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Characterisation of the role of embryonic stem cells in the therapeutic treatment of tendon injury

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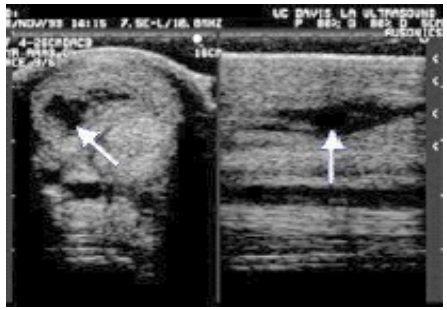
HBLB VAC Reporters: R Pilsworth & CM Marr

Tendon injuries

- Tendon injury is very common in racehorses, accounting for approximately 46% of all injuries.
- Because the tendon heals by inelastic 'scar' tissue, it is never the same again following injury.
- This results in a very high re-injury rate of almost 70% following rehabilitation and treatment



Stem cells

- There has been a lot of interest in the possible use of stem cells in treatment of tendon injury
- Stem cells are ‘multipurpose’ cells which can turn into any other specialist type of cell when placed in the right environment
- We can collect stem cells from the injured horse as there are some available in the bone marrow (autogenic use)

Stem cell treatment

- But, there are relatively few stem cells in bone marrow.
- For clinical use they have to be multiplied up in tissue culture, which takes a long time, and all that time the tendon is laying down scar tissue as it starts to heal.
- The ‘holy grail’ would be a source of stem cells which would be immediately available for treatment, ‘off the shelf’.

Embryonic Stem Cells

- Stem cells can also be derived from embryos.

- But, just as human organ transplants can be rejected by the recipient, there is a risk that cells from an embryo might be rejected if they were transplanted into another horse.

How can stem cell behaviour be studied?

- Because of differences in the 'marker' proteins on the cell surface, it should be possible to distinguish between embryonic and bone marrow-derived stem cells after they have been implanted, and use these markers to follow their migration and survival.

- These markers could be used to study:
  - how well each type of cell performs in aiding repair;
  - how well they survive after injection;
  - whether or not it is possible to use cultured cells from one horse in the treatment of another (allogenic use).

Reason for Research

- This project studied those factors, and assessed:
  - how well the cells survived?
  - where they went to in the tendon once injected?
  - and whether injecting the cells derived from one horse or embryo into another horse caused any problems?

Differentiating stem cells:
(aka mesenchymal stromal cells, MSCs)

Common "markers" of equine MSCs from 2 tissue sources were defined.

	CD90	CD44	CD29	CD14	CD79α
Bone marrow	+	+	+	-	-
Cord blood	+	+	+	-	-

Differentiating stem cells:
(aka mesenchymal stromal cells, MSCs)

MSCs from different sources have some differences, including different expression levels of molecules on their surface that comprise the Major Histocompatibility Complex (MHC).

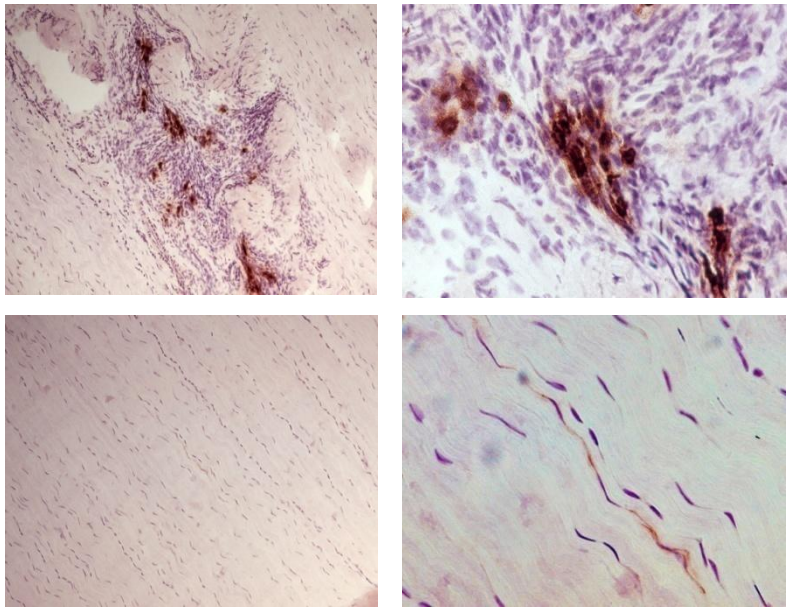
	Control		IFN-γ treated	
	MHC I	MHC II	MHC I	MHC II
Bone marrow	+	-	++	+
Cord blood	+/-	-	++	-

