

A New Generation of Regeneration

Lucy Wilson unravels the latest technological advancements in the veterinary world with the introduction of a new technique that has been a massive leap forward for regenerative science

You might think of stem cell therapy as being a pretty exclusive and expensive treatment for major tendon injuries, but in reality this branch of medical science has many subdivisions. For over three decades, bone marrow formed the basis of treatment for human patients suffering from conditions such as Leukaemia and Lymphoma. Other developments went on to include the management of acute graft vs host diseases in children called Prochymal, based on allogeneic stem therapy based on Mesenchymal Stem Cells (MSCs) derived from adult donors of bone marrow. Hematopoietic stem cell treatments, derived from umbilical cord blood, have also been approved by the FDA (Food and Drug Administration of America). In the 90s, scientists successfully grew an ear on the back of a mouse and the possibility of regenerating tissue from stem cells seemed endless, despite concerns about the ethics of such practice. Ten years ago, regenerative science wasn't a huge part of a veterinary student's curriculum, whereas now there are plenty of textbooks and research to be poured over in the libraries. Data, published in the 2015 edition of the *Equine Veterinary Journal*, revealed the annual incidence of injuries in polo ponies was 10.6% (2), and veterinary surgeons have been searching for the best way to deal with these, often career ending, injuries.

What are stem cells and how do they work?
Stem cells fall into three different categories:



Professor Carlo Tremolada demonstrating how to harvest adipose tissue

Autologous Mesenchymal, Allogeneic and Xenogeneic. The definitions and uses of all these cells are lengthy, so for the purpose of understanding this new technology we will focus on Mesenchymal Stem Cells (MSCs) and the latest understanding of their infinite possibility for polo ponies.

It isn't often that the basis of understanding in a scientific technique is turned upside down on its head, but a second more unusual pathway is that the latest discovery in regenerative science is seeing a shockwave run through not only human medical world, but also the veterinary world. New research and

understanding of MSCs throws out the historical understanding of their capabilities, where adult stem cells were seen as having limited use in repair and regeneration. It was thought that the embryonic stem cells were the only kind that reproduce into any type of cell in the body.

In a recent talk given by Dr. Arnold Caplan, a professor of Biology and General Medical Sciences and Director of Cellular and Molecular Basis for Aging Training Program of Case Western Reserve University in Cleveland, Ohio at The Orthobiological Institute in June 2016 he blew all previous misconceptions about MSCs' capabilities out of the water.



Lipogems canister containing the MSCs, which will be filtered to create Lipospirates

His research shows that all vascular structures, from arteries to capillaries and microvessels, are surrounded by Pericytes. These are the key components of regeneration it seems. Caplan emphasised that MSCs can be taken from multiple tissue sources and that, as all tissues sources contain vasculature structures, Pericytes will be present. MSCs in vitro (cultured research in petri dishes) showed multi-potency, whilst in vivo (in the body) they were less potent. This is where the magic of the Lipogems technique, created by a plastic surgeon from Milan, Dr. Carlo Tremolada, has caused the biggest stir in the application of regenerative medicine. Caplan also explained in his speech that MSCs are akin to the body's management system for regeneration, repair and response to the body's immune system. It is in vivo that this management of how the Pericytes perform to regenerate the damaged cells is the key. It is proof that implanting a high concentration of MSCs in a specific area will promote the needed repair. So far there are 615 MSC clinical trials being conducted worldwide, with the greatest concentration

of research in America, Europe and Asia. These studies include research specifically aimed at diseases such as MS, ALS, Chronic Heart Failure, Rheumatoid Arthritis, Lupus, Chrono's Disease and even Kidney Failure

His most important discovery was that MSCs taken from Pericytes should be renamed as Medicinal Signalling Cells as they manage the body's ability to regenerate an injury and, for the medically minded, they display trophic immunomodular activity.

Equine application of stem cells

In a research paper *Beneficial Effects of Autologous Bone Marrow-Derived Mesenchymal Stem Cells in Naturally Occurring Tendinopathy* (1) published 25 September 2013, "Twelve horses with career-ending naturally-occurring superficial digital flexor tendon injuries were allocated randomly to treatment and control groups. 1X10⁷ autologous BM-MSCs suspended in 2 ml of marrow supernatant were implanted into the damaged tendon of the treated group. The control group received the same volume of saline. Following a six month

exercise programme, horses were euthanised and tendons assessed for structural stiffness by non-destructive mechanical testing and for morphological and molecular composition."

