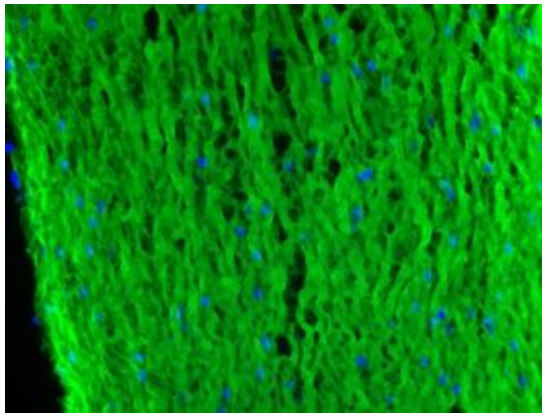
Comparing the ability of horse stem cells to make tendons

Dr Debbie Guest



Using a laboratory system to optimise the use of stem cells for tendon repair in horses

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Comparing tendon generation by equine pluripotent stem cells

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Tendon injuries

- Occur commonly in racing Thoroughbreds
- **Repair** through scar tissue formation. This is inferior to normal tendon tissue and pre-disposes horses to high re-injury rates (up to 67%).
- Tendon injury is the number one reason for retirement from racing.
- Stem cells may aid the **regeneration** of healthy tendon tissue to reduce re-injury rates.

Horse pluripotent stem cells

- Pluripotent = can turn into all cell types of the body
- Can be derived from early embryos (embryonic stem cells, ESCs)
- Can be derived by "reprogramming" adult cells to induce pluripotency (induced pluripotent stem cells, iPSCs)
- Previous work demonstrated that horse ESCs can turn into functional tendon cells that may have the potential to aid tendon **regeneration**.
- The ability of iPSCs to form tendon was unknown.

Understanding tendon formation and regeneration

- During development a protein called scleraxis is required for normal tendon formation.
- Scleraxis levels increase following a tendon injury and scleraxis is produced by ESCs when they turn into tendon cells but it was not known if it was required for this process.
- Understanding the process for making healthy tendon tissue will help to optimise future therapies for tendon regeneration after an injury.

Objectives

1) To determine if a growth factor (TGF-β3) can drive iPSCs to turn into tendon cells.

2) To determine if iPSCs-derived tendon cells are functional and can generate "artificial" tendons in the laboratory following culture in a 3-dimensional (3D) system.

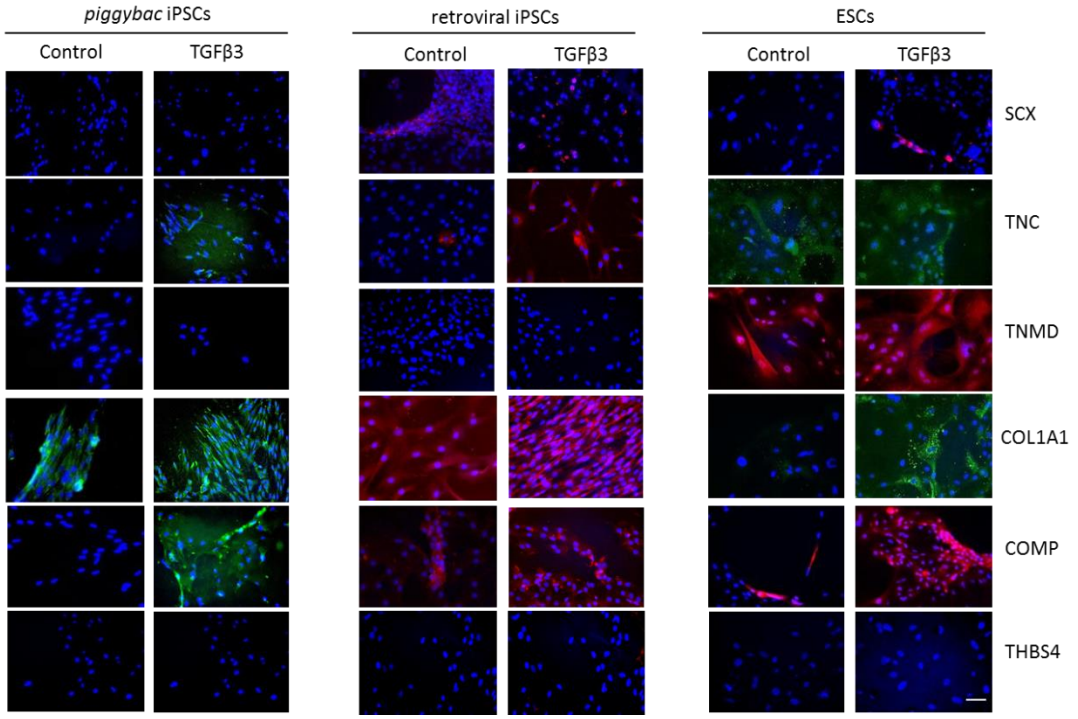
3) To determine if scleraxis is required for turning pluripotent stem cells into tendon.

