Reviewer’s report

Title: Continuous Low-Intensity Ultrasound Attenuates IL-6 and TNFα-induced Catabolic Effects and Repairs Chondral Fissures in Bovine Osteochondral Explants

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Reviewer: Mikko Lammi

Reviewer's report:

Despite contradictory results, pulsed ultrasound stimulation has long been suggested to have positive effects on cartilage and chondrocytic metabolism. This study gives further evidence on the positive effects of LIUS on cartilage. The findings related to apposition and catabolic effects may be valuable for cartilage repair studies.

However, the complicated geometry of the in vitro experimentation has been well presented (Leskinen and Hynynen, Ultrasound Med Biol, 2012). There are also quite high temperature rise variations due to sound absorption, sound reflections and inadequate heat transfer during pulsed LIUS at 1.035 MHz (Olkku et al Bone, 2010; Leskinen et al IEEE Trans Biomed Eng, 2014). Therefore, I am surprised that seemingly stronger US treatment does not induce adverse heating of the samples. Therefore, US conditions are likely to be different in monolayers vs. tissue explants. This should be discussed in the manuscript.

Line 157: What criteria was used to confirm representative image?

Parametric test was used for the statistical analyses, was normal distribution certified?

Compared with my experience, the live/dead analysis (Fig. 2) shows surprisingly few dead cells in the incision area. More importantly, is a image Fig 2F representative (cLIUS+TNF-alpha), it seems to have non-stained empty areas. Another thing the authors do not mention is the apparent difference in cell morphology into elongated one in Fig 2L (cLIUS+TNF-alpha). Is this indication of fibroblastic transformation?

Presentation of Fig 3 raises an idea how representative are the Figures shown. In 3A, there is clear incision, while the inset does not seem to have it, same is true for 3C, while inset in 3L has incision in inset, but not in higher magnification image.

Line 239: The authors write that there is increase in apposition from 3.32% to 58.04% with significance of 0.012, while the Figure seems to show 0.027, which one is correct?

The authors tell that there is depletion of proteoglycans in the samples treated with IL-6 for 28 days and strong staining in cLIUS-treated samples. In the methods, it was not mentioned whether all the samples were stained at the same time, perhaps staining at different time could explain the difference in staining intensity.
How was the specificity of the antibody checked, there is no indication of negative controls?

For qPCR, how was the variation in controls determined? I doubt a bit such a strong significance for MMP13 and ADAMTS4. A mistake often done with controls is that they are adjusted into 1, which gives no variation, and therefore any other comparison gives significant difference. For instance, in my group we never experience practically zero variance for type 2 collagen. All the control should be analysed in the same PCR run.

Line 362: Where is the data on GAG loss measured by DMMB? It should be presented if mentioned.

Minor points

In lines 132 and 135, many of readers are certainly not be familiar with the abbreviation Vpp, please write open.

The values and units should be separated with space, please correct those missing the space.

Line 155: who is Dr. S.H.?

Style in reference list is variable, please correct the title styles, journal names (abbreviations), and issue numbers to consistent form.

If the length of scale bars is presented in Figures I think they should be readable, which is not really the case now.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?  
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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