Stem Cell Therapy in the Horse

Kyla Ortved, DVM, PhD, DACVS, DACVSMR
Assistant Professor of Large Animal Surgery
New Bolton Center, University of Pennsylvania

What is a stem cell?
- Undifferentiated cell that has 2 properties:
  1. Self-renewal
  2. Potency

Stem Cells: Self Renewal
- Ability to undergo cell division (mitosis) without differentiation
- Maintains the stem cell population

Stem Cells: Potency
- Totipotent: can differentiate into all embryonic and extra-embryonic cells
  - Cells present in morula immediately after fertilization
- Pluripotent: can differentiate into any of the 3 germ layers (endoderm, mesoderm, ectoderm)
  - Embryonic stem cells (ESC), induced pluripotent stem cells (iPS)
- Multipotent: can differentiate into multiple, but limited cell types
  - Hematopoietic stem cell (HSC), mesenchymal stem cell (MSC)
- Oligopotent: can differentiate into only a few cell types
  - Lymphoid or myeloid progenitor cells
- Unipotent: can differentiate into only 1 cell type
  - Osteoblast, hepatoblast
### Types of Stem Cells

**Embryonic stem cells**
- Totipotent or pluripotent depending on time of harvest

**Fetal stem cells**
- Similar to adult but less mature

**Adult (somatic) stem cells**
- Multipotent
- Reside in “stem cell niche”

**Induced pluripotent stem cells**
- Reprogrammed adult cell that is pluripotent

### Types of Adult Stem Cells

- **Hematopoietic**
- **Mesenchymal**
- **Neural**
- **Neural crest**
- **Endothelial**

### Mesenchymal Stem Cells (MSCs)

- Most common stem cell used in regenerative medicine
- Multipotent cells capable of trilineage differentiation

**Criteria for Human MSCs:**
1. Trilineage differentiation
2. Adherence to plastic
3. Cell surface markers
   - Positive = CD73, CD90, CD105
   - Negative = CD11b or CD14, CD34, CD45, CD79α, HLA-DR

### Equine Mesenchymal Stem Cells (MSCs)

- Often defined by plastic adherence & morphology alone
- Trilineage differentiation more difficult
- Still no consensus on cell markers in the horse
  - Positive: CD29, CD44, CD90, CD105, MHCI
  - Negative: CD34, CD45RB, CD79α, MHCII?

### Sources of MSCs in the Horse

**Adult**
- Bone marrow
- Adipose
- Synovium
- Articular cartilage
- Tendon

**Fetal**
- Umbilical cord blood
- Umbilical cord tissue
- Amniotic membrane

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![Image](https://www.eurostemcell.org)
**BM-MSCs: Harvest**

Obtaining sternal bone marrow aspirate from a standing horse.

Lisa A. Fortier
Lauren V. Schnabel

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**Cultured BM-MSCs**
- Density centrifugation
- 2-4 weeks for expansion
- High density of MSCs allow for several rounds of injection

**Bone marrow aspirate concentrate (BMAC)**
- Patient-side
- Low density of MSCs

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**AD-MSCs**
- 15 to 20 grams harvested and collagenase digested

**AD-stromal vascular fraction (ADSVF)**

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**Mechanism of Action**
- Previously thought MSCs engraft, differentiate and then regenerate into native tissue
- More likely to modulate the environment through paracrine effects and promote appropriate healing

- Immunomodulatory
- Angiogenic
- Anti-inflammatory

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**Mesenchymal Stem Cells (MSCs)**

**Clinical Uses of MSCs**
- Tendonitis / Desmitis
- Laminitis
- Joint Disease
- Cartilage Lesions
Ultrasound-guided Injections

- Sterile prep on limb, sterile glove on ultrasound probe
- Local anesthetic skin bleb or perineural analgesia
- ~ 10-30 million cells
- 20-33g needle to minimize damage to tendon & cells
- Alcohol on skin
- Needle parallel to probe & within plane of ultrasound beam
- Watch needle enter lesion
- "Fill" core lesions
- Repeat every 3-4 weeks for 3-4 treatments

RPL for extensive lesions?

