Author's response to reviews

Title: An Ultrasound Study of Altered Hydration Behaviour of Proteoglycan-degraded Articular Cartilage

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Version: 2 Date: 2 September 2013

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The Biomed Central Editorial Team

Object: MS: 1500130610101120 - An Ultrasound Study of Altered Hydration Behaviour of Proteoglycan-degraded Articular Cartilage. Dr Qing Wang et al.

Thank you for consideration of our manuscript for publication in your journal. The clarification of ethical approval and competing interests and authors’ contributions have been added in page 6 and page 14 of the revised manuscript, respectively.

And thanks for the reviewers’ constructive comments and suggestions. We have revised the above manuscript according to the reviewer’s comments and made responses to the reviewers’ comments as follow.

Reviewer's report

Title: An Ultrasound Study of Altered Hydration Behaviour of Proteoglycan-degraded Articular Cartilage

Version: 1 Date: 18 July 2013

Reviewer: Hairong Zheng

Reviewer’s report:
Minor Essential Revisions
1. Does Trypsin destroy any other components besides PG? Does PG loss or other factors related to the destructed surface of cartilage tissue and the cavities shown in figure 5? I think this should be discussed.

Response: Besides decomposing PG macromolecules, the trypsin enzyme causes minor degeneration of the collagen network (Harris et al 1972). The minor damage in collagen network especially in the superficial layer might increase the evaporation of water during dehydration. However, the effect of minor damage in collagen network on the osmotic stress in the cartilage tissue is not as great as the PGs, because the changes in the collagen network could not be qualitatively measured in polarized light microscopy images (Nieminen et al 2002). In addition, we found that the trypsin treatment might destruct the local chondrocytes to formed the cavities (Figure 5B). However, most PGs were degenerated by the enzyme and thus the effect of the destructed chondrocytes on PG synthesis was slight.

The possible effect of these factors on the hydration behaviour of the trypsin-treated cartilage samples has been discussed in the revised manuscript. Please refer to the 2nd paragraph in page 11.

Harris ED Jr, Parker HG, Radin EL, Krane SM: Effects of proteolytic enzymes on structural
2. In OA, what causes the degeneration of PG? Mechanical injury or chemical environment? I suggest to describe this more clearly.

Response: In natural OA, several factors are involved in the progress of the PG degeneration. First, the abnormal loading on the cartilage matrix can cause quick failure of articular cartilage. The high magnitude of the imposed stresses and the sustained stress peaks may trigger an imbalance of chondrocyte anabolic and catabolic activities, leading to the abnormal metabolism of PG. Without the normal function of chondrocytes, the decomposition of PG is more than the composition of PG, thus the cartilage matrix greatly loses the PGs (Little et al. 1996). Secondly, some enzymes such as metalloproteinase including trypsin and aggrecanase digest the PGs in the cartilage matrix causing the degeneration of PG (Lark et al. 1997). Thirdly, the structural damage of the PGs and collagen fibrils triggers immunological reaction and consequently the degradation of PG is accelerated (Neame and Sandy 1994).

We added the above description in the revised manuscript. Please refer to page 4-5.


3. You mentioned that "The increase of water content, the degeneration of PGs and the damage of collagen network are main characteristics of early OA." Why in the experiment, it was found that the specimens with degeneration of PG corresponding to a less hydration ability?

Response: Thanks for the reviewer’s comment. There is no conflicts between our finding and previous results. As mentioned in the answer to comment2, the natural OA is a quick complicated procedure with many factors involved in. Therefore, in OA, the increase of water content is related to the damage of PG and collagen network as well as synovial fluid (Mow and Hung 2001).

In our experiment, PG is the target to be degenerated by trypsin and the external bathing solution is not changed. After the decomposition of PG, the PG macromolecules become small sections and the noncovalent bonds along PG are
broken. Therefore, the hydration function of PG decreases. For the dehydrated samples, the normal cartilage could recover due to the intact structure and normal distributed composition in the matrix, whereas the PG-degenerated cartilage could not recover due to the loss of PG. We added the above discussion in the revised manuscript. Please refer to the last paragraph in page 10 and the 2nd paragraph page 11.


4. What's the PRF of your ultrasound P/R systme in this study? This may be related to the temporal resolution of your monitoring of the swelling.

Response: The PRF of our ultrasound P/R system was set at 200 Hz.

5. In Fig. 4, the x-label should be "Group".

Response: Done.

Level of interest: An article of importance in its field

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
analyzes the function of proteoglycans on cartilage hydration. The results of this study provide useful information in the biomechanical property of articular cartilage in early osteoarthritis. However, the reviewer has several concerns as follow.

1) Articular cartilage has free water and bound water. Both are related to proteoglycans. Why the authors only discussed free water in hydration? Please provide a clearer discussion on this issue.

Response: Thanks for the reviewer’s comment. Proteoglycans (PGs) are related to the intrinsic water of articular cartilage. Free water depends on osmosis pressure to permeate in or shift out of the cartilage tissue. Bound water is related to noncovalent bond along the PGs and collagen network. We have added a discussion in revised manuscript. Please refer to the first paragraph of Discussion section in page 10.

2) In this study, the authors used the method of exposure-to-air to induce dehydration of the cartilage tissue. The degree of dehydration of the tissue is easily affected by the environment. Please tell how to control the environmental factors such as temperature and humidity.

Response: All the tests were performed at a room temperature of 25°C±1°C and humidity of 65% ± 5%.

Specific minor comments:
3) Page 1, There is inconsistent in the marker of corresponding author

Response: The marker of corresponding author has been added behind the name of the first author. Thanks.

4) Page 4, Line 4 from the bottom: “(Mankin et al., 2000)” should be deleted.

Response: Done.

5) Page 9: “%” should be added behind the values of strains.

Response: “%” has be added behind the values of strains as the reviewer indicates.

6) Page 10, Line 5: “(Gu et al.,1998)” should be deleted.

Response: Done.

7) Font color should be consistent in Figure 1a.
Response: The figure has been revised.

8) Better add the definition of ‘**’
Response: The definition of “***” in Figure 4 has been added in the caption of Figure 4 in page 17. ** in Figure 4 denotes significant difference at \( p < 0.01 \).