

**Could stem cell techniques be used to
treat the different stages of equine
laminitis and aid rehabilitation of affected
animals?**

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PASS WITH DISTINCTION

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Abstract

In this paper I will begin by discussing the principles of stem cell research and its relevance to medicine, by looking at developments in stem cell research and current research into possible medical uses for stem cells. Then I will move on to describe the ways in which I believe stem cell research could provide treatments for horses suffering from laminitis. In this section I will cover the techniques already in place for the application of stem cell therapies to treat diseases in animals that inspired me to do my research, then I will outline the treatment processes for using stem cells to treat laminitis. I will also cover the ethical issues surrounding embryonic stem cell research. In my conclusion I will summarise my ideas, discuss any problems with them, and think of ways in which any problems might be overcome.

Introduction

Stem cells are undifferentiated cells that have the potential to develop into several different kinds of cells in the body. They are capable of dividing rapidly to replace damaged and diseased tissues in our bodies. When a stem cell divides, it can either remain as an undifferentiated stem cell, or it can develop into a more specialised cell type, for example a brain cell or a bone marrow cell. Although there are three main types of stem cells - adult stem cells, embryonic stem cells and foetal stem cells - embryonic stem cells have attracted the most attention from scientists and researchers due to their ability to develop into almost any type of tissue in the body. These cells come from embryos that are usually about four to five days old.

This knowledge has led scientists to explore the factors and conditions which cause embryonic stem cells to keep dividing into undifferentiated cells, until they are needed to repair a tissue in the body. If scientists could discover how undifferentiated embryonic stem cells become differentiated, significant steps could be made within stem cell research. It is already known that cell differentiation is controlled by genes. Abnormal cell division and differentiation is central to some of the most serious diseases such as birth defects and cancer, (Sell - 2006) and University of Gothenburg - 2009). With more information about the genetic controls of differentiation, progress could be made towards finding new treatments for these diseases.

Discovering how these stem cells differentiate would enable scientists to control stem cell differentiation, permitting the growth of specific types of cells. If the correct physical environment could be stimulated, scientists could use embryonic stem cells to grow different types of tissues and organs, to replace diseased ones. Mayhall et al. (2004) proposed that this kind of technology could provide a cure for life-threatening diseases such as Parkinson's, Alzheimer's, heart disease and diabetes, and could treat conditions like spinal cord injuries, strokes and arthritis. Research done by Oreffo and Triffitt (1999) describes how mechanical contraptions such as joint replacements could be replaced by organs grown from stem cells.

For example, an article by the NIH (2008) refers to recent studies which show that it may be possible to grow heart muscle cells from embryonic stem cells or adult bone marrow cells, and then transplant them into a person suffering from heart disease. The article also points to research in rats and mice which shows that when bone marrow stem cells are placed into a damaged heart, they can generate heart muscle cells, leaving the animal with a healthy, functioning heart once more.

Adult stem cells are the undifferentiated cells found in a tissue or organ. These cells can divide to renew themselves, or can differentiate into the different cell types of that tissue or organ. For example bone marrow or Mesenchymal stem cells can into cartilage, fat and fibrous connective tissue. Recent research by Kuehnle and Goodell (2002), has discovered that adult stem cells have a greater plasticity - the ability of adult stem cells from one tissue to differentiate into cells from another tissue when placed under the right conditions- than was originally thought. Recently stem cells from a patient's bone marrow were used with epithelial cells from the patient's bronchus, to grow another bronchus successfully - Smith (2008).

Stem cell therapies using adult stem cells are being used routinely to treat diseases and are referred to by the Genetic Science Learning Centre (2009). The bone marrow transplant is perhaps the most commonly known, used to treat leukaemia, some types of cancers and blood disorders. This involves

the patient's bone marrow stem cells being replaced with those from a matching donor. If the transplant is successful, the new stem cells released into the blood stream will travel to the patient's bone marrow and will begin producing new, healthy tissue. This in turn will regenerate the immune and blood system. This tendency for stem cells to migrate to areas of abnormal or damaged tissue can be used by scientists to great advantage. Veterinarians have developed stem cell therapies to treat several conditions in animals, some of which are already successfully in use. Since 2002, Vet-stem have successfully treated horses and dogs suffering from ligament and tendon injuries, and osteoarthritis. Their treatments use Adipose Derived Mesenchymal Stem Cells AD-MSCs either to re-grow the cartilage worn away as a result of osteoarthritis, or to repair damaged tendons and ligaments (Black et al (2008)). This involves a small fat sample being taken from the animal, then in the laboratory the stem cells are isolated from the adipose tissue and processed. Then the veterinarian simply injects the cells directly into the injured site. These therapies already in use are the first steps towards the use of stem cells throughout medicine and veterinary medicine.