- Sole et al. 2012: Good MSC migration with IV or IA perfusion under GA
- Spriet et al. 2015: Limited MSC migration with IV perfusion in normal standing horses
- Espinosa et al. 2016: Intra-arterial MSC injection of allogeneic cells in standing horse
  - Good distribution but some venous thrombosis

MSCs for tendonitis / desmitis: Clinical Evidence

- Collagenase-induced ISDF tendonitis
  - Injected with 10 million AD-MSCs labeled with iron-oxide or saline
  - Labeled cells tracked with MRI and histology over 24 weeks
- Persistence of MSCs in lesion (~2.5% total cells) and presence of MSCs in saline treated lesion
### MSCs for Tendinopathy / Desmitis: Clinical Evidence

**Abstract**

Tendon disease is a common age-related degenerative condition, frequently with a recurrence of injury. Tendon repair often includes collagen (including collagen fibrils) and ground substance which contain proteoglycans, provides a supporting structure for tendons and ligaments. These structures are often used in the treatment of surgical tendinopathies and injuries by providing support, allowing continued movement and functionality and normal quality of life. This disease also occurs naturally in horses, with many similarities to human tendinopathy. The use of autologous mesenchymal stem cells (MSCs) has been shown to reduce inflammation and improve collagen organization and function in vitro and in vivo. The aim of this study was to determine the potential of MSCs to repair naturally occurring tendinopathies.

#### Methods

- **Implantation of MSCs**: Bone marrow-derived MSCs were implanted into the superficial digital flexor tendon of horses at the Department of Biotherapeutics, University College London, Stanmore, United Kingdom.
- **Evaluation**: The evaluation included histology, histochemistry, immunohistochemistry, and semi-quantitative scoring of the MSC-treated tendons compared to ungrafted control joints.

#### Results

- **Histology**: MSC-implanted tendons had lower structural stiffness (p<0.05) although no significant difference in calculated modulus of elasticity, similar histological appearance, and improved collagen organization compared to ungrafted controls.
- **Semi-quantitative scoring**: The MSC-implanted tendons showed significant improvement in many parameters compared to ungrafted controls.

#### Conclusion

MSCs have the potential to improve tendon healing and function in both pre-clinical and clinical settings. Further studies are needed to fully understand the mechanism of action and to optimize the use of MSCs in tendon repair.

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**Table 1**: Selected Histologic Scores for MSC and Control Implanted Cartilage Defects. Scores are Graded (0-3), with 0 representing normal tendon and 3 representing fibrous scar tissue.

<table>
<thead>
<tr>
<th>Score</th>
<th>MSC Implanted</th>
<th>Control Implanted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal tendon</td>
<td>Normal tendon</td>
</tr>
<tr>
<td>1</td>
<td>Mild fibrous</td>
<td>Moderate fibrous</td>
</tr>
<tr>
<td>2</td>
<td>Moderate fibrous</td>
<td>Severe fibrous</td>
</tr>
<tr>
<td>3</td>
<td>Severe fibrous</td>
<td>Severe fibrous</td>
</tr>
</tbody>
</table>

**Figure 4**: Histological assessment of MSC and control implanted cartilage defects. (A) MSC-implanted defects had improved collagen organization compared to control defects. (B) Increased tissue volume in the defect (A) and white "cartilage-like" material on the surface of the graft (B). (C) Improved collagen organization in MSC-implanted defects (D) compared to control defects (E). (F) Improved collagen type II in MSC-implanted defects (G) compared to control defects (H).
MSCs for cartilage repair: Pre-clinical and clinical evidence

Evaluation of Articular Cartilage Progenitor Cells for the Repair of Articular Defects in an Equine Model

- Autologous BM-MSCs improved healing over fibrin and control
- Allogeneic did not improve repair, led to mild inflammation

Fig. 5-B
Photomicrographs of a representative histologic section from each treatment group as well as a normal reference (hematoxylin and eosin).

MSCs for bone. The values on the ruler are 0.0, 0.5, and 1.0 mm.

Allogeneic did not improve repair, led to mild inflammation

Autologous cells improved healing over fibrin and control

Fig. 5-B
Mean total histology score (and standard error). Groups labeled with the same letter did not significantly differ from each other.

→
60-63%

MSCs administered via regional limb perfusion as early as possible in acute phase

More perfusions at varying intervals

May modulate pro-inflammatory state in laminae

No pre-clinical or clinical evidence yet

MSCs for joint disease: Pre-clinical and clinical evidence

Clinical Outcome After Intra-Articular Administration of Bone Marrow Derived Mesenchymal Stem Cells in 33 Horses With Stiffe Injury

- All horses underwent arthroscopy for various stiffe injuries
- All horses had IA injection of BM-MSCs post-operatively
- Overall 43% returned to previous work & 33% returned to work
- 75% of horses with meniscal damage returned to work
- Arthroscopy alone → 60-63% return to previous work

MSCs for joint disease: Pre-clinical and clinical evidence

- BM-MSC joints had less synovial effusion and less inflammatory cells in the subchondral bone reaction, and Outerbridge score. Quantitative magnetic resonance imaging datasets were analyzed with statistical methods.

