Regenerative therapies in practice

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examines various treatments in equines, such as stem cell therapy, that aim to improve recovery time and reduce re-injury

THE aim of regenerative medicine is to facilitate repair of an injury by restoring tissue architecture and function. This means replacing the injured tissue with that of the same, rather than fibrous, scar tissue, which is usually of inferior quality. In turn, this should result in a better functional repair, with reduced risk of re-injury after returning to work.

The use of biological autologous products means you are utilising the horse's own ability to regenerate or repair tissue to treat the injury. Biological medicine is based on provision of a scaffold for support of healing tissue, provision of growth factors to positively influence the healing and provision of cells to populate the injured area.

Various regenerative therapies provide some or all of these components in differing ways. Remembering which of these components a therapy provides will help the veterinary surgeon select the most appropriate treatment for the injury incurred and the future athletic function required of the horse. For the budget-conscious owner, it must be remembered all tissues heal to some degree with time and rest alone, so these treatments only aim to improve the quality of the repair or increase the speed of return to athletic function.

This article will examine the commonly available regenerative therapies, including stem cell therapy, bone marrow aspirate concentrate (BMAC), use of interleukin-1 receptor antagonist protein (IRAP) or autologous-conditioned serum (ACS) and platelet-rich plasma (PRP).

Mesenchymal stem cells

Mesenchymal stem cells have the potential to differentiate into tenocytes, among other cell types, and are usually isolated from bone marrow, although they can also be cultured from adipose tissue. Originally, infiltration of injured tendon tissue was done using bone marrow aspirated from the sternebrae and then directly re-injected into the tendon. Although this technique was low cost and easily performed, there are very low numbers of stem cells within the aspirate and there is potential for mineralisation of the injured area after infiltration.

This technique has been refined and it is usual to collect stem cells from bone marrow aspirate and send them to an external lab for incubation and expansion of the appropriate cell line (Smith). This process takes about three weeks, resulting in a purer population of mesenchymal stem cells. This cell population is then resuspended in bone marrow supernatant for intralesional injection. The supernatant provides growth factors. This technique obviously has increased financial implications and takes time for completion, but does provide a scaffold, growth factors and a cell population for optimal healing.

Mesenchymal stem cell therapy is most commonly used for treatment of core lesions within the superficial digital flexor tendon, although it has also been tried in other soft tissue injuries and as an intra-articular therapy. In the case of tendon or ligament lesions, this treatment is only appropriate if certain parameters are met; a core lesion should usually be greater than 10 per cent of the cross sectional area of the tendon and an intact paratenon is vital (Godwin et al, 2012).

It is worth remembering smaller core lesions are often not suitable for this costly treatment as they will have filled in with tissue by the time the stem cells are cultured and ready for re-implantation. If the lesion is smaller, yet still suitable for implantation, it is possible to request a small volume/high concentration preparation. Ideally, the mesenchymal stem cells are re-injected into the injured area after the inflammatory phase is complete, but prior to the commencement of fibrosis. In practice, this is very difficult to achieve, but taking the bone marrow and sending it for culture as soon as possible after diagnosis should help.

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Once the diagnosis is made, bone marrow is harvested from the fifth sternebra under ultrasound guidance in a standing sedated horse. It was suggested two sample sites should be used; however, one site is now sufficient as advised by the company most commonly used in the UK for stem cell preparation. The fifth sternebra is easily accessed, although it is imperative to use ultrasound guidance as there is a small, but significant, risk of cardiac puncture if the Jamshidi needle is incorrectly placed. An alternative site is the tuber coxae, but this site is physically harder to access and produces variable results. After collection of the bone marrow aspirate into heparinised containers, it is sent to an external laboratory for culture.

Once the cell population is ready for re-implantation, the lesion is scanned again and the area of skin correlating to the lesion is prepared in a sterile manner. It is necessary for the horse to be sedated before implantation to facilitate accurate placement. Using ultrasound guidance, a needle is inserted into the lesion and visualised. Using a spinal needle may help maintain sterility and be easier for some clinicians to position. After needle placement is confirmed, the lesion can be infiltrated with the cell population. It is important to bandage the area after implantation to minimise haemorrhage, maintain the cells in the correct area and keep it warm (Godwin et al, 2012). At the author's clinic, the leg will be kept in a partial Robert-Jones bandage for two weeks, followed by stable bandages.

