Author's response to reviews

Title: Viscoelastic properties of bovine knee joint articular cartilage: dependency on thickness and loading frequency.

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Author's response to reviews: see over
TITLE:
Viscoelastic properties of bovine knee joint articular cartilage: dependency on thickness and loading frequency.

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The authors thank the reviewers for their comments and address each point raised (below). All changes made to the manuscript have been highlighted in the text.

EDITORIAL REQUIREMENTS

1. Context information has been added to the background section of the abstract (page 2, lines 2-5).

REVIEWER: Jessica Deneweth

1. Cartilage regions showing lesions were not tested. However, as joints were not osteoarthritic, large scale damage was not evident and it was always feasible to identify a region of interest for testing. In some cases this meant testing near but not on a lesion. Clarification has been added to the text (page 5, lines 12-15).

2. A new limitations section has been added to the manuscript discussion for clarity (page 14, lines 1-19).

3. We are unsure as to the symbol referred to by the reviewer. In all tables superscripts \( A,B,C \) have been defined to indicate significantly different groups. However, the symbol \( \wedge \) does not appear in our version the document. Could it be a low resolution \( \wedge \) to which reference is being made by the reviewer? Regardless, further explanation has been added to aid interpretation of superscripts (page 7, lines 9-14).

Minor essential revisions
1. The text has been amended (page 8, lines 6 & 24, & table 2).

REVIEWER: Cathy Holt

1. A new discussion section has been added to the manuscript, relating the heel-strike transient frequency, DMA and OA (page 13, lines 4-25).

2. The use of a Bovine model is discussed in a new limitations section (page 14, lines 12-19).
3. The statistics section of the methods has been amended to aid the reader in interpreting statistical differences presented in tables (page 7, lines 9-14).

REVIEWER: X. Lucas Lu

1. The results presented are the storage and loss stiffness for the cartilage. In this case loss stiffness includes any physical mechanism which leads to dissipation of energy and, thus, results are measurements of cartilage mechanical behaviour under loading. The mechanisms by which they occur are of interest, as are the mechanisms by which the gel-collagen interaction leads to such energy storage and dissipation. Discussion has been added to the text (page 13, lines 18-21).

2. The reviewer’s paradigm ignores the need for precycling in cartilage and its role in knee dynamics, also we have addressed this point in previous publications. Further reference is now made to the two studies in which the procedure used is validated (page 6, lines 4).

3. Our study demonstrates how frequency alone can alter mechanical properties of cartilage. The feasibility of the method used has been previously published. Further reference is now made to the two studies in which the procedure used is validated (page 6, line 4).

4. In a previous study we found the order not to affect results, i.e. the role of precycling. The feasibility of the method used has been previously published. Further reference is now made to the two studies in which the procedure used is validated (page 6, lines 4).

5. We have demonstrated that dynamic mechanical properties of cartilage are significantly correlated with its thickness. This is consistent with studies on static loading [11] and studies that have investigated changes to knee cartilage ultrastructure and thickness [14]. This is now clarified in the text (page 12, lines 15-18).

6. We do not measure or report the properties of OA cartilage. However, the knee joint cartilage has been shown to demonstrate signs of early onset OA [14], thus, it has often been used as a model for early onset OA. To clarify this point we have amended the title of the relevant discussion section (page 12, line 6).

7. This is the first study to correlate cartilage thickness to its dynamic properties. Given that novel is generally defined as meaning new: the finding is novel. More importantly, it provides a link between an easily measured parameter clinically (cartilage thickness) and dynamic properties. The novelty has been clarified in the conclusion (page 14, lines 22-23).

8. The reviewer has answered his own question: the indenter in contact with cartilage had a diameter of 5.2 mm. Further reference is now made to the two studies in which the procedure used is validated (page 6, lines 4).
9. A flat region was tested, with underlying bone cut parallel to the cartilage surface. Clarification has been added to the manuscript (page 5, lines 15-16).

REVIEWER: Deva Chan

1. The keyword has been changed (page 2, lines 21).

2. Reference is made to our previous publications where the difference has already been explained (page 6, lines 23-24).

3. Reference is made to our previous publications where the difference has already been explained (page 6, lines 23-24).

4. The word has been corrected (page 8, line 5; page 12, line 15).

5. Reference is made to our previous publications where the difference has already been explained (page 6, lines 6-7).

6. We did not measure strength, however, evidence in the literature supports our statement, a relevant reference has been added to the conclusion (page 15, line 4-5).

Minor essential revisions:
1. The abstract has been amended (page 2, line 6 & line 20).

2. We disagree with the reviewer on this point based on our collective experience in publishing over 300 papers and book chapters in the field.

3. We disagree with the reviewer on this point based on our collective experience in publishing over 300 papers and book chapters in the field.

4. Reference is made to our previous publications where further explanation is available (page 5, lines 10).

5. Reference is made to our previous publications where further explanation is available (page 6, lines 23-24).

6. The range is similar to our previous studies to which readers are now referred to in the text (page 5, line 25 to page 6, lines 1).

7. Results at 1 Hz results are consistent with results at other frequencies, it is also not feasible to add 24 additional tables to the manuscript. Discussion on the relevance of medial-lateral comparisons has been added to the manuscript (page 14, lines 8-11 & 17-19).
8. This study compares cartilage from different knee joint regions which was not performed in the previous study. This is already described in the manuscript introduction and has been clarified in the conclusion (page 14, lines 22-23).

9. Discussion has been added (page 12, lines 15-18).

10. Discussion has been added (page 13, lines 4-25).

11. We did not measure strength, however, evidence in the literature supports our statement, a relevant reference has been added (page 12, line 15-16).

Discretionary reviews:
1. See response to Minor-essential revisions #7.

2. We disagree with the reviewer on this point based on our collective experience in publishing over 300 papers and book chapters in the field.

Statistical review:
All three authors, and the three other reviewers, all disagree with Reviewer-Deva Chan.