The potential for stem cell treatments is wide and awe inspiring. If scientists could control embryonic stem cell differentiation and create the right conditions for these cells to grow, alongside developing the therapies they already have using adult stem cells, then they would have truly harnessed the remarkable ability of the stem cell to regenerate and repair almost any kind of damaged and diseased tissue.

Discussion

My proposal for a future use of stem cell research will be for the treatment of laminitis. Acute and recurring cases can put an end to the working life of a horse, and too often cases of laminitis result in the horse being humanely destroyed. My proposal is that stem cell therapy could provide a cure for this disease, and could return the hoof to its correct, healthy state.

The Normal hoof

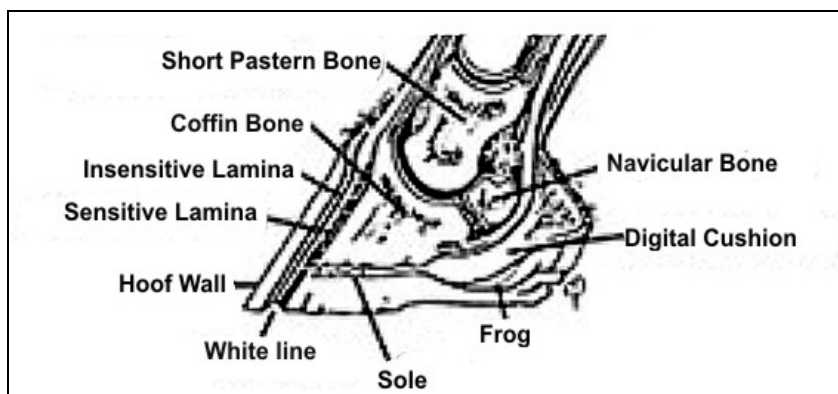


Fig 1

Anatomy of the foot as described by Rooney (1974), (see Fig 1) The weight of the horse is passed down the bone column to the coffin bone that is suspended inside the hoof by the laminae. These laminae are divided into the sensitive laminae attached to the coffin bone, and the insensitive laminae of the hoof wall. The laminae act as slings suspending the coffin bone inside the hoof and securing it to the hoof wall in such a position that it is held parallel to the hoof wall.

Explaining laminitis

Laminitis is caused by a failure of the laminae that attach the coffin bone to the hoof wall (Eustace - 1992),. The first stage is an interruption in blood flow to the sensitive laminae resulting from damage to the arteries in the hooves, whereby blood enters and leaves the foot without passing through the laminae. This inschaemia, can be caused by the following: Toxaemia (researched by MacLeod (2007)) resulting from overeating or a retained afterbirth; Mechanical trauma from working on hard ground; Obesity - insulin resistance; Inadequate foot trimming that makes the laminae tear because of increased strain

The extent of the damage to the sensitive laminae depends on how much of their area is affected by inschaemia, and for how long. In mild laminitis cases, where only a small area is affected for a short time, the damage usually repairs itself, and the horse is recovered. However, if reduction in blood supply is severe and long standing, more laminae are destroyed and there is an insufficient area of healthy sensitive laminae left to support the coffin bone. This causes the coffin bone to rotate down through the foot towards the sole. This is known as founder, and is shown in Fig 2, diagrams showing the different degrees of rotation of the coffin bone. Note the changing position of the navicular bone shown in green, this illustrates the extent of the mechanical change in the hoof caused by the dropping of the coffin bone. In acute founder cases, the separation between the hoof wall and the coffin bone in contributed to by pressure from fluid released from the damaged blood vessels.