In collaboration with Mr Smith (Newmarket Equine Hospital) and Dr Ousey (Animal Health Trust). Stem Cells Cloning Ad. Appl. (2008) 1, 1-9.

Autologous and allogeneic MSCs can both be used in the injured tendon

Lesion

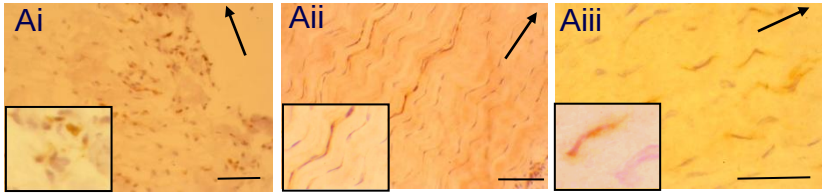
Healthy tendon

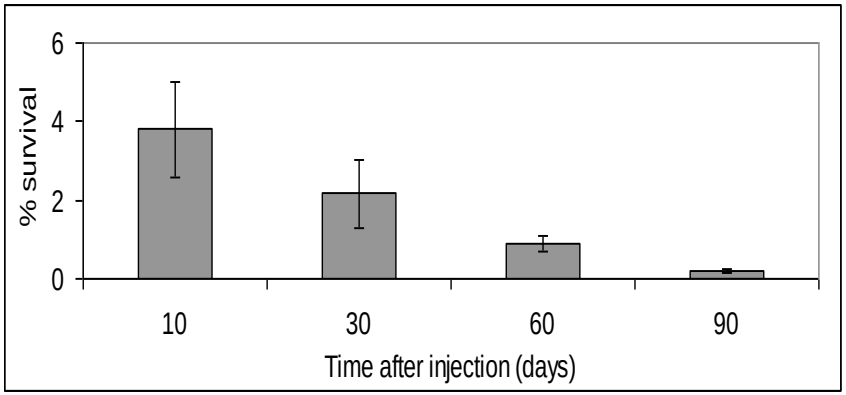


There are no differences in the survival or distribution of autologous (i.e. cells from the same horse) and allogeneic MSCs (i.e. cells from a different horse).

Cells from embryos and bone marrow have different survival rates and migration patterns in the damaged tendon

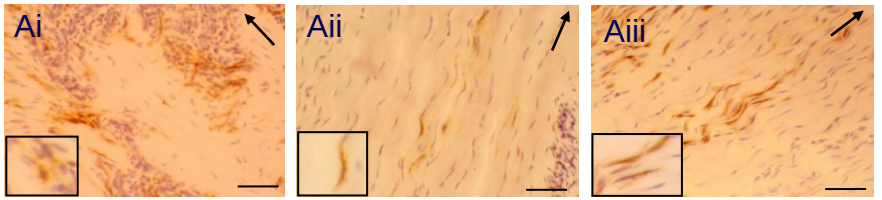
MSCs (bone marrow)

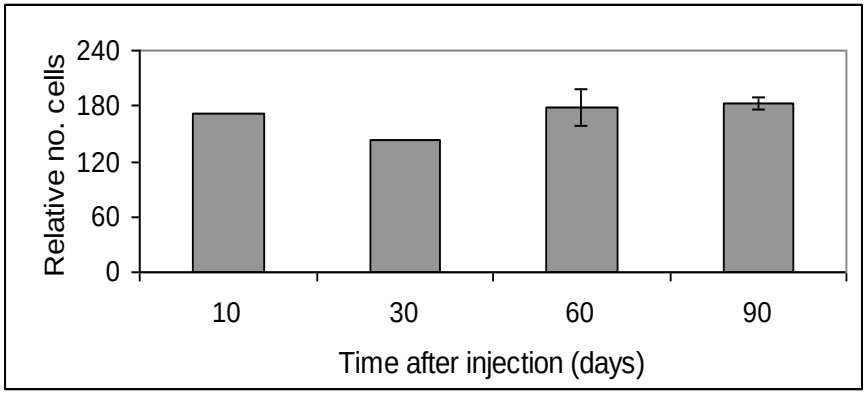




Only found at site of damage into which they were injected

ESCs (embryo)