Significance was assessed to determine percentage fill, synovial reaction, and subchondral bone reaction for arthroscopic scores at twelve weeks postoperatively, gross and microscopic scores at twelve months.

Mean total histology score (and standard error). Groups labeled with the same letter did not significantly differ from each other.

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MSCs for joint disease: Pre-clinical and clinical evidence

Overall 43% returned to previous work & 33% returned to work

All horses had IA injection of BM-MSCs post-operatively

All horses underwent arthroscopy for various stiffe injuries

75% of horses with meniscal damage returned to work

Arthroscopy alone → 60-63% return to previous work

MSCs administered via regional limb perfusion as early as possible in acute phase

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May modulate pro-inflammatory state in laminae

No pre-clinical or clinical evidence yet

MSCs for joint disease: Pre-clinical and clinical evidence

Bone Marrow Aspirate Concentrate (BMAC)

Designed by Harvest Technologies

- Similar disposable cup as PRP but different shelf density
- Same centrifuge as PRP

- Contains small quantities of MSCs
- Concentrates platelets like PRP

BMAC: Administration

60 mL sternal aspirate

15 units heparin/mL aspirate

10:1 BMAC : thrombin (3300 U/mL)

14 minute centrifugation

SmartPrep II

7 mL BMAC

Supernatant
BMAC Grafting

HA BMAC Graft

Pluripotent Stem Cells

Embryonic stem cells/embryonic-like stem cells (ESCs)

- Cultured from inner cell mass (ICM) of blastocyst

- Equine different from mice, primates, humans?
  *Unable to form teratomas in SCID mice

Induced Pluripotent Stem Cells

Induced Pluripotent Stem Cells

Yamanaka et al. 2006 → (programming of adult fibroblasts with 4 transcription factors converts cells into pluripotent cells)

- Oct4, Sox2, cMyc, Klf4

Induced Pluripotent Stem Cells (iPS)

iPS cells generated from adipose stromal cells reprogrammed with lentiviral vector

- Cells showed improved muscle healing and regeneration in mice with injured gastrocnemius muscles
Conclusions: All MHC-mismatched recipient horses produced anti-ELA-A2 antibodies following injection of ELA-A2 MSCs and developed a wheal at the injection site that persisted for the duration of the experiment. Anti-ELA-A2 antibody responses were varied both in terms of strength and timing. Four recipient horses had high-titered anti-ELA-A2 antibody responses, with one horse having undetectable antibody titers.

Methods: Preparation and intradermal injection of donor MSCs: Donor MSCs, which were previously isolated and immunophenotyped, were used as MHC-matched controls. MHC homozygote horses of known ELA-A2 haplotype were used as MSC donors, and unrelated MHC type recipients were identified. The ELA-A2 haplotype was confirmed in the recipients by genotyping. Horses were injected intradermally with 2 million donor MSCs, and antibody responses were monitored every 7 days for the duration of the study.

Tables:

<table>
<thead>
<tr>
<th>Breed</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Horse</th>
<th>MHC Status</th>
<th>MSC Passage</th>
<th>Number of MSCs Injected</th>
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<tbody>
<tr>
<td>Thoroughbred</td>
<td>14</td>
<td>Gelding</td>
<td>9</td>
<td>P5</td>
<td>Passage 2</td>
<td>6 x 10^6</td>
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<td>P3</td>
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<td>6 x 10^6</td>
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<td>Thoroughbred</td>
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<td>Gelding</td>
<td>4 (2)</td>
<td>P4</td>
<td>Passage 2</td>
<td>8 x 10^6</td>
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<tr>
<td>F</td>
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<td>Gelding</td>
<td>4 (1)</td>
<td>P4</td>
<td>Passage 2</td>
<td>8 x 10^6</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>Gelding</td>
<td>9</td>
<td>P5</td>
<td>Passage 2</td>
<td>6 x 10^6</td>
</tr>
</tbody>
</table>

Questions?

Gaps in Our Knowledge:
- Optimal source of MSCs
- Optimal cell number
- Differentiation status of cells at implantation:
  - Naive
  - Directed differentiation
  - Growth factors
- Need for matrix to contain cells
- Autologous vs. allogeneic

Allogeneic Stem Cells:
- Ideal cell source to use patient-side without lag time
- Fetal cells may have greater differentiation potential
- Safety and efficacy remains unknown
- All horses produced antibodies to allogeneic cells and local inflammatory response
- May limit effectiveness of cell therapy

Need for matrix to contain cells
- Optimal cell number
- Differentiation status of cells at implantation:
  - Naive
  - Directed differentiation
  - Growth factors
- Need for matrix to contain cells
- Autologous vs. allogeneic