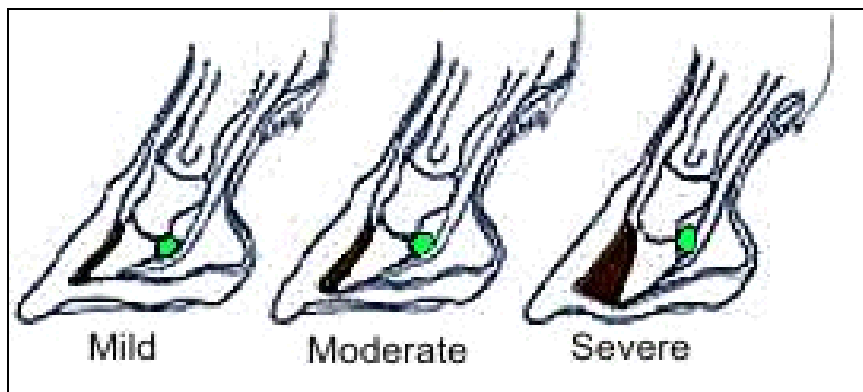


Fig 2

In very serious cases, the coffin bone becomes completely detached from the hoof wall and horses suffering from this are called sinkers. Figs 3&4 show the difference between a normal foot (4) and a sinker (5), where the single white lines indicate the hoof wall, and the drawing pins indicate the sole. The orange arrow in fig 2 shows where the coffin bone is resting on the sole of the hoof. At this stage the coffin bone is no longer aligned parallel with the hoof wall, the laminae have all been destroyed.

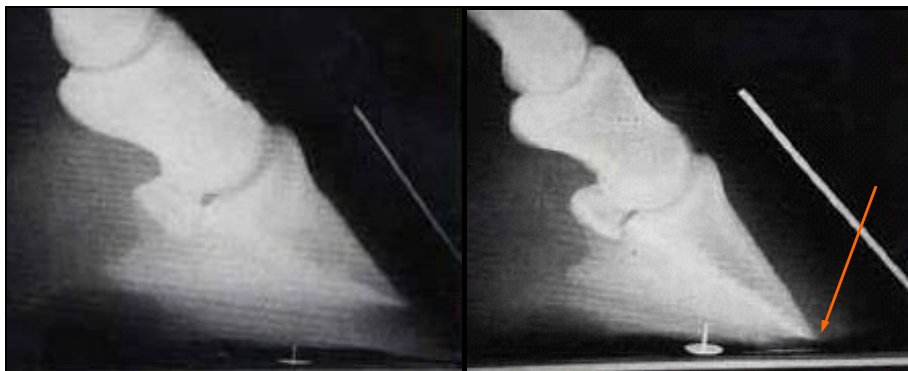


Fig.3

Fig 4

Stem cells and laminitis

Given that laminitis is a disease involving the damage of tissues - the laminae - within the foot, and that stem cells are non differentiated cells with the potential to replenish various kinds of different cell tissues within the body, it is possible that stem cell therapies could be used to regenerate these damaged tissues inside the hoof, as an aid during the rehabilitation of animals suffering from laminitis. If the right conditions could be created in order to induce stem cells to develop into laminar tissue, then this would facilitate the application of stem cell therapies allowing the hoof to return to its former condition.

The laminae are a fibrous connective tissue. In the introduction it was explained that bone marrow or Mesenchymal stem cells are cells that develop into cartilage, fat and *fibrous connective tissue*. However, bone marrow extractions are a rather too intrusive and time consuming way of treating this disease, so I propose using another source of Mesenchymal stem cells - AD-MSCs (see Fig 5). These can be collected in far greater concentration than stem cells derived from bone marrow, and have a diverse plasticity (Vet-Stem - 2007). AD-MSCs secrete growth factors that support numerous processes including tissue remodelling, promoting anti-inflammatory healing, making these stem cells ideal for regenerating the laminae inside the horse's hoof.

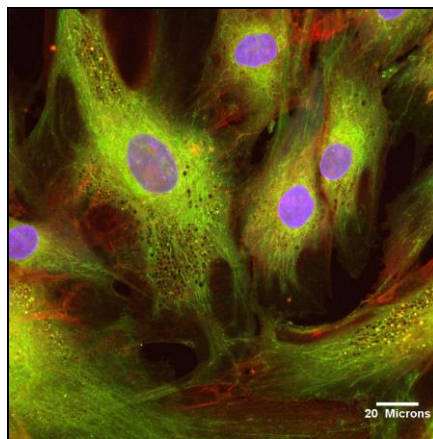


Fig 5

This idea has developed from the current techniques covered in the introduction used for treating arthritis and tendon injuries in dogs and horses currently used by companies like Vet-Stem. My research into these AD-MSC technologies have led me to conclude that they could be a successful way to treat laminitis for the following reasons. Firstly, the laminae have similar properties and functions and are put under the same kind of strains as tendons and ligaments i.e. they are fibrous, their function is to connect two structures together and they too must support the animals weight when it moves. Secondly, horses recovering from mild laminitis can heal the laminae themselves. This shows that when the laminae are not under too much stress, they have a capacity to regenerate themselves. Combine this factor with the tendency of stem cells to be attracted to and to migrate to areas of damaged or diseased tissue, this demonstrates there is potential for stem cells to help the horse heal itself. It is proposed that these technologies could be applied to growing laminar tissue from AD-MSCs, and that the AD-MSCs would not only successfully develop into laminar tissue but would also continue to grow into laminae within the horse's hoof.