iPSCs turn on tendon genes and proteins after exposure to TGF-β3

Tendon genes and proteins are turned on in iPSCs after exposure to TGF-β3, but they do not turn on as quickly as they do in ESCs.

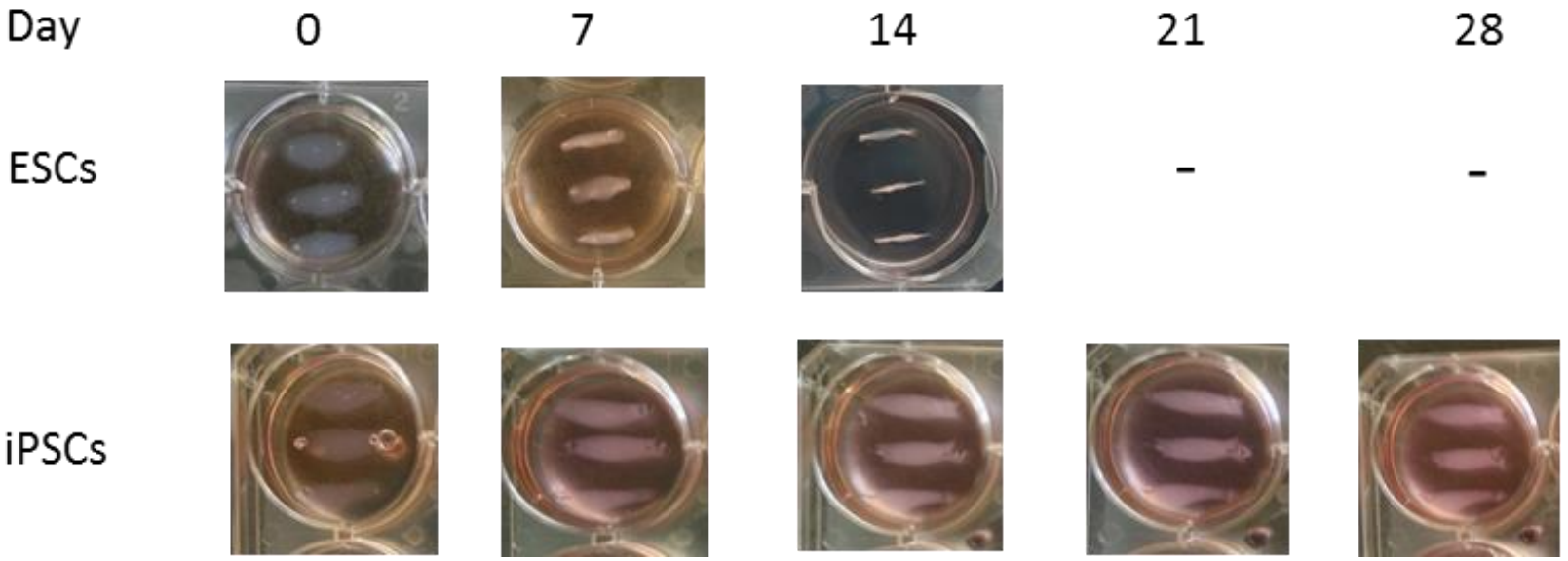
Image shows staining for a range of tendon-associated proteins in iPSCs made using two different methods and in ESCs after 7 days of culture.