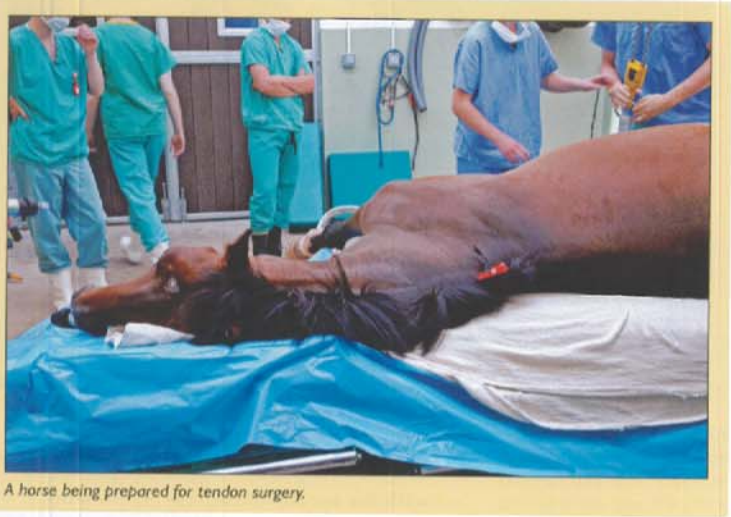
After implantation, the horse is required to follow a strict rehabilitation programme of box rest, in combination with controlled exercise, with the aim of returning to full athletic work approximately a year after implantation. It has been shown use of stem cells in the treatment of core lesion tendon injuries results in a significantly lower re-injury rate (Godwin et al 2012).

Embryonic stem cells may be beneficial as they show some ability to migrate to areas of injury, yet this is practically difficult to achieve within the equine population (Guest et al, 2010).

Although use of mesenchymal stem cells has been studied extensively in equine superficial flexor tendon injuries, they are being used in other soft tissue injuries and even for some joint pathologies in practice.

BMAC

BMAC is formed after collection of bone marrow using the standard procedures. The aspirate is then centrifuged in a commercial system that effectively concentrates the mononuclear cells, including the mesenchymal stem cells.



A horse being prepared for tendon surgery.

This product therefore provides growth factors, together with some mesenchymal stem cells and a scaffold effect (Fortier and Smith, 2008). This procedure is less expensive than stem cell cultures and also is able to be completed at the time of diagnosis of the injury.

Again, BMAC was initially used in tendinous injuries as a slight refinement of the technique of removing bone marrow, then injecting it directly into a tendon lesion. This product has been used for the treatment of ligamentous and articular pathologies, although there is little evidence-based medicine regarding its use. The use of BMAC has been described in the arthroscopic treatment of bone cysts (Sparks et al, 2011) and found to be beneficial when used in combination with microfracture techniques in experimentally induced cartilage lesions (Fortier et al, 2010).

IRAP/ACS

Interleukin-1 is the inflammatory cytokine believed to play a significant role in the formation of an arthritic response. In a healthy joint, this cytokine exists in balance with interleukin-1 receptor antagonist protein. To reduce the induction of inflammation and subsequent arthritic changes within a joint, antagonism of the interleukin-1 requires a greatly increased concentration of interleukin-1 receptor antogonist protein. Unfortunately, the exact ratio of IL1Ra: IL1 required is yet to be determined in equine joint therapy.

There are two different systems used for harvesting IRAP peptides or autologous conditioned serum. The conventional system is known as IRAP and the newer system is known as Arthrex or IRAP II. There may be a slight difference between these products in vitro (Hraha et al, 2011), although whether there is any clinical difference is yet to be fully examined. Whichever method of harvesting is used, blood should always be taken in a sterile manner from the horse to be treated, as there will be no preservative or antibacterial properties within the preparation. After incubation, the collection is usually split into 3ml to 5ml aliquots for repeated injections. After preparation, any unused aliquots can be carefully labelled and frozen for use at a later date.

IRAP peptides should be viewed as having a primarily anti-inflammatory effect and are used for intra-articular therapy. After harvesting of the ACS, the affected joint should be prepared aseptically and then the preparation injected into the joint. There is no set protocol regarding number and frequency of injections.

Most clinicians will use three intra-articular injections spaced seven to 14 days apart in the initial treatment of a symptomatic joint problem and save any further aliquots for treatment one to six months later, or when the horse next becomes symptomatic. As this product is of the horse's own blood products, it is not restricted for competition use, although care must be taken to abide by the FEI rules regarding use of intra-articular injections and any sedatives required.

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Use of ACS has been shown to have a chondroprotective effect in experimental osteoarthritis models (Caron et al, 1996) and has improved lameness when used for carpal chips (Frisbie et al, 2007). When the treatment first became available, many horses were treated if their joint pathology was non-responsive to conventional therapies (Ferris et al, 2001). IRAP is now often used as a type of prophylactic treatment, especially after arthroscopic investigation of a joint or if synovitis is suspected.

