

Is Regenerative Medicine the Answer to Canine Osteoarthritis?

Veterinarians across the globe are constantly being challenged to improve their treatments for diseases such as osteoarthritis, but what criteria should they use to make decisions between promising new treatments and tried and tested practices? In recent years, regenerative medicine has been lauded as a 'cure' for osteoarthritis and many other inflammatory diseases but also labelled as the 'new snake oil'. Which is true and how do we know?

Osteoarthritis is a progressive, inflammatory disease that affects approximately one in five of all domestic dogs^{2,3}. Current treatments rely primarily on symptom relief using non-steroidal anti-inflammatory drugs^{4,5}, weight loss programmes and nutraceuticals such as chondroitin sulphate and glucosamine. None of these prevents the progression of the disease so that ultimately surgery becomes the only option. This is expensive, traumatic for the dog and not always effective, especially in the case of elbow osteoarthritis. It would therefore be of great benefit to veterinarians and their patients if regenerative treatments were effective.

What is Regenerative Medicine?

Regenerative medicine has been defined as 'the branch of medicine that develops methods to regrow, repair or replace damaged or diseased cells, organs or tissues'⁶. Living organisms have their own inbuilt ability to regenerate tissue after damage. Natural wound healing involves cells from the immune system, mesenchymal stromal cells, growth factors and anabolic cytokines and scaffolds such as fibrin clots in a coordinated sequence of repair. Regenerative medicine uses various combinations of these factors to encourage healing in areas that are not able to heal fully. The main sources of these factors are blood (as a source of platelet rich plasma), bone marrow and adipose tissue (as a source of mesenchymal stromal cells).

Growth Factors

Platelet Rich Plasma (PRP)

PRP is a useful source of growth factors contained within the platelets and also contains fibrinogen which will clot upon injection to form a tight mesh of fibrin fibres entrapping and activating the platelets. This provides a natural scaffold which releases growth factors and paracrine signals that act as chemo-attractants to migratory cells such as mesenchymal stem cells.

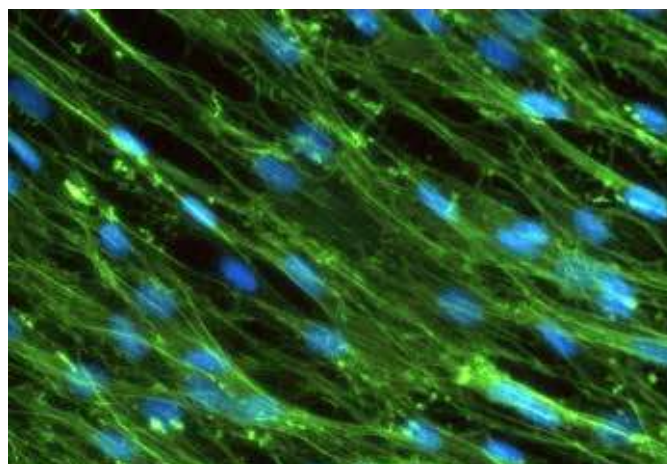
PRP can be prepared from an anti-coagulated blood sample. There are many PRP preparation systems available on the market: one uses a selective filtration mechanism but most use a simple two-stage centrifugation which allows the removal of most of the red and white blood cells and leaves a platelet concentrate suspended in plasma. The relative concentrations of platelets and other blood cells vary considerably according to which system is used to prepare the PRP^{7,8} which could go some way to explaining the variability of clinical results reported for the use of PRP to treat osteoarthritis^{9,10,11}. Whilst the Cook and Fahey studies both found significant improvements

in OA compared with the placebo control, the Franklin study did not find any significant improvements. There are many more less well controlled studies and case series that have been published, but until a consistent method of PRP preparation and growth factor level estimation has been established, it will be difficult to ascertain whether PRP on its own is sufficient to treat OA effectively.

There is an increasing trend to use PRP in conjunction with mesenchymal stem cells (MSC) to treat OA. This combination has the advantage of implanting large numbers of MSCs within a growth factor rich scaffold and should therefore prime the MSCs for action.

Mesenchymal Stromal Cells (MSCs)

MSCs are a type of multipotent adult stem cell that is derived from tissues such as bone marrow, adipose tissue and other tissues of mesodermal origin. They are most abundant in adipose tissue, making up 1–5% of all the nucleated stromal cells (excluding adipocytes) extractable from canine adipose tissue^{12,13,14}. They have regenerative, anti-inflammatory, immunomodulatory and trophic functions¹⁵ but due to their lack of telomerase activity, they do not undergo transformation even after prolonged culture expansion¹⁶. MSCs are capable of differentiating into various mesodermal cell types such as chondrocytes, osteocytes and adipocytes, but it is their paracrine ability that is now thought to be key to their anti-inflammatory activity¹⁷. They are able to secrete a wide range of bioactive factors that allow them to be attracted to the site of injury, reduce pain and inflammation and contribute directly to tissue repair¹⁸ and have now been shown to adapt their secretory activities according to their changing environment¹⁹. It is no wonder that there are now more than 500 listed clinical trials involving MSCs on the US National Institutes of Health clinical studies website (<http://clinicaltrials.gov>)!

