Author’s response to reviews

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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Author’s response to reviews:

Dear Dr. Byrne: Thank you for the peer-review of our manuscript. We thank the reviewers for their comments and critiques. We have provided an itemized response to the reviewer comments.

Reviewer reports: Mikko Lammi (Reviewer 2):

Comment 1: The authors have sufficiently responded to my comments, although there is still some inconsistency in the style of reference titles. The scale bars are now fine, although the Figures 2 and 5 still give the length of scale bars in font size that is not readable.

Response 1: Thank you for your comments and suggestions. Inconsistencies in the reference styles were corrected. The font size in the scale bars of Figures 2 and 5 have now been modified to ensure readability.

Comment 2: I sorry to present one more comment on Figure 5 B and C. They should present Safranin O staining, which gives red color on the sections, now these two parts of the Figure looks more like Alcian Blue-stained. Are the authors sure they have the correct figures here. I am sorry this question was missing in my original comments.

Response 2: Thank you for the comment. In this study, CONTROL refers to specimens that were maintained in culture medium, but not exposed to LIUS. Panels 5A, 5B and 5C represent the following osteochondral specimens:
5A: Osteochondral specimen, Control (no LIUS) and no cytokines (no IL-6 or no TNFalpha)

5B: Osteochondral specimen, Control (no LIUS) plus IL-6.

5C: Osteochondral specimen, Control (no LIUS) plus TNFalpha.

Figure 5A represents the safranin O stain of explants in control conditions for 28 days. Figure 5B and 5C are representative of the explants that were treated with IL-6 or TNFα for 28 days; no LIUS was employed so that comparison can be made with 5A. The lack of red/brown color is attributed to GAG loss as demonstrated by lack of Safranin O stain (it is not an Alcian Blue stain). We observe that in in-vitro culture, when explants are exposed to cytokines, there is GAG loss and similar observations were made by Uddin et al., (Osteoarthritis and Cartilage 2017). Figure 5 shows Safranin O staining (red) for GAG in the cartilage part of the osteochondral explants, with Fast Green as counterstain. The regions that were positive for GAG stained red whereas the regions where GAG was lost did not stain red and only picked up the counterstain (blue-green) (Figure 5B, C).

For the sake of clarity, insets of the whole osteochondral sections imaged at 2X magnification were included in Figure 5.