Assuming that there is potential for AD-MSCs to re-grow the laminae, process of extracting and growing these stem cells must be explored. The first step would be to extract a small amount of fatty tissue from the horse. Then the AD-MSCs would need to be isolated from the adipose tissue. This is where my proposed methods differ from those already used by companies like Vet Stem, who at this stage re-administer the stem cells in their undifferentiated state back to the patient. Given that the time taken before treatments are started is the main factor deciding whether a horse will or will not recover from laminitis, it is proposed that the stem cells should be administered to the horse in their differentiated state to re-build the laminar tissue. The stem cells would be exposed to specific growth conditions in the laboratory inducing them to develop into the early stages of the desired laminar tissue. At this stage - they are now the differentiated stem cells - they would be harvested and injected back into the horse's hoof just above the coronet band – the soft line where hair growth stops and hoof growth begins - into the space between the hoof wall and the coffin bone. This procedure is shown by *Fig 6* the orange arrow indicates where the cells would be injected, and the picture shows how the coronet band is directly above the space between the coffin bone and the hoof wall, where the laminae need to be. Once inside the hoof capsule, these cells would continue to grow, repairing or replacing the laminae, so that the coffin bone is once more attached to the hoof wall.

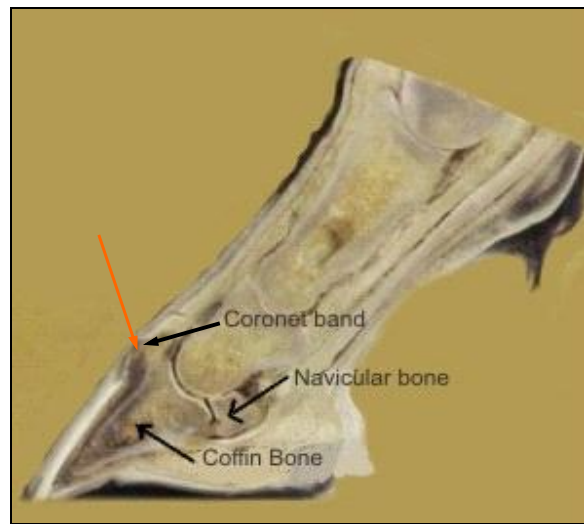


Fig 6

Stem cells alone cannot cure laminitis. It is proposed that stem cells are used alongside corrective trimming and hoof support, as detailed by Eustace et.al (1992) in order to ensure a much quicker recovery than current treatments can give. The shorter timescale that I think stem cell treatments could provide would be extremely beneficial to the horse's long term health. Any long-standing lameness causes the horse to compensate for the mobility it has lost by overloading the other limbs, or moving in an such a way that less weight is taken by the painful areas, and because the horse is such a large animal, this can cause a number of serious joint and back problems. I believe that with a shorter rehabilitation period, the potential for a horse to develop these problems resulting from laminitis would be a lot less.

At the moment it takes a horse about two months to recover from an attack of laminitis. (Laminitis clinic) This is because although the horse may appear sound, the laminae are still inflamed and have lost some of their strength, so if the horse moves around too much then what was a laminitis case can easily progress to founder. It makes sense to take every possible measure to prevent this from happening, so I suggest that the cultured stem cells are injected into the hoof, so as to encourage the damaged laminae to heal faster and stronger. Dogs and horses treated with AD-MSCs have shown improvement within two weeks, (Vet-Stem) and I believe this is achievable for horses suffering from laminitis, with perhaps a total of a month before the horse can start gentle exercise again.

With a horse that has foundered, the coffin bone and the hoof wall must be realigned to their original positions in order to return the foot to its former condition. The biggest problem facing veterinary surgeons when dealing with a laminitis case is that even though these processes are carried out, the horse returns to founder at some stage during rehabilitation. My proposal is that by providing a

supportive connection between the hoof wall and the coffin bone in the form of the regenerated laminae all the way through rehabilitation, this situation could be avoided. Therefore I propose a treatment regime as follows. First, to determine the degree of rotation of the coffin bone, X-rays are taken and following this hard plastic glue-on shoes are fitted to support the heels, relieve pressure at the toe and support the sole. Now is the time to inject the first treatment of differentiated stem cells, which will begin building up the connection between coffin bone and hoof wall. The next stage is to stabilise the hoof by shoeing and/or trimming, involving shortening the toe, lowering the heel and supporting the sole. In these cases, where rehabilitation is a longer process, I think that the differentiated stem cells should be injected at regular intervals to speed up the horse's recovery and to create a stronger laminar bond between the hoof wall and coffin bone than a horse with already compromised health might have created on its own.