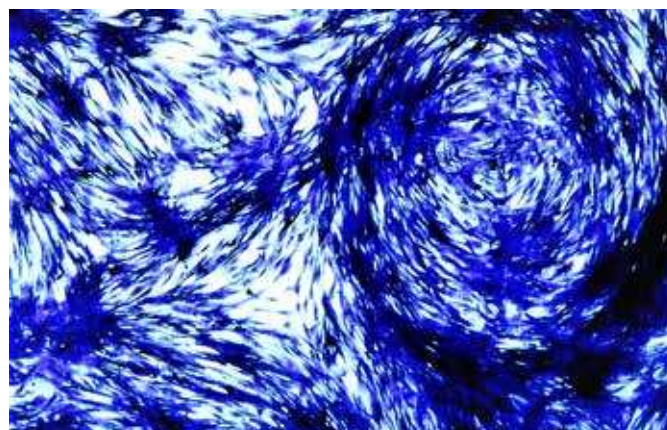
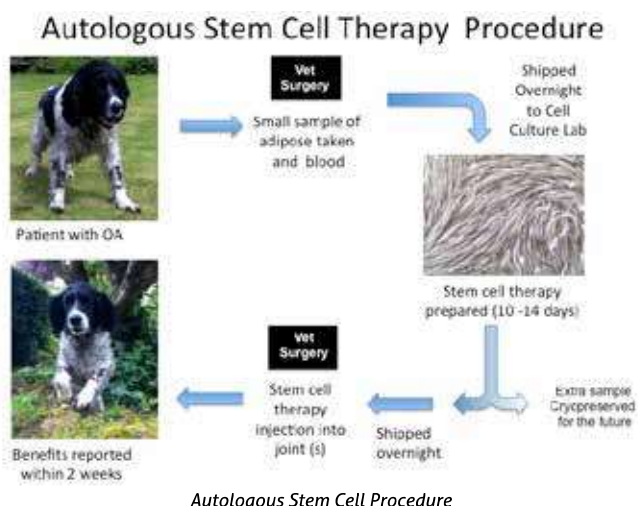


Canine MSCs in culture

MSCs can be prepared from the animal that is to be treated (autologous) or harvested from one animal and used in another of the same species (allogeneic).

Autologous MSCs

Veterinary medicine most commonly uses autologous MSCs because of the perceived risk of immune rejection and, in the



Canine MSCs in culture (CV stain)

UK and many other countries, allogeneic cell implantation requires either an animal test certificate (ATC) or full market authorisation (MA) as the product is considered to be a drug. In the US, an investigative new animal drug application (INADA) has to be in place before culture expanded autologous or allogeneic MSCs can be administered to animals. For this reason, in the US, it has become of particular interest to regenerative medicine companies to develop patient-side preparation devices that separate the stromal vascular fraction (SVF) cells from adipose and bone marrow aspirate concentrate (BMAC) without the need for culture expansion. These preparations are not considered to be 'more than minimally manipulated' and therefore are not controlled by the FDA. Unfortunately, SVF contains a very small proportion of MSCs and a very large proportion of other cells, including 20% endothelial cells, 45% hematopoietic cells²⁰, and a large amount of cellular debris and collagen¹⁴. BMAC has an even lower proportion of MSCs, estimated to be less than 0.01% of the nucleated cells in the preparation²¹. Given that the proportion of MSCs is so low in these preparations, they should not be considered to be 'stem cell treatments' and should be used with caution, especially since it would not be possible to test the injectable product for microbial contamination within the short time frame.

Culture expanded autologous MSCs, on the other hand, can be prepared by authorised veterinary cell culture labs in the UK (under the Veterinary Medicines Directorate ESCC authorisation). The great advantage of culture expansion is that the MSCs can be prepared as doses of many millions of cells per joint and any of the original hematopoietic and endothelial cells will have been washed away before the final cell harvest. Further to this, the cells can be cryopreserved, a sample can be tested for microbial contamination, and a final available cell count can be made before supply to the veterinarian ready for implantation.

Autologous culture expanded MSCs are generally supplied as minimally expanded cells because they only need to be expanded enough to treat one animal. Allogeneic culture expanded MSCs have to undergo many more population doublings before being prepared for implantation because they have been derived from only one original sample and are required to treat many thousands of animals.

