Author's response to reviews

Title: Alterations in the vimentin cytoskeleton in response to single impact load in an in vitro model of cartilage damage in the rat

Authors:

Frances M D Henson (fmdh1@cam.ac.uk)
Thea A Vincent (theavincent@hotmail.com)

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Author's response to reviews: see over
Reply to reviewers

We very much welcomed the comments from the reviewers and feel that their criticisms have been used constructively to improve the manuscript.

Referee 1

Major revisions

1. Labelling of figures now corrected.
2. The Maximum Modified Mankin score is 15 because, as shown in Table 1, Structure has a maximum score of 6, cellularity of 4, matrix staining of 4 and tidemark integrity of 1. Totalling 15. There are not, as the referee states, 3 categories with a maximum of 6.
3. Q re: 72 samples This is an error in the manuscript – 84 samples were used (42 rats) – each was cultured in 2 x triplicate (giving 6 sections in total). This has been amended in the manuscript.
4. Q re: processing/fixing. Samples were snap frozen (as stated in the manuscript) and fixed in 4% paraformaldehyde - this has now been included in the manuscript.
5. Q re: link Fig1 and text. The link between the figure and the relevant score has been added in the text.
6. Q re: figure labelling discrepancy. This discrepancy in Figure labelling was due to the uploading system which necessitates each figure to be loaded separately. Figure 2 has now been presented as a composite figure. The scale bars had been lost in the uploading process and are now replaced. The error in scale bars in Figure 4 has been corrected.
7. Q re: Mankin score results. This confusion has been amended in the text.
8. Q re: Figure 3b. This has been amended in the text.
9. Q re: ERK/pERK data has now been added as a new Figure – Figure 6.
10. Q re: proteoglycan loss. The reference to proteoglycan loss has been more fully discussed in the text.
11. Q re: integrin mediated mechanisms. The reference to integrin-mediated mechanisms has been removed and more text included to discuss this result.
12. Q re: nuclear localisation of vimentin – this has been amended in the text.
Minor revisions

1. Q re: Secondary antibodies – these have now been included
2. Q re: t = 0. This has now also been included in the Material and Methods section.
3. Q re: Labelling of axis. The loss of labels from the axis is an uploading problem – they are clearly visible on the figures submitted – hopefully they will be on the re-submitted figures.
4. Q re: Significance markers on Figure 5. These have been added.
5. The manuscript has been read carefully and hopefully all errors removed.

Referee 2

Major revisions

1. Q re: Growth plate. The rats used are young adults. In this species the growth plate never closes and so this we do not understand the relevance of this question as there will always be growth plate in every rat joint.
2. Q re: Vimentin labelling. Our preliminary studies did not show an alteration in vimentin labelling between zones and all zones were included i.e. full thickness cartilage was scored. This scoring was performed blindly. These points have been added to the text. The cartilage sections were 7µm thick (as stated in the text). With regard to the methodology this study was a description of the distribution of vimentin – not a quantitative study of the amount of vimentin. Therefore the cell size is not relevant as the distribution of the stain is clearly graded regardless of cell size. The cells given a score of 3 were evenly distributed through the cartilage and we are not mistaking a small cell with cytoplasmic stain for a large cell with perinuclear stain.
3. Q re: In this study we report that 4 ‘control’ sections had some damage. The Modified Mankin score is an extremely ‘strict’ scoring system, with even a tiny fibrillation on the cartilage surface scoring a 1. This finding of a very low level of damage is considered to represent dissection and handling damage, for example, touching the cartilage surface with forceps to pick up piece of cartilage. If is not of significance in this experiment and does not represent ‘diseased’ cartilage.
4. Q re: proteoglycan loss. This has been amended in the revised manuscript.
5. Q re: Figure legends. These have been made more informative where possible.
6. Q re: Significant differences. These have been added to all the graphs.
7. Q re: Scale bars. Many of these were lost in the Uploading process – they have been put back in.
8. Q re: Text missing. This text has been added.
9. Q re: Significance of the animal model. The reviewer quite rightly states that we must be careful about a comparison between this model and human OA. Indeed, throughout this manuscript we had tried to be very careful NOT to attempt to compare this model with human OA. Our only comparisons have been with other animal models for OA and other rat models of joint disease. Indeed our final paragraph states “In conclusion SIL causes cartilage damage in the rat that is similar to that reported in rat in vivo OA models . . .” and does not mention the human disease. It would be very helpful if the reviewer would clarify where he feels we have made too close a comparison with the human pathology and we will amend the manuscript accordingly.
10. Q re: Rapid clustering. We are disappointed that the reviewer finds the appearance of chondrocyte clusters at an early time point ‘worrying’ – we found it exciting and challenging. We present both histological and quantitative data to back up the observation. We have amended the text to include other possible explanations of how this might be occurring.
11. Q re: We state in our manuscript that we do not see any significant evidence of re-assembly of vimentin through the culture period. However there is a trend towards re-assembly – disassembly peaks at 2 hours and then declines (Figure 5), the p value of t = 2h vs t = 48 hours in p = 0.59, which is not statistically significant at the p<0.05 level and thus not stated as such, but is interesting nevertheless. Therefore we do not think that our work differs from the results of Durrant et al 1999, rather that we cannot prove statistically that is agrees. This has been amended in the manuscript.
12. Q re: We have not included a diagram to show the loading equipment at this stage as we do not feel that this is essential and was not asked for by the other reviewer.
13. Q re: Where OA has been referred to as a disease, this has been replaced with ‘disorder’.
14. Q re; statistical analysis – now in text and removed from legend.