PRP

PRP describes a plasma-based preparation that contains an increased concentration of platelets. This term does not describe the other components present or the concentration of platelets within the plasma. There are several commercially available systems available for production of this product, using either centrifugation or filtration. It is recommended platelets should be concentrated three to five times normal; however, this is not yet proven to be the optimal concentration (McLellan et al, 2011).

Platelet concentration techniques result in a simultaneous concentration of white blood cells, which may or may not be desirable. White blood cells may release pro-inflammatory cytokines upon infiltration of the damaged area, yet this may also provide some degree of protection from infection.

PRP provides growth factors to stimulate cell proliferation, promote vascularisation and aid synthesis of extracellular matrix (Fortier and Smith, 2008). It will also provide a small amount of scaffold effect to influence healing (McLellan et al, 2011). Upon infiltration and subsequent activation, platelets degranulate to release cytokines, including platelet-derived growth factor. The PRP also clots upon activation, which may help provide a form of fibrin scaffold for tissue repair (Fortier and Smith, 2008).

Ultimately, the aim of using PRP is to increase speed and quality of repair, but as it does not contain a cell source, it may

continued on page 24

REGENERATIVE THERAPIES IN PRACTICE
– from page 22

promote scarring and fibrosis, rather than replication of the original tissue. Initially, PRP was used for treatment of tendonitis and then became more commonly used in cases of suspensory ligament desmitis. Infiltration of a core lesion, whether it is tendinous or ligamentous, has the same requirements as that of stem cells – a definable lesion contained within an intact paratenon or capsule to contain the preparation.

Preparation of PRP takes a short time and can be done as soon as diagnosis is made. It is also relatively inexpensive when compared to use of mesenchymal stem cells. The method of implantation is the same as for stem cell implantation and bandaging after implantation is still advisable. After this, the horse will again enter a period of box rest and controlled exercise, although this is usually much shorter than the protocol after stem cell implantation.

Using computerised analysis, it has been shown there is a significant difference between end-stage healing of tendon injuries treated with PRP versus those treated with a placebo (Bosch et al, 2010). The safety of different PRP preparations has been investigated (Textor and Tablin, 2013) and is now commonly used as an intra-articular therapy, especially post arthroscopy, with the aim of providing growth factors to stimulate healing post debridement of intra-articular lesions.

Summary

There are a myriad of different regenerative therapies available for use in soft tissue or articular pathologies. With the exception of mesenchymal stem cell use in superficial flexor tendinitis, there are limited studies regarding the use of different regenerative medicines, so the recommendations for their use will continue to evolve. At present, selection of a procedure frequently depends on the type of injury, treatment availability, clinician's experiences, finances and the owner's preferences.

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SPOTLIGHT

This article is part of our regular Spotlight series of clinical and topical features aimed specifically at the equine veterinary practitioner

Study sheds light on human drug-resistant *Salmonella*

A NEW study has shown the contribution of local animal populations to human drug-resistant *Salmonella* infections may have been overstated.

The study – *Distinguishable Epidemics of Multidrug Resistant Salmonella Typhimurium DT104 In Different Hosts* – was published online in the *Science Express* (September 12, 2013) and draws its conclusions from a genomic study of more than 370 *Salmonella* samples collected over a 22-year period.

It shows local domestic animals are unlikely to be the major source of antibiotic resistant *Salmonella* in humans.

By studying the genetic variation in the *Salmonella* bacteria and their drug resistance genes, researchers at the Sanger Institute in Cambridge found distinguishable bacterial populations exist in human and animal populations living side by side.

Comparing the genomes of *Salmonella* in humans and animals showed the *Salmonella* bacteria largely remained within their original host populations and second, there were more varied combinations of drug resistance in the human-infecting bacteria.

"For the first time, we've determined in detail and on a large scale how *Salmonella* strains taken from humans and animals in the same setting and over the same time period relate to each other," said Alison Mather, first author on the study, from the Wellcome Trust Sanger Institute.

Large scale

The team sequenced DNA from 373 human and animal samples infected with *Salmonella typhimurium* DT104 over a 22-year period, mainly from Scotland, but also from other countries, making it the largest study of its type.

The scale of the work allowed scientists to show populations of *Salmonella* in humans and animals were distinguishable and also that the estimated number of times the bacteria had jumped from animals to humans (and vice versa) was low.

"This is a study that uses the latest genomic approaches and a unique collection of samples to address a significant public health problem," said Nicholas Thomson, senior author from the Wellcome Trust Sanger Institute.

"Our data provide a very simple message, challenging the established view that local animals are the predominant source of *Salmonella* infections in Scotland. This finding will reinvigorate discussions on the sources of antibiotic resistant *Salmonella* infections in humans in other environments," Prof Thomson added.