Allogeneic MSCs

Allogeneic MSCs have the considerable advantage that they are 'off the shelf treatments'. If an animal is injured, it will be possible to implant a ready-made MSC preparation without having to take a sample of adipose tissue or bone marrow and without having to wait while the cells are culture expanded.

There are not yet any market-authorized canine allogeneic MSC treatments available in the UK, EU or US but there is now an equine allogeneic MSC treatment that is manufactured by Global Stem Cell Technology in Belgium.

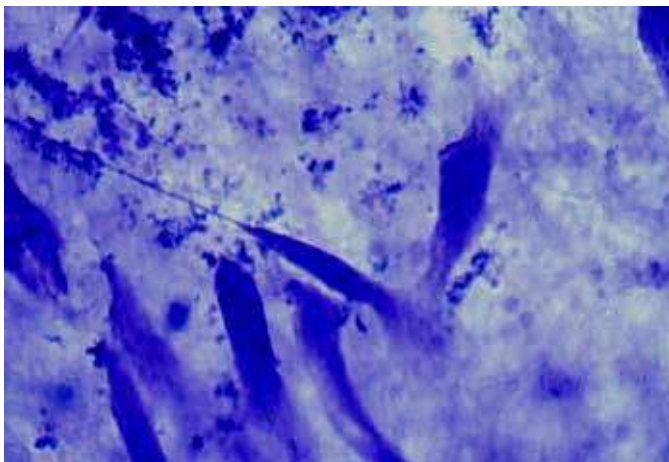
Because MSCs are considered to be immune privileged cells, it is assumed that it is not necessary to match the recipients but there have been some studies that have found transient inflammation²² and adverse reactions following repeated intra-articular injection of allogeneic MSCs in horses²³. It is therefore not yet certain whether repeated injection of allogeneic MSCs will be possible in the treatment of osteoarthritis.

What is the Clinical Evidence of Efficacy?

The gold standard of clinical trials is a randomised, placebo-controlled study with sufficient animals in both groups to provide significant differences. One such study, treating canine OA using a single intra-articular injection of allogeneic adipose derived MSCs compared with saline as the placebo has been published by VetStem²⁴. The study aimed to establish both safety and efficacy of the treatment and was successful in both aims, with significant clinical improvements recorded by both validated owner questionnaires and veterinary global outcome measurements. Recently a similar study by Shah et al. in Australia came to similar conclusions¹⁸ and in addition showed that both the quality of life and osteoarthritis grade (measured by radiographic changes) were improved following mesenchymal stem cell treatment.

A recent review entitled 'Cell-Based Therapies for Animal Joint Disease' states that 'there are a significant number of studies that show improved functional outcomes after treatment with adipose derived MSCs for naturally occurring canine coxofemoral, cubital and scapulohumeral joint OA'²⁵. The studies she refers to are placebo-controlled, blinded and randomised, and include large enough animal numbers for the statistical analyses to be meaningful^{26,27,12,24}. Bogers also notes that the therapies have shown a 'large effect size on lameness' measured both objectively and subjectively with client satisfaction questionnaires. Further evidence of the cartilage regenerative effects of MSCs has recently been published by Li et al.²⁸, in which a beagle model of cartilage damage was treated with either MSCs in hyaluronic acid (HA), HA alone or saline. The dogs were sacrificed after 28 weeks and extensive histology, immunocytochemistry and MRI measurements showed that the MSC group had significant improvements in cartilage defects compared with the other two groups. There are also many other similar studies which do not include placebo controls but nonetheless contribute to the increasing body of evidence that canine OA can be effectively treated by intra-articular injection of MSCs^{18,29}.

Combinations of MSCs with PRP



Canine MSCs in PRP clot

One of the difficulties when comparing the efficacy of regenerative medicine treatments is that each study uses a different combination of cells, growth factors and in some cases scaffolds. A frequently used combination is MSCs injected with PRP. This combination has shown promising results^{26,27,30} and is increasingly becoming the optimal approach. It has been shown that platelets release factors that recruit MSCs towards the PRP clot^{31,32}. MSCs are able to proliferate within PRP clots and preliminary studies have shown that they are able to maintain their biological functions and three months after implantation showed evidence of osteogenesis in patients with non-union fractures³³.

Is Regenerative Medicine 'Snake Oil'?

In the past, many veterinarians have considered regenerative medicine to be an alternative and unproven treatment that has no place in modern clinical medicine. This position has now been changed to the point that the topic is included in veterinary training and the majority of veterinary practices now offer some form of regenerative medicine alongside more traditional approaches to treating osteoarthritis. The clinical evidence of efficacy of mesenchymal stem cell treatment (with or without other scaffolds and growth factors) is mounting rapidly.

The total regenerative medicine market (human as well as veterinary) is predicted to be worth \$38.7 billion by 2021 and is expanding at a rate of 23.6% per annum, it seems unlikely that this is all in pursuit of 'snake oil'.

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