STEM CELLS AS A TREATMENT FOR OSTEOARTHRITIS

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Stem cells are receiving a great deal of scientific attention as well as coverage in the lay press. One of the many reasons for the attention stems from the potential of these cells to regenerate tissues without the production of scar tissue that is generally associated with healing processes. With any new technology comes a myriad of terms, many of which are poorly defined with regard to stem cells. One of the most difficult distinctions when discussing stem cells is defining what is a "stem cell". The first distinction to be made is between embryonic and adult stem cells. Adult stem cells are those which arise or are obtained from any post-natal source. Embryonic cells arise from an embryo, often in an 8 cell or fewer stage. Embryonic cells that are capable of generating an entire organism are referred to as "totipotent". A more restricted subset of cells that is capable of forming tissues from each of the germ layers is referred to as "pluripotent" cells or, when generating an even more restricted subset of cells, called "multipotent". It has long been thought that each tissue type had a resident population of adult stem cells present to maintain the tissue. The recent idea of plasticity suggests that adult stem cells can de-differentiate then re-differentiate down another cell lineage or transdifferentiate to another lineage. An example would be a hematopoietic stem cell (mesodermal in origin) that becomes neuron (ectomdermal origin).

Many of the early reports used bone marrow as a source of stem cells, but other sources of mesenchymal stem cells (MSC's) have been more recently demonstrated. For example muscle, cartilage and adipose tissue all have been shown to contain multipotent MSC'S.

Isolation of MSC's from bone marrow or digested tissue extracts is most commonly achieved by simple adhesion and proliferation of MSC's to tissue culture surfaces. This crude technique does not ensure a homogenous population of MSC's because cells such as fibroblasts may likewise readily adhere and proliferate. While non-progenitor cell contamination may be an expected outcome of the adhesion sorting technique, the extensive volume of literature detailing bulk multipotent behavior of adherent MSC's populations demonstrate the presence of a significant, if not a homogenous, MSC's population. In fact, near-homogenous MSC'S populations have been reported from adhesion sorting.[1] Researchers are currently working on more rigorous methods of identifying stem sells through the use of cell surface antigens such as cluster differentiation (CD) factor 34 and 44. There is still significant research to be done in this area, and a consensus on the exact antigen profile of a MSC'S has not been reached.

Most of the research aimed at clinical treatments has been carried out using autologous MSC's, mainly from bone marrow.[2] Specifically, bone marrow derived stem cells have been used in vitro to generate bone, cartilage, tendon, ligament, meniscus, intervertebral disc, fat, muscle, and nerve.[2] Because of the availability of adipose tissue, it too has received a fair amount of recent research as a source of MSC's.[3] A clear delineation of the pros and cons of fat derived verses bone marrow derived MSC's is lacking. Ease of collection procedure, number of stem cells recovered, capacity and efficiency to differentiate into various mesenchymal tissues, as well as morbidity associated with the collection procedure are all important points to consider when discussing bone-marrow versus adipose derived stem cells. Because MSC's treatments are

being used from both fat and bone marrow, it is important to point out that few direct comparisons have been published, and at this point a definitive answer is lacking on which population of cells is better. The following section will detail some salient points regarding the use of MSC's in veterinary medicine, including comparisons of the two commonly used tissue sources (bone marrow & adipose), supporting research for the intraarticular use of stem cells, and some early follow-up on MSC'S treatment in equine joints.

Typically, aspirated bone marrow is described to contain 40 million nucleated cells, of which 2,000 are stem cells per milliliter (or 1 stem cell per 20,000 cells). In contrast, fat is far less cellular (approximately six million cells per cubic centimeter of tissue compared to 40 million in bone marrow aspirates), but the prevalence of stem cells in fat has been described as high as one per 4000 cells, which is higher than that in bone marrow.[2] Corrected for tissue volume, a bone marrow aspirate would contain 2,000 versus 1,500 stem cells in a fat aspirate per cubic centimeter. Most studies agree that if bone or fat derived stem cells are culture expanded, the fat derived cells appear to have a faster doubling rate, by about 4 days.[4]

Some studies have suggested that fat-derived and bone-marrow-derived cells are similar.[5,6] Others have shown a decreased osteogenic and chondrogenic potential in fat when compared directly to bone marrow derived stem cells.[2,7,8] Work in the authors' laboratory (DDF & JDK) also suggested a decreased chondrogenic potential of fat versus bone derived stem cells, even when harvested from the same patient (horse).[9] The authors have recently completed one of the few studies making a direct comparison *in vivo* where fat or bone marrow derived stem cells were injected directly into the joint to assess an anti-arthritic potential. While neither cell source demonstrated overwhelming effectiveness, better results were observed with the bone marrow derived cells.[10] For the time being, the information needed to correctly manipulate adipose derived cells is lagging behind that amassed for bone marrow derived cells. Those studies published on bone marrow derived MSC'S should not be considered interchangeable when fat is the tissue source.

