

Reviewer's report

Title: Stochastic Amplitude-Modulated Stretching of Rabbit flexor digitorum profundus Tendons reduces Stiffness compared to Cyclic Loading but does not affect Tenocyte Metabolism

Version: 3 **Date:** 8 August 2012

Reviewer: Brendan Harley

Reviewer's report:

Major Compulsory Revisions

- 1.) Please discuss the choice of the rabbit flexor digitorum profundus as a model system.
- 2.) Please provide details for any animal protocols used for the harvest of tissue.
- 3.) Did the media contain any serum? Please specify.
- 4.) How did you choose the mechanical loading times (only 1 h/day for half of the days of the study)?
- 5.) Why wasn't collagen content measured (hydroxyproline) along with GAG and DNA content? The collagen content will have a far greater impact on mechanical properties than GAG content.
- 6.) The formatting of Table 1 makes it difficult to read, please revise.
- 7.) The conclusion that "stochastically stretched tendons suffer from increased mechanical microdamage, relative to cyclically loaded ones", while possibly correct, cannot be drawn based on the data presented here. Statements like these should either be removed or the required analysis should be performed. SEM or histology as suggested by the authors would be a good start, but polarized light microscopy or second harmonic generation to look at collagen organization would be preferred.
- 8.) The gene expression data covers a wide range of targets, although some of the choices are peculiar. COLII expression is not expected to be detected in the tendon proper, and while aggrecan is a component of tendon other proteoglycans like decorin and biglycan make more sense as targets. More complete review of each target based on the literature should be done (similar to what was done for COL I). For example, MMP-1 and -13 are up-regulated in diseased tendon while MMP-3 is actually down-regulated (Clegg et al. 2007). Additionally, the measurement of phenotypic markers like scleraxis, tenomodulin, or tenascin-C would have considerably strengthened the profile of markers assessed here. The authors should at least discuss why they chose certain markers and the importance of other targets often found in the literature.
- 9.) There are some writing errors that arise, especially in the discussion section, that make this manuscript a bit sloppy and difficult to read:
 - a. Page 2: "Muskuloskeletal" should read "Musculoskeletal"

b. Pages 8-11: Multiple parts of the discussion are duplicated. Please correct/combine accordingly.

Minor Essential Revisions

N/A

Discretionary Revisions

N/A

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests'