Author’s response to reviews

Title: A comparison of the stem cell characteristics of murine tenocytes and tendon-derived stem cells

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Version: 1 Date: 21 Mar 2018

Author’s response to reviews:

Manuscript review response letter

We thank the reviewers for their interesting and useful comments, which we will address in order as listed below.

Reviewer 1

Comment 1:
From a clinical point of view IT is not clear how the results could be translate in a human setting and how they could be useful for treating human tendon diseases.
Can the Authors more specify these aspects in the discussion and conclusions paragraphers?

Response 1:
We have now included a section on the clinical relevance of the research for human tendon repair in the discussion (page 15, line 333-339) and conclusion (page 16, line 347) sections.
Reviewer 2

Comment 1:

How were TDSCs and tenocytes distinguished in the original isolation? It appears that this was only done by seeding density. How do the authors know that the differences seen aren't the result of de-differentiation, for example? What about the difference in seeding guaranteed stemness/progenitor status? For both seeding densities, how long were the cells cultured before next trypsinization? Also, at next passage were they seeded? Same densities? Addressing this is essential for the methods but also the "Tenocyte and TDSC morphology and colony formation" section of the results. Honestly, the whole study hinges upon better clarification of these points.

Response 1:

We based our cell isolation procedures on previously published methods as described in the discussion section (page 13, line 285). These papers describe low density plating as an effective method for isolation of tendon-derived stem cells, which is due to cell contact causing differentiation. Fibroblasts require cell contact to proliferate and differentiate, therefore a high seeding density was selected, references are provided in the methods section (page 4, line 98-95). It is possible that the TDSCs may have de-differentiated however they expressed stem cell and tenogenic markers as well as demonstrating multipotency, therefore their phenotype suggests that they are a form of progenitor cell of tendon origin, so de-differentiation is unlikely. TDSCs were cultured for 6-8 days before passaging, whereas tenocytes were cultured for 2-3 days. For subsequent passages the cells were split 2:1. We have now included this information in the methods section (page 4-5, line 96-97).

Comment 2:

In the cell proliferation assay, was this the first passage of the cells (or each cell type as the authors propose)? It is not indicated.

Response 2:

For the cell proliferation assay the cells were used at passage 2, we have now included this information in the methods section (page 5, line 102).
Comment 3:
For the colony forming assay, was this the first passage of the cells (or each cell type as the authors propose)? It is not indicated.

Response 3:
For the colony forming assay the cells were used at passage 2, we have now included this information in the methods section (page 5, line 109).

Comment 4:
This lack of chondrogenic success for TDSC needs to be better addressed, particularly in the discussion as a limitation. Others have had great success. The authors should compare chondrogenic differentiation media, technique, seeding density used and comment. Addressing the failure there is more useful then the authors realize.

Response 4:
To clarify, we didn’t observe a lack of chondrogenic differentiation for TDSCs, but were unable to perform the assays due to low cell numbers as chondrogenic differentiation assays require a large number of cells. Therefore we do not know if TDSCs are able to differentiate down the chondrogenic lineage. This limitation has been outlined in the results section (page 11-12, line 233, 237 and 247) and the discussion section (page 13-14, line 293-297).

Comment 5:
Remember GAGs are also found in tendons, maybe not because of aggrecan but with SLRPs. How do the GAG levels that measured here compare to other studies performed in regard to chondrogenesis? This could be addressed in the results in the discussion to discuss how robust the assay used here was. That is, not all chondrogenic assay strategies are created equal, particularly depending upon species. A comparison of these results versus other studies numbers could both help you understand what happened and distinguish species differences. This would be very useful to someone exploring the literature for TDSC vs tenocyte differences. Are your
GAG levels cartilage or fibrocartilage or tendon or engineered/culture tissue levels? This is a good opportunity to expand upon your interpretation.

Response 5:

GAGs are present in tendon, predominantly associated with SLRPs with only small amounts of aggrecan present, whereas in cartilage aggrecan is the predominant proteoglycan. Aggrecan contains considerably more GAGs than SLRPs, therefore the GAG assay used in this study is more reflective of the level of aggrecan present, rather than SLRPs, and indicates the level of chondrogenesis. To our knowledge no other studies have analysed GAG levels in murine tendon cells (tenocytes or TDSCs) therefore an informative comparison cannot be provided, however one study found increased production of GAGs in human TDSCs compared with our study (Stanco et al, 2014). As suggested, this is likely due to species variation as well as differences in tendon type. In addition, increased GAG levels have been observed in murine Achilles tendon (Mikic et al, 2009), however this discrepancy is likely due to the use of tissue rather than cells. These details and references have been included in the discussion section (page 14, line 297-301). We wished to determine if murine tendon cells had the ability to differentiate down the chondrogenic lineage, we did not investigate the type of cartilage formed.