Early work using labeled MSC'S has shown that they do have an affinity for damaged joint tissue. More recently, *in vivo* studies have confirmed their ability to localize and participate in repair of damaged joint structures, including cruciate ligaments, menisci, and cartilage lesions.[11] Most of the *in vivo* studies utilizing MSC'S has focused on meniscal repair, in some cases using MSC'S in a carrier or scaffold while in others utilizing direct injection into the joint.[12-14] These studies have shown good support for use of bone marrow derived cells for treatment of meniscal damage. The degree of damage has ranged from experimental meniscal lacerations treated with bone marrow aspirates, separating and utilizing only the nucleated cells,[15] to total medial meniscectomy treated with injection of bone marrow derived culture expanded MSC's.[14] With respect to cartilage healing, early work indicted that the use of MSC's deposited in a fibrin matrix would be useful in improving cartilage healing. Although a recent equine study demonstrated early benefit, no significant differences were noted when MSC's plus fibrin was compared to fibrin alone at eight months.[16] Based on this work, it appears likely that modulation of the matrix or cells will need to be accomplished to observe long term benefit of MSC'S for cartilage repair.

The previously mentioned goat study, while showing regeneration of the meniscus, was aimed at evaluating the in vivo effects of intraarticular stem cell injection on decreasing the progression of OA.[14] This study used a medial meniscectomy and cranial cruciate transection model to induce OA. The investigators concluded that the decrease in OA seen in the study appeared to be secondary to the regeneration of the medial meniscal tissues, which was dramatic

in 7 of 9 cases. However, the design of the study did not lend itself to determining if the stem cells had a direct effect on the articular cartilage and progression of OA. Thus, the authors completed an equine study that used an osteochondral fragment with bone and cartilage debris to induce OA, unlike the study by Murphy et al., which relied on joint instability (medial meniscal model) to create secondary OA. The results of this study indicated nominal improvement in symptom or disease modifying effects with bone or adipose derived cells.[10] The results of this study and Murphy et al. combined suggest that the regeneration of the medial meniscus in Murphy et al.'s study may have in fact been the reason for less OA progression. Furthermore, these studies also suggest that MSC's by themselves do little to counteract the progression of acute OA mediated by enzymatic degradation and joint debris. It would appear modification of the MSC's is needed if they are to be useful in treating the OA. Treatment timing in relation to the degree of pathology could also be a factor contributing to the insignificant results of the equine study. Specifically, because MSC's appear to have a tropism for damaged cells, including fibrillated articular cartilage, it may be that at day 14 (day of treatment) the degree of fibrillation was not great enough for an effect of MSC'S treatment to be realized. Evaluation in cases with more advanced fibrillation would need to be conducted to answer this question. Because significant improvement in acute OA could not be demonstrated following intraarticular treatment using either bone marrow derived culture expanded stem cells or adipose derived stromal vascular fraction, these treatments can not be recommended at this time for use in clinical cases of acute OA.

The authors did conclude that the use of MSC's appears to be indicated with loss of soft tissue structures leading to instability, such as with meniscal damage and have pursued this treatment modality clinically in a specific multicenter trial. Cases were selected to receive intra-articular MSC as a treatment when the condition was considered to have a poor prognosis with other conventional treatment modalities and concurrent intra articular soft tissue involvement. Conditions treated included subchondral bone cysts, cartilage damage and/or loss, and torn menisci. There were 15 horses that had at least 6 months post treatment follow up ranging from 6-18 months. Joints treated included 12 stifles, 2 coffin joints, 1 hip, and 1 fetlock. The range of ages for the horses treated was 1-16 years of age with a mean of 5 years. Follow up information was obtained by one of the authors either by examining the horses or by telephone conversation with owners and trainers. In horses that became sound, the average days post treatment with stem cells before they became sound was 78 days with a range from 30-240 days. 10 of the 15 horses that became sound returned to their previous level of work in the discipline they were used for prior to treatment. Given the fact that all horses in the treatment group had a poor prognosis prior to treatment, MSC therapy has yielded favorable results. Cases that did not respond favorably had joint surfaces devoid of cartilage with evidence of gross damage to subchondral bone at arthroscopy and radiographic signs consistent with moderate OA. Significant cartilage loss in full weight bearing areas such as distal MCIII and the medial femoral chondyle remain a difficult challenge. Earlier treatment with MSC before complete cartilage loss and partial joint collapse may be the key to preventing failure.

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