In some acute founder cases, where there is a build up of fluid in the hoof capsule, the front of the hoof needs to be removed (Dorsal wall resection, see fig 7) to drain the fluid, thus releasing the painful pressure on the laminae. The new hoof will then grow down at the correct angle. This process demonstrated by Eustace (1992) also allows any infections to be addressed and dead laminar tissue can be removed. I believe that stem cells could be used to go about treating the horse after it has had a section of the hoof removed using a similar procedure as a bone marrow transplant. Dead, diseased laminar tissue would be removed and the differentiated stem cells injected into the foot as before, where they will begin producing new, healthy tissue. As the new hoof grows down, inject differentiated stem cells to create and improve the laminar bond.



Fig 7

Why use adult stem cells rather than embryonic stem cells?

Using adult stem cells avoids the fiercely debated ethical issues involving the use of embryonic stem cells to carry out stem cell treatments and research. Embryonic stem cell research presents us with a moral dilemma because it asks us to decide which is the most important – the prevention of death and suffering, or the value of a potential human life, this is covered in a paper by Rickard (2002). Many believe that the embryo is the beginning of a new life. This makes embryo destruction equivalent to murder. The opposing argument is that embryos are mere body parts that have the potential to save thousands of lives. Therefore, although embryonic stem cells are the most versatile, one of the reasons I have chosen this disease for my research project is that I believe it is possible to treat it without using embryonic stem cells, but by using adult stem cells instead. These kind of technologies enable scientists to research and experiment without such tight restrictions, thereby increasing the amount of information we have on stem cells, and moving us ever closer to curing hundreds of diseases.

Conclusion

In my discussion I have examined the issues that so many veterinarians are faced with when dealing with a laminitis case. Too often vets come to the end of possible treatments, resulting in an animal being euthanised. New treatments are needed, and I believe that my proposals for the treatment of laminitis using stem cell therapies could provide an answer.

To summarise, my proposal for a future use of stem cell therapies is to use them to repair the laminar tissue destroyed by laminitis. I have established that the horse's body has a capacity to heal these structures, but that when the foot is put through the mechanical changes caused by laminitis, this is much more difficult. I have compared the sensitive laminae to tendons and ligaments, and by researching technologies already in place for treating tendon and ligament damage I have come to the conclusion that similar principles can be applied to suggest ways in which veterinarians could go about treating laminitis with stem cells. This will involve injecting differentiated stem cells into the space between the hoof wall and the coffin bone, both as an initial treatment and a repetitive treatment during rehabilitation of laminitis.

However, new treatments always present problems. In this case, the first problem is the cost. Similar treatments from companies such as Vet-stem are available from around two thousand pounds. Whilst paying this much may be worthwhile for a very valuable animal that has the potential provide its owners with an income, treatments costs need to fall dramatically before they can be used commonplace to treat ordinary horses. I think this problem could be overcome as stem cell therapies become more and more common in veterinary medicine. As technology progresses, it is likely that more efficient and cheaper methods of carrying out stem cell therapies will follow. While these treatments may be expensive to start with, as they become more widely used they should be easier and cheaper to obtain.

The second problem is the way in which to go about stimulating the undifferentiated stem cells to differentiate into the desired tissue. In the case of the bronchus transplant covered in the introduction, stem cells were put with base donor cartilage from a bronchus to stimulate them into differentiating into bronchus tissue. If similar techniques needed to be applied to differentiate AD-MSCs into laminar tissue, then this presents scientists with a problem. While laminar tissue could be removed using a syringe, it does not make sense to extract a sample of laminar tissue from the horse's hoof for this purpose if the horse's laminar tissue is diseased, and using donor tissue result in tissue rejection. This is where embryonic stem cells come into the equation. In the future, if scientists have mastered both controlling embryonic stem cell differentiation, and controlling tissue rejection, then medicine and veterinary medicine could move on to the stage where the differentiated cells of hundreds of different tissues including laminar tissues are banked, ready to be administered when needed.

If these problems could be overcome, my vision is that stem cell therapies for laminitis would be a widely used treatment that would make the difference between a horse having to be euthanised, and a horse returning to its former working life.

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