Stem Cells in Small Animal Orthopaedics

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In 1909 Alexander Maximow, a Russian academic, introduced the concept of “multipotent” blood stem cells when he addressed the Berlin Haematologic Society. Decades later, in 1976, another Russian, Alexander Friedenstein, was investigating haematopoetic stem cells in mice when he discovered a population of cells harvested from bone marrow that were adherent to culture vessels, mesenchymal (fibroblast-like) and colony forming with high replicative capacity (Friedenstein et al., 1976). These cells became known as "mesenchymal stem cells" (MSCs). As well as their discovery, Friedenstein is credited with foresight into the clinical application of MSCs, particularly in the field of orthopaedics. He performed detailed studies with Gabriel Ilizarov, who any budding orthopaedist will recognise as a pioneer of external fixation and limb-lengthening methods. MSCs are currently in clinical veterinary use for fracture management, osteoarthritis (OA) and tendon injury.

The defining characteristics of an MSC are those of self-renewal (the ability to go through numerous cycles of cell division whilst maintaining their undifferentiated state), and of multipotency (the ability to generate progeny of several distinct cell types). MSCs offer potential as they have the property of multipotency, but with fewer ethical considerations than embryo-derived stem cells. In fact, the scientific and public interest has grown exponentially. Figure 1 illustrates the number of papers published in each of the last twenty years that show up in a PubMed search for “mesenchymal stem cells”: sixty in 1993, 5118 in 2013.

A surge in interest also followed the discovery, made in 2002, that MSCs could be derived from adipose tissue (Zuk et al., 2002), the harvest of which is easier and associated with less morbidity than that of bone marrow. The extraction of MSCs from fat and their expansion is now commercially available to vets in the UK via
The Veterinary Tissue Bank. Extraction-only services are also advertised for MSC isolation. These services are quicker as they do not require the expansion of MSCs. However, the extraction process yields a heterogeneous population of cells called the *stromal vascular fraction (SVF)*. Only an estimated 1 to 10% of the SVF are actually MSCs (Mitchell et al., 2006; Oedayrajsingh-Varma et al., 2006; Zhu et al., 2008).

![Figure 1: Papers published each year that appear in a PubMed search for “Mesenchymal Stem Cells”](image)

**Bone Healing**

A canine fracture gap model has been used to demonstrate beneficial effects of autogenous (Bruder et al., 1998) and allogeneic (Arinzeh et al., 2003) MSCs on bone healing. In 2007 Bajada and colleagues, from the Robert Jones and Agnes Hunt Orthopaedic Hospital in Oswestry, reported the successful management of a tibial fracture in a man that had been refractory to six attempts at surgical management over a nine-year period. Calcium sulphate pellets combined with
cultured, autogenous, bone marrow-derived MSCs were implanted.

However, as the expansion (culture) of MSCs takes approximately two weeks, MSCs are unlikely to replace autogenous bone graft, or off-the-shelf osteoinductive products such as demineralized bone matrix (DBM) or recombinant human bone morphogeneic proteins (rhBMP), in the management of routine fractures. They might, however, prove useful in planned revision surgeries of non-unions. The authors are aware of an unpublished case of autogenous MSCs being used to successfully treat a delayed union in the tibia of a cat.

**Osteoarthritis**

The therapeutic rationale for MSCs is a shifting paradigm. The original hypothesis was that MSCs, or other stem cells, could be injected or implanted, either systemically or locally, into a patient, and that the cells would find their way to the damaged tissue, differentiate into the needed cell-type, and assist repair. Indeed, a current trend in cartilage defect treatment is the implantation of stem cells, often on a synthetic scaffold, with the aim of tissue regeneration (Emadedin et al., 2012; Haleem et al., 2010; Kasemkijwattana et al., 2011).

However, it is increasingly recognised that MSCs may have modes of action that are paracrine in nature. That is, they may alter the environment of a diseased tissue or organ, such that reparative processes are augmented. In a caprine model of OA (involving complete excision of the medial meniscus and resection of the cranial cruciate ligament) a single injection of an expanded population of autogenous bone marrow-derived MSCs resulted in reduced cartilage degradation, osteophytosis and sub-chondral sclerosis compared to controls. Yet, there was no evidence of MSC engraftment in the cartilage of the treated joints, engraftment was only high in the synovium, fat pad and lateral meniscus (Murphy et al., 2003).
Tendon Injury
In equine orthopaedics there is considerable interest in the use of MSCs to treat tendon injury.

In a tendon gap model, using rabbits’ Achilles tendons, MSC treated tendons had better mechanical properties than operated controls (Young et al., 1998). Re-injury rate of National Hunt horses with overstrain injuries of the superficial digital flexor tendon treated with intralesional MSC injections have been reported as 25.7%, which is significantly lower than those treated by other methods (Godwin et al., 2011).

Summary
Interest in MSCs is set to continue. In small animal orthopaedics at least, perhaps the most promising application is in the treatment of joint disease. Anecdotal reports of response to intra-articular injections of MSCs for the management of OA are encouraging. It is anticipated that reports concerning the safety and efficacy of MSCs will be forthcoming in the near future.
References


