How to Inject Bone Marrow–Derived Mesenchymal Stem Cells Into Tendons and Ligaments

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1. Introduction
Bone marrow–derived mesenchymal stem cells (BMDMSCs) or progenitor cells have been used for treatment of many musculoskeletal diseases in the horse. Diseases most commonly treated with the use of this therapy in the horse are tendonitis, desmitis, and joint disease.1–4 Bone marrow is an excellent source of progenitor cells, and, although mesenchymal stem cells harvested from fat can also provide a source of progenitor cells, bone marrow–derived progenitor cells appear superior in their ability to heal musculoskeletal tissues.5–9 The technique of harvesting BMDMSCs from ilium or sternum has previously been described.10

The objective of this presentation is to familiarize practitioners with the technique of injecting these cells once they are culture-expanded and sent back to the practitioner. Specifically, this presentation will review the thawing, preparation, and ultrasound guidance of injecting these cells into tendon and ligaments.

2. Methods
Sedation should be performed using detomidine hydrochloride at 0.02 mg/kg and butorphanol at (dose) intravenously. Flunixin meglumine should be administered before injection at a dose of 1.1 mg/kg intravenously to decrease any inflammation associated with the injection process as well as to minimize the potential of a “flair” associated with the stem cell injection, which is reported with treatment using other biologics as well. The area to be injected should then be clipped, and an ultrasound examination is performed. The affected area should be visualized in both the horizontal and longitudinal planes. The area of the limb should then
be locally anesthetized using peripheral nerve blocks with mepivacaine hydrochloride above the region of the tendon or ligament defect to minimize any movement of the limb while the ultrasound-guided injection is being performed. Alternatively, a subcutaneous local block may be performed; however, this may not adequately locally anesthetize the area, adding increased risk to the ultrasonographer, and a subcutaneous bleb of fluid may decrease the visibility of the needle passing into the lesion, using ultrasound guidance due to the fluid or potential gas.

After administration of local anesthesia, the frozen vial(s) of cells are removed from the packaging. The handler should wear latex gloves and place the vials (while grasping the tops so as not to submerge the entire vial) in a warm water bath of approximately 37°C. The cells should be thawed rapidly and at this temperature should thaw in 2 to 4 minutes (Fig. 1). The outside of the vials should then be dried. Lactated Ringer's solution (LRS) should

Fig. 1. The frozen cells are removed from the packaging and thawed in a warm (32°C) water bath for thawing. Thawing is usually complete in 2 to 4 minutes for complete thaw.

Fig. 2. Lactated Ringer's solution should be used to dilute the cells. Photo shows 1 mL of lactated Ringer's, solution being drawn into a syringe for dilution of the stem cells.

Fig. 3. The cells are drawn up into the syringe that has 1 mL of lactated Ringer's solution. The cryovials have 1 mL (or less) of frozen cells. Concentration of cells is usually 10 millions cells/mL. If 5 million cells are present, then it will be in a volume of 0.5 mL.
then be used to dilute the cells in the desired volume (Fig. 2). The LRS should be drawn into the syringe. The top of the vial should then be removed and the LRS added in dropwise fashion to the thawed stem cells (Fig. 3). Usually there is 1 mL of stem cell solution, and the volume should be doubled (although concentration as well as thawing techniques may vary, depending on the company that prepares the suspension). If 1 mL of solution is present in each vial, then 1 mL of LRS should be used to dilute the cells. Antibiotics should not be placed into the cell solution and do not need to be administered unless the clinician believes there is an ongoing septic process.

Before aseptic preparation, the ultrasound machine setting should be adjusted to maximize visualization of the area and specifically the lesion. The needle path should then be selected to avoid other structures whenever possible. Vasculature and nerves should also be identified and avoided. The area is then aseptically prepared, and ultrasound gel is applied to an ultrasound probe; the probe is then placed in a sterile glove or sleeve. Alcohol is then placed (ideally sprayed) on the area of injection to maximize visualization of the tendon or ligament structure.