Can migrate to other sites of damage in the same tendon

Implications of this project

1. Stem cells derived from tissues can now be identified using a set of markers we defined.

2. Although this set of markers is common in cells derived from different tissues, there are other differences which are innate to the cells and may affect their clinical use.

3. Stem cells derived from another horse can be used in the damaged tendon without generating a cell mediated immune response.

Implications of this project

4. Whether from the same or a different horse, the bone marrow stem cells have a very poor survival in the tendon.

5. Embryonic stem cells have a high survival rate in the tendon, they do not generate a cell mediated immune response or form tumours. They may therefore provide a source of “off the shelf” stem cells for future use in aiding tendon regeneration in the horse.

Commentary on conclusions

- In contrast, stem cells derived from embryos survived in much greater numbers, and also migrated to other injury sites in the tendon, spreading out the repair process.

- No problems were seen following placement of embryonic stem cells into another horse, they appeared not to be 'rejected', a common problem when 'foreign' organs or tissues are transplanted from one animal or person into another.

Commentary on conclusions

- This project offers great hope for the future:

- Embryo derived cells could be produced in tissue culture in the laboratory in huge numbers, and could therefore be available for commercial use in future for the treatment of injured tendons in the racehorse.

Find more information about tendon injuries in racehorses

- Musculoskeletal disease and injury, now and in the future. Part 2. Tendon and Ligament injuries, PD Clegg:

  http://onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.2012.00563.x/full

Scientist's summary

- Tendon injury is common in racehorses and competition horses. Tendons are elastic in nature, and after injury heal slowly with scar tissue. This scar tissue makes the tendon less elastic and less strong, and further tendon injuries are common.

- Stem cells are different from the normal cells in the body in that they can renew themselves and differentiate into a wide range of specialised cells. The two types of stem cells are embryonic stem cells (ESC) isolated from embryos, and adult stem cells found in adult tissues such as bone marrow and the umbilical cord at birth (known as mesenchymal stem cells –MSCs).

Scientist's summary

- The first objective of the project was to isolate and characterise MSCs from the horse's own, and other horses, bone marrow and the umbilical cord. Isolation was successful.

- Characterisation consisted of observing, by cell culture in the laboratory, growth and differentiation into bone, fat and cartilage cells, with measurement of the expression of genes and other proteins by the cells.

- Overall MSCs from different sources varied in growth, differentiation and gene and protein expression.

Scientist's summary

- The second and third objectives were to determine what happens to stem cells when they are introduced into damaged tendons in horses. So that introduced cells could be distinguished from existing cells, ESCs and MSCs were differently labelled by adding 'marker' genes.

- After injection of ESCs and MSCs into the tendons MSCs did not survive in any significant numbers for any significant time, whereas ESCs survived in significant numbers for an extended period, and migrated to other damaged areas of the tendon.

Scientist's summary

- Despite the fact that ESCs are obtained from other horses they did not cause an immune response and tumours (a possible side effect) were not seen.

- The fourth objective was to understand how ESCs change when injected into tendons. It would appear that contact with the other cells and tissues of the tendon is associated with the ESCs themselves differentiating into tendon cells.

Scientist's summary

- In conclusion, ESCs have been shown to have the potential to be used in tendon injury, but clinical studies that look at return to function are still required, and there are potential ethical and scientific issues from the current source of supply of ESCs.

To find out more about HBLB's research go to:

"HBLB's Advances in equine veterinary science and practice"

- http://onlinelibrary.wiley.com/journal/10.1001/%28ISSN%292042-3306/homepage/hblb_virtual_issue.htm