Reviewer's report

Title: Second harmonic generation analysis of early Achilles tendinosis: identification of tenocyte-associated collagen degeneration in response to mechanical loading in vivo

Version: 1 Date: 10 October 2010

Reviewer: Margaret Smith

Reviewer's report:

Major concerns.
With respect to the title of this paper, the authors have shown a reduced density of collagen around the tenocytes, but not that this is due to degeneration. It could be increased hydration, fatty infiltration, increased hyaluronan, increase in cell size (with cell rounding), decreased synthesis/turnover etc. The title is, therefore, misleading.

The goal is clearly stated but with the technique used, only half of the goal can be accomplished.

The concern I have with this paper is that the final images of the SHG signal do not visualise the tenocytes at all – they are presumed (not unreasonably) to be in the spaces between fibers. This means that, in normal tendons, they cannot be visualised using this technique. The visualisation has a surprisingly low resolution if the figures supplied are representative.

The SHG images are not as well resolved as those obtained using polarised light microscopy on formalin-fixed picrosirius red-stained sectioned tendon. With this light technique, images can be taken at 16 – 400x magnification under polarised light to see the collagen fibers and then a matching image taken without moving the section under nonpolarised light to view the tenocytes. This technique, as you suggest for SHG, does not highlight regions of provisional matrix.

Suggested revisions
This reviewer suggests you attempt this polarised light technique as a comparator. It would strengthen the validation of SHG as an appropriate method for studying tendon collagen.

Figure 1 B and 1D do not seem to be highlighting all the collagen present. Are you suggesting your technique only presents aligned collagen fibres? Despite the perceived failings of immunohistochemistry, a comparator immuno-stain for collagen type I would have confirmed this.

Minor revisions.
You cannot say that blood flow was increased without evidence of angiogenesis without looking for such evidence. Did you look for vascular elements in the H&E sections? This may provide some data.
“Tendon blood flow is regulated by many locally produced, diffusible factors in tendon which can result in the dynamic regulation of smooth muscle tone, including Cox-2 and nitric oxide.”

Please provide reference(s) for this statement.

The parametric t-test is not suitable for ultrasound scores. These are ordinal data, not continuous.

Significance was predetermined if alpha was less than 0.05.

Figure 3 is poorly rendered.

More results figures would be beneficial. Ultrasound pictures, H&E sections to view the tenocytes etc.

There should be a section in the discussion on how this technique compares with other used in the existing literature.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**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