In the conclusion of the paper the authors, including Dr Roger Smith, the leading veterinary surgeon based at the Royal Veterinary College, concluded that, "The data presented here has supported the hypothesis that MSC implantation with BM supernatant results in a tissue more like normal tendon matrix rather than the fibrous scar tissue formed after natural inflammation and repair."

Why isn't stem cell therapy widely used in equines?

Stem cell therapy comes at a high financial cost and many veterinarians have been disappointed with outcomes. One reason for this may be that the process of culturing these stem cells simply selects cells that are good at growing in a laboratory rather than those that can differentiate into strong and healthy tissue. There is also a significant time delay, often of several weeks, between collection of stems cells and re-injection. Other regenerative therapies have been developed to help fill these gaps, with platelet-rich plasma and autologous conditioned serum being the best known. These are simpler, quicker and cheaper techniques, with treatment completed at the side of the horse within several hours or a day. While they do not offer a rich source of stem cells for tissue repair, these are now much more frequently used by equine vets than laboratory-cultured stem cells.

Lipogems and the revolution of Pericytes for equines

How does Lipogems work?

Lipocast Biotech UK has brought the technology of Lipogems to the UK veterinary scene with already astonishing results. It works by micro-fragmenting adipose tissue (tissue harvested from fat) obtained from Lipoaspirates, through a non-enzymatic, mechanical processing using the closed system and disposable device. Adipose tissue is harvested using a vacuum syringe from around the tail head, after the region has been anaesthetised by local infiltration with sterile saline and lignocaine. The aspirated fat tissue is washed in saline and gently agitated so that the all-important Pericytes detach from small vessels and activate. They are then isolated along with the stromal vascular structure of adipose tissue, which acts as a local scaffold to

Case study 1

11-year-old polo pony

Injury: Superficial flexor tendon left fore, zones 1a to 2b and lateral branch suspensory ligament right fore

Prognosis after treatment: Outstanding results as the scans show significant change in just four weeks. Pony is now back in exercise.



Before treatment



After treatment

Case study 2

12-year-old polo pony

Injury: Early osteoarthritis in both front fetlocks and desmitis of the branch of the suspensory ligament

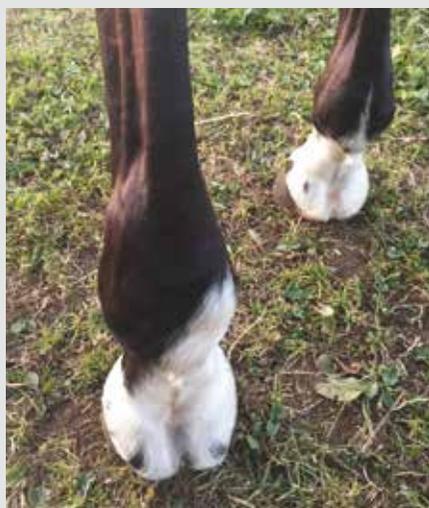
Prognosis after treatment: Sound with less inflammation in fetlocks two weeks after treatment



X-Ray of the nearside fore before treatment



X-Ray of the nearside fore 11 days after treatment



Swollen and hot fetlock joints in the nearside fore



Less swelling and less heat in both front fetlocks

maintain regenerative activity for many months. The whole procedure from harvesting to the injection is completed in less than 30 minutes in a stable. One of the important points about this procedure is that it is quick to perform and promotes healing as early as three weeks after treatment and involves very little requirement for drug usage other than sedation and Lignocaine. Ultrasonography and radiographic evidence of tissue repair has been identified within three weeks after treatment.

Why is this technique revolutionary for regenerative therapy?

The Lipogems technique gently reduces the size of adipose tissue cell clusters, while rinsing by counter-flow, the naturally floating adipose tissue from cellular debris, blood and emulsified oil residues. The process therefore results in a reduction of the pro-inflammatory contaminants of the micro-fragmented adipose tissue product preserving the stromal-vascular niche. Engraftment of adipose tissue clusters are favoured by their reduced size and the final tissue product is primed by the mechanical processing, enhancing its immunomodulatory and tissue regeneration/repair properties.

What can this therapy be used on?

It is increasingly used in human surgery for the treatment of orthopaedic conditions, poor wound healing and faecal and urinary incontinence, as well as in sports medicine and reconstructive surgery. For equines it can be used on a range of problems including; osteoarthritis lesions of the superficial and deep flexor tendons; suspensory ligament desmitis; and chronic granulating distal limb wounds. The treatment also has long-term anti-inflammatory and substantial anti-bacterial and analgesic effect and to date no adverse reactions have been recorded either at the site of the adipose harvest or the injection.

How was the technique discovered?

Lipogems technology was developed by the Swiss-Italian plastic and maxillofacial surgeon Dr. Carlo Tremolada MD, EBOPRAS, who was looking for a better way to create adipose grafts for his maxillofacial procedures. Unexpectedly his patients experienced a significant reduction in the bruising and inflammation normally associated with such surgery. Scientists at a number of leading universities subsequently identified unique regenerative

characteristics associated with the activation of specific cells called Pericytes within the Lipogems Lipoaspirates. Pericytes are small cells wrapped around capillaries and react to signals associated with tissue injury or damage by transforming into Mesenchymal Stem Cells. As these cells divide they produce substances that guide the development of resident stem cells into the various cell types required for tissue regeneration and natural healing.

Where is this treatment available in the UK?

At a recent seminar at Cirencester Park Polo Club led by Professor Tremolada himself, Dr Tim Watson, as the lead Lipogems veterinary surgeon, and four other leading equine vets were trained and accredited as the first Lipogems veterinary practitioners authorised to perform the procedures throughout the UK. Since the seminar, Tim at his Waterlane Equine Veterinary Practice in Gloucestershire, in collaboration with Lipocast Biotech UK Ltd, has performed a number of procedures on polo ponies and racehorses that have demonstrated safety and evidence of efficacy.

What do the vets think?

**Dr Clive Hamblin BVET MED MRCVS,
National Trainers Federation (NTF)
Veterinary Advisor**

“Lipogems is a new regenerative therapy for use in horses, utilising fat tissue derived Mesenchymal Stem Cells taken from the tail head of the patient and prepared using a stable-side kit, enabling the treatment to be carried out on site and without delay. It has been delivering extremely encouraging results in human medicine and similarly in a large number of case studies carried out so far in horses.

“It is used principally for the treatment of soft tissue injuries (tendons and ligaments) by local infiltration into the damaged tissue and is also used as a treatment for joint problems by intra-articular injection. It is proving to be a revolutionary new mode of treatment for a group of injuries that have previously been difficult to treat and often with an unfavourable outcome.

“Experimental work is showing that it is easy to freeze the Lipoaspirate, for future use in the horse, to treat a specific injury or potentially for systemic use, following extreme exercise, in order to enable the equine athlete to rapidly recover from its exertions. This has great potential in many equestrian disciplines such as racing, eventing and polo.”

Murray Shotter BVSc MRCVS, Shotters & Byers Equine Veterinary Services

“Lipogems allows the harvesting and transplanting of stem cells from fat tissue in the horse within 20-30 minutes of harvesting.

“On the ponies I have done so far the results look very promising, but it is still early days. The idea that you can transplant in the field rather than send it to a hospital is great news.

“This technique of injecting freshly harvested Lipoaspirate into a tendon or suspensory ligament is interesting and no rejection has been seen in the cases I have done so far. There has been little to no swelling five days after transplanting and even two weeks after.”



Dr Tim Watson BVM&S PhD MRCVS, Waterlane Equine Vets

“While the science behind Lipogems is exciting, the results are even more remarkable than this promise suggests. Having used the procedure in a range of sites and pathologies, we are seeing healing at a rate and of a quality that you simply don’t see with bone marrow-derived stem cell or platelet-rich plasma therapies. Also, as the technique is fine tuned we are now collecting 20-30ml of Lipogems in a single harvest, bringing the prospect of being able to freeze a bank of material for repeat or future treatments.”

Case study 3

Nine-year-old polo pony

Injury: Deep laceration to the bone that resulted in extensive scarring and mobility issues

Prognosis after treatment: Back playing polo within two months



Deep laceration to the bone



Huge inflammation, lame in trot and scar tissue granulation



No heat, no swelling and playing medium goal polo two months later

(1) Roger Kenneth Wheelands Smith, Natalie Jayne Werling, Stephanie Georgina Dakin, Rafiqul Alam, Allen E. Goodship & Jayesh Dudhia. Department of Clinical Sciences and Services, the Royal Veterinary College, University of London, Hatfield, United Kingdom, Department of Biotherapeutics, National Institute for Biological Standards and Control, South Mimms, United Kingdom, Institute of Orthopaedics & Musculo-Skeletal Science, University College London, Stanmore, United Kingdom

(2) Polo pony injuries: player-owner reported risk, perception, mitigation and risk factors. C. M. Inness and K. L. Morgan. Version of Record online: 17 JUL 2014

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