignment, with one being the best condition. At two months, the tendon fibers showed improvement at a grade 1 and a decreased lesion size. At four months, the tendon fibers were completely realigned (Guercio et al., 2015). Of the nine athletic horses, seven of them returned to previous levels of activity after seven to nine total months of therapy and have not relapsed (Guercio et al., 2015). Two of the horses did relapse, but both cases were unrelated to the treated injury. One occurred on a different location on the tendon than the original injury, while the other most likely relapsed because the owners did not follow the recommended recovery period. The results of the therapy also showed that the new tissue closely resembled the tissue size and alignment of normal, healthy tissue (Guercio et al., 2015). The healing mechanism of the stem cells greatly improves the chances that the horses are able to return to normal athletic abilities without a relapse in the SDFT.

The platelet rich plasma that was used in this study is also another type of regenerative medicine that can be paired with stem cells during therapy. PRP has several important growth factors and two to four times the amount of platelets as normal blood

(Textor, 2011), which help supplies the area with factors that the tendon would be deprived of due to lack of blood. The growth factors include platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-B1), insulin-like growth factor, and vascular endothelial growth factor (VEGF) (Hamilton, 2015). Together, these growth factors contribute to increasing the type 3 collagen, increase expression of genes for extracellular components and protecting extracellular components by decreasing certain enzyme expression (Amable et al., 2013). It is also thought that the PRP may recruit stem cells to the injury site, so the combination of extra growth factors and additional stem cells could be advantageous to use in junction with stem cell therapy.

Regenerative medicine and stem cell research have shown that stem cell therapy is a highly effective way to manage tendon injuries, one of the most common being the superficial digital flexor tendon injury. The ability of the cells to differentiate into several types of cells, including muscle and tendon cells, gives the injured tendon the opportunity to return to being fully functional. The additional benefits that the stem cells provide to the injured tissue include growth factors and immunomodulatory paracrine effects, which help with the overall healing process. Stem cell therapy has already been able to improve the number of horses able to return to full athletic ability when compared to mechanical or surgical methods, regardless of the differences between adipose and blood marrow types of stem cells. Although adipose tissue stem cells are more accessible and have been shown to differentiate better, blood derived stem cells also provide an effective and successful resource for regenerative medicine. Used with other therapies, including platelet rich plasma, stem cell therapy is a viable option for horse owners wanting to treat horses that are athletically advanced and get better results than those by conventional or surgical methods. If more research is done and approved, stem cell therapy could improve injuries or diseases over several different species, possibly even humans.

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