THE USE OF INTRAARTICULAR STEM CELLS IN HORSES
David D. Frisbie, DVM, PhD, DACVS, DACVSMR
Equine Orthopaedic Research Center, College of Veterinary Medicine and Biological Sciences, and Molecular, Cellular & Tissue Engineering, Department of Mechanical Engineering, School of Biomedical Engineering, Colorado State University, Fort Collins, Colorado

Key Points
- The use of bone derived cultured expanded mesenchymal stem cells (MSC) in addition to surgery appears to improve return to function compared to historic studies with surgery alone in the equine stifle.
- The use of intraarticular injection of MSC appears to have better long term results compared to delivery with fibrin.

In the last decade researchers have been looking into the use of stem cells to aid in various joint pathologies. These can loosely be broken down into 3 categories; 1) cartilage resurfacing, 2) osteoarthritis and 3) damaged intraarticular soft tissue structures like the meniscus. It must be realized that joint disease usually has some component of all three categories. To date the equine peer reviewed literature has been focused on autologous cells. This has been for two main reasons; 1st the immunogenicity of allogeneic equine stems cells has yet to be established in the peer reviewed literature and 2nd the legality of regulatory pathway for commercial utilization of allogenic cells. Thus this manuscript will focus on autologous cells.

Researchers from Cornell University have been assessing autologous stem cells mainly for cartilage resurfacing techniques. They have been utilizing fibrin as a scaffold to hold the mesenchymal stem cells (MSC’s) in cartilage defects. While some clinical success has been reported by Nixon, Wilke et al in a controlled experimental study using autologous culture expanded cells did not demonstrate long term beneficial effects.1 Researchers at Colorado State University’s (CSU) Orthopaedic Research Center have taken a different approach, injecting MSC’s into the joint space 30 after lesion creation without the use of a scaffold. Following a year of strenuous exercise some softening of the microfracture alone (HA only) repair tissue was noted but the repair tissue in the MSC treated joint remained firm as compared to a 6 month time point. Further the degree of aggrecan staining was improved in the MSC treated repair tissue when compared to microfracture alone at the 12 month time point.2 Based on this work and that of Lee et al who also administered MSC’s to the joint space in a porcine model it would appear that direct intraarticular administration of MSC’s may have long term advantages compared to delivering them in fibrin for cartilage resurfacing techniques at this time.

Using an established model of OA bone derived culture expanded MSC’s or adipose derived stromal vascular fraction (SVF) were evaluated side by side for treatment of acute equine OA.7 The results of this study indicated significant improvement in synovial fluid prostaglandin E2 (PGE2) levels in response to treatment with bone derived cells although significant differences were not demonstrated in other key parameters (clinical lameness, radiographic, histologic and biochemical). The study also demonstrated an increase in synovial fluid tumor necrosis factor (TNF) concentrations in response to adipose derived cells, which would be interpreted in a negative light. The conclusion of the study is that nominal improvement in symptom or disease modifying effects were seen with bone derived stem cells. Further that the administration of adipose derived SVF upregulated the production of proinflammatory cytokines.7
Trying to simulate the beneficial results seen by Murphy et al\(^6\) for healing meniscal tissue researchers at CSU have been treating various clinical cases of joint disease that have arthroscopic confirmation using a combination of HA and autologous MSC’s, with special interest in those diagnosed with meniscal injury. A 6 month follow-up pilot project involving 15 cases\(^8\) showed promising results and lead the authors to expand the study into multicenter trial. The results of this prospective multicenter trail were also very promising.\(^9\) Specifically, 39 cases were treated with intraarticular (IA) administration of autologous bone marrow derived MSC’s and having a mean follow-up period of 21 months. It is noteworthy that cases selected for this trial were meant to have failed routine treatments, be moderate to severely affected, and have surgical confirmation of the diagnosis. Seventy seven percent returned to some level of work. Thirty six percent (14/39) returned to or exceeded their prior level of work, 36% (14/39) returned to work at a lesser level or required some level of additional medical treatment in the affected joint to maintain soundness, and 28% (11/39) did not achieve work status prior to follow-up.\(^9\)

Stifle injuries comprised 29 of the 39 cases with 20 having a primary diagnosis of meniscal damage. Interesting differences were noted when these data were compared to published studies on meniscal damage with surgery alone.\(^10,11\) With surgery alone, grade 3 meniscal tears had a 0 and 6%\(^10,11\) return to work compared with the current study with MSC’s and surgery having a 60% return to work. The intraarticular treatment with autologous cells had an 8% incidence of acute inflammation or “flare”, which given the concurrent administration of HA (12% flare incidence reported) would be expected or lower than expected incidence. Thus it appears in clinical cases of joint disease, especially the more severe; the addition of stem cells may improve the horse’s ability to return to work over surgery alone. The next logical step would be a placebo controlled clinical trial, this is unlikely because of difficulties gaining owner compliance for such a study design, however, a positive controlled clinical trial may be possible and shed more light on the most useful treatment applications for cell based therapies.

In Conclusion using endogenous cells stimulated through subchondral bone microfracture appears to be the state of art for equine cartilage resurfacing. It appears that this technique can be significantly augmented with the addition of MSC’s to the joint space. There is evidence that MSC’s do not work in all cases of stable OA but we do not have enough information to make a definitive conclusion on all types of OA. Finally there appears to be evidence that augmenting surgery with MSC administration improves the return to work of horses suffering from severe meniscal injury. In summary it is early in the use of MSC’s for the treatment of joint injury but it appears that they will provide benefit to our equine patients but we have much to learn.

Acknowledgement
Dr. Frisbie is a shareholder in Advanced Regenerative Technologies (ART), 200 West Mountain Suite A, Fort Collins, CO 80521, (970) 222-9831, www.art4dvm.com