Author's response to reviews

Title: The three-dimensional microanatomy of the chondro-osseous junctional region of the normal human knee joint.

Authors:

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Author’s response to reviews: see over
Dear Editorial Team,

Please find below a point-by-point response to the comments.

Reviewer Cicuttini:

Major Compulsory Revisions
Specific comments:
1. The authors make a case that previous studies have involved human material from advanced OA and animal models. However, it is very unclear how the investigators collected their study samples. We are told that 10 necropsy cases (aged 27-70 yr) and a further 37 cases (51-83 years) were used in this study. The authors then evaluated samples which we are told were all normal. This seems rather odd in view of the generally older age, where the prevalence of knee OA is getting quite high, and the fact that the 37 cases were undergoing arthroplasty. Even if some sections of the cartilage looked normal, OA tends to be a generalized disease so could the findings be due to very early OA?
Response
The wording of the manuscript has been altered in the Background and Methods sections to clarify the exact tissue used. This was young and completely normal so the comments on older or diseased tissue do not apply.

2. It is not clear from the methods section how the samples from individual subjects were used in the analyses. There are no individual data shown, yet a large number of subjects were included. The methods and results sections need more detail. Are the results of one subject only? Which subjects were included or excluded? Are they composite results of all subjects? If so, was there any individual variation?
Response
More detail has been added to the relevant sections to clarify which tissue was used and to which the results apply.

3. What effect might age, gender, possibly BMI have on the results? Could some of the changes simply be a feature of age and/or early OA?
Response
The specific features described in the manuscript related to young totally normal tissues. This means that they are not a feature due to aging or disease.

4. This manuscript needs a clearer description of the actual methodology and analyses so that the data presented can support the conclusions and extensive discussion presented by the authors.
Response
As much detail as possible has been added to the Methods and Results sections. This is an observational study and so the methods are limited.

5. Some consideration of alternative explanation of their results needs to be presented and also potential methodological limitations of the study.
Response
The study records some straightforward observations and attempts to illustrate these in as simple a way as possible. Methodological limitations could depend on the resolution of the photographs but those that we obtained have sufficient detail to support the arguments given.
Reviewer: Quinn

Minor Essential Revisions

1.) Abstract and Results – “virtual islands”: it becomes clear in the course of reading the paper that these are islands of uncalcified cartilage surrounded by a sea of calcified cartilage. At least, that’s what I understood. However, this is not obvious when this phrase is first encountered in the Abstract and Results. It seems for example that the “island” is made of calcified tissue, within a sea of uncalcified cartilage. For example, in the Results, we have the sentence “Chondrocytes adjacent to these prolongations appeared … to be entombed in calcified matrix (virtual islands).” Do you mean “adjacent to” or “within”? Is the island calcified or not (vice versa for the sea)? The way this phrasing is handled throughout the paper is confusing.

Response

The wording of the descriptions of the anatomical structures has been revised to clarify what was observed.

Discretionary Revisions (which the author can choose to ignore)

1.) What do these findings change regarding interpretations of previous data? I was surprised that so little data was reviewed in the Intro to motivate the study. It’s not clear what difference this investigation will make if one does not see the relevant questions clearly in advance.

Response

The observed features were found when the tissues were investigated as part of a larger study. There has been no previously published record of these. We believe they are of importance as a contribution to the general understanding of this important part of joints.

2.) One thing that occurred to me is that solute transport might occur from below the cartilage in addition to from the synovial fluid, as described in the Conclusions. But beyond this, biomechanical effects on transport would be different because the uncalcified protrusions would likely not deform much during tissue loading, since they are surrounded by stiffer calcified tissue. So there would be less fluid flow, and solute transport “from below” would be less influenced by compression-induced fluid flows. Biomechanically, it’s very different, and it highlights the changing depth-dependent biomechanical environment of chondrocytes, related to the depth-dependent function of cartilage (in this case, “interfacial function”). It contrasts very strongly with the articular surface interface, where solute transport occurs in the presence of large matrix deformations.

Response

This could well be true but we did not want to over interpret the biochemical or biomechanical relevance without further specific studies to address these matters.

3.) There are lots of samples used for these studies but no insights emerging from the sampling groups. Were there differences between the necropsy vs arthroplasty samples? Between men and women?

Response

The actual tissue used has been explained more clearly. It is not possible to make these comparisons.
4.) No quantitative data are presented. This would require a lot more work, but this would greatly increase the paper’s likely “longevity” in the literature. What are the characteristic sizes of the protrusions (how long and how wide)? What is the volume fraction of these protrusions? Do these data change between men and women, healthy and diseased tissue, or among different joint locations (okay that’s future work)? There are stereological methods available to measure these things, and if you could attach some numbers to your observations it would be a great help to interpreting the importance and implications of your findings.

Response
Quantitative data was not available from the techniques used here. As much detail as possible was added to the results but this manuscript describes these features for the first time – the rest, as the reviewer says, is future work. Measurements of the proportion of the boundary area between calcified cartilage and bone taken up by uncalcified cartilage contact would be interesting.

5.) Figures 8 and 9 are not easy to interpret. Furthermore, it’s not clear what they add to the study, beyond the illustrations of light micrographs already presented.

Response
The labelling on these figures has been modified for clarity. These figures are an integral part of the study. They are needed to illustrate the contact between uncalcified cartilage prolongations and bone.

With regards to ethical approval of the study:
The tissues were collected and processed over twelve years ago at a time when the University of Manchester Research Ethics Committee allowed a generalised use of such materials (obtained with consent) for histopathological research without approval of specific projects.

Yours sincerely,
Sheena McClure (for all four authors)