The clinician should have both hands sterile, and the ultrasound probe is held in one hand while the needle is positioned with the other hand (Fig. 4A). It is not recommended that a separate person place the needle because it makes alignment of the needle and ultrasound beam much more difficult. However, in select cases, it is necessary for the ultrasonographer to have a separate individual hold or guide the needle. The first example (Fig. 4) demonstrates injection of a deep digital flexor tendon at the level of the midmetacarpus. It is important to steadily hold the probe onto the limb and direct the needle (no smaller than 22 gauge) with the opposite hand and insert the needle in the plane parallel with the long axis of the probe and the ultrasound beam. The probe should be held in the area of the lesion, and the needle should be identified as it is advanced into the lesion. In Figure 4, the needle is being inserted from the lateral aspect so as not to pass through any other structures except skin, subcutaneous tissue, and affected tendon (Fig. 4B). It is vital that the tip of the needle be visualized the entire time it is being advanced. Once the end of the needle is visualized in the lesion, the stem cell solution can be injected either by the ultrasonographer or another individual if the ultrasonographer prefers to either visualize the injection while holding the probe and needle in place. If the lesion does not extend more than 2 to 3 cm in length, then the injection is often performed at one location. Multiple locations proximally and distally may be used, however, or the needle can be slightly redirected without actually completely removing the needle from the skin.

Another example of injecting the deep digital flexor tendon is shown in Figure 5, A and B. The thin, soft tissues in the area of the pastern region does not allow much variation in needle placement.
palmarly/plantarly to dorsally. Therefore, the transducer should be placed in a transverse plane relative to the limb and the needle placed perpendicular to the ultrasound beam angle from either the medial or lateral aspect of the limb. Small manipulations of the hub of the needle can then be performed to direct the needle palmarly or dorsally, and the needle can be angled superficially or deep, depending on the lesion.

Injection of the forelimb suspensory ligament is usually performed with the leg in flexion, allowing the suspensory ligament to “relax,” and the superficial and deep digital flexor tendons can be moved away from the injection site with the probe. Figure 6 (A and B) reveals the technique and the ultrasound appearance of injection of a suspensory ligament.

After injection, the area is bandaged with a pressure bandage for 2 to 3 days. We recommend administration of nonsteroidal anti-inflammatory drugs (NSAIDs; phenylbutazone, flunixin meglumine) for 2 to 3 days after this injection process.

3. Results
Approximately 1,500 cases of BMDMSC injections have been performed at the Orthopaedic Research Center and Veterinary Teaching Hospital at Colorado State University and collaborating practices with Advanced Regenerative Therapies, Inc. (Fort Collins, CO). Approximately 60% of those have been for tendon or ligament injuries and 40% for joint disease. When these cells are injected into joints, the cells are prepared in the same fashion and injected into joints as the practitioner would inject/aspirate any joint and hyaluronic acid (20 to 22 mg) is often injected before or after. We do not typically use ultrasound injection for joints, although postinjection recommendations remain the same as for tendon and ligament.

The expanded cells are frozen in 95% of the horse’s own serum and 5% dimethylsulfoxide (DMSO) to preserve the cells. Rarely are “flairs” observed (less than 2% of injections), but owners are warned that this is a potential side effect. Flunixin meglumine intravenously and phenylbutazone or other NSAIDs for 3 days after injection may decrease the potential of this as well as decrease any inflammation observed with the injection.

4. Discussion
Ultrasound-guided injection of BMDMSC is the ideal technique to inject these cells because the lesion is visualized and accurate injection of the lesion is ensured. The most important aspect of the technique is to visualize the needle, using the ultrasound probe, and to adjust the direction of the needle to insert the end of the needle into the area of lesion. The volume of cell solution used should not exceed the volume of the lesion because this may result in damage of the surrounding tissues.

The technique described above is easy and practical for clinicians. With practice, the clinician can become very effective at guiding the needle with the ultrasound probe into the tissues. Once the cells are thawed, the clinician should not allow these cells to exist at room temperature for longer than 15 to 20 minutes because the DMSO that is present in the thawed solution can damage cells. The clinician should work expeditiously to inject the cells once thawed. Furthermore, the authors believe that it is important to administer NSAIDs before injection and for 2 to 3 days after injection to reduce any inflammation associated with injection and also to apply a pressure bandage for 1 to 2 days.

Presently, very little information exists as to ideal numbers of cells or whether multiple injections into lesions improves healing and prognosis. When cells are expanded, typical yields are 10 million to 30 million stem cells. For a lesion in a deep digital flexor tendon, superficial digital flexor tendon (SDFT), or suspensory ligament that is 1 cm in diameter, 1 million to 3 million cells might be injected (1 to 3 million cells/cm). Future research will guide
clinicians in determining ideal cell numbers and appropriate numbers of injections over time.

References

Fig. 6. A, Injection into the proximal suspensory ligament on a forelimb. The leg is flexed, the probe is placed on the caudal aspect of the limb, and the needle is directed into the suspensory ligament by aligning the needle parallel to the ultrasound probe. B, Ultrasound appearance of the suspensory ligament lesion (green arrow) and needle (red arrow) as it is placed into the lesion. The volume of the lesion should not exceed the volume of the solution of stem cells injected.