Cell nuclei are shown in blue, tendon proteins in red and green.

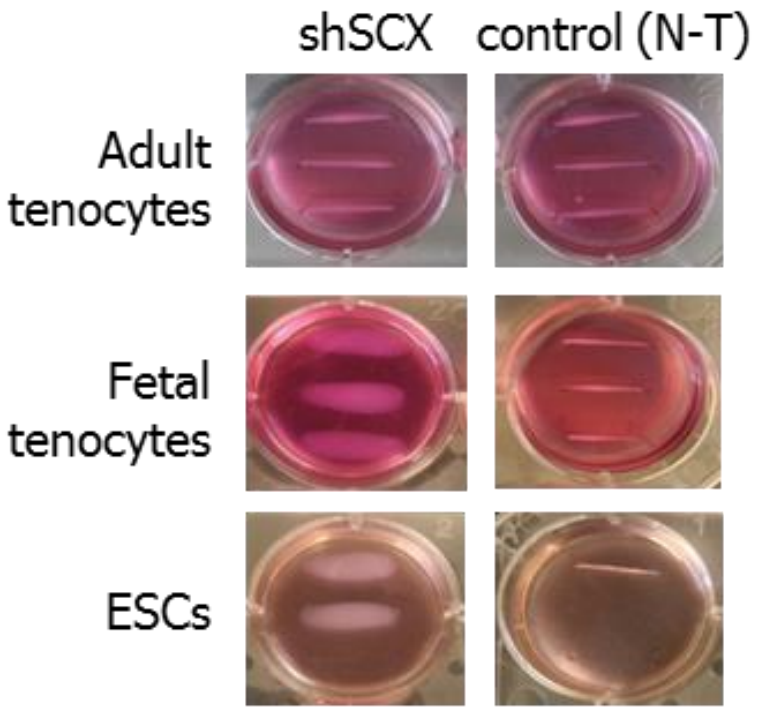


iPSCs do not produce artificial tendons in 3D culture

- ESCs produce artificial tendons within14 days in 3D culture, but iPSCs fail to generate artificial tendons over 28 days.
- The iPSCs have a similar survival to ESCs but remain in a stem cell state.



Scleraxis has different roles at different stages of development



- A short hairpin RNA to scleraxis (shRNA) was used to block its expression
- Loss of scleraxis has no effect on adult tendon cell function in 3D culture
- Loss of scleraxis completely prevents the formation of artificial tendons by fetal tendon cells and ESCs
- Loss of scleraxis has significant effects on tendon gene expression in fetal, but not adult, tendon cells in 2D culture

Conclusions

- iPSCs are not as efficient as ESCs at generating **functional** tendon cells.
- Scleraxis is required for turning ESCs into functional tendon cells.
- Scleraxis is required for the function of fetal, but not adult, tendon cells.
- Our 3D culture system provides an important test of the functionality of tendon cells.

Impact for the Thoroughbred

- To ensure that the clinical application of novel pluripotent stem cell based therapies can be optimised, we must understand the mechanisms by which they function.

- An optimised and effective cell therapy for tendon injuries would help to reduce re-injury rates and allow more Thoroughbreds to return to racing following an injury.

Potential next steps

- To understand what differences exist between iPSCs and ESCs to result in our observed differences in their capacity for tendon regeneration.

- Fetal tendon can regenerate without forming scar tissue. Adult tendon undergoes repair through scar tissue formation. A better understanding of the role of scleraxis in fetal and adult tendon cells and ESC-derived tendon cells would enable us recapitulate regeneration, rather than repair, following the use of a cell based therapy.