Author’s response to reviews

Title: Fibroblast-like synovial cell production of extra domain A fibronectin associates with inflammation in osteoarthritis

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Author’s response to reviews:

Dear reviewers and editor,

Thank you for taking your time to thoroughly read our manuscript. We appreciate the relevant comments and suggestions made by the reviewers. We agree with all suggestions made by the reviewers and have made corrections. We believe the paper has been greatly improved. Thank you!

On behalf of the authors,
Tue W. Kragstrup

Technical Comments: None.

Editor Comments: None.

Reviewer reports:
Mohammed Sharif (Reviewer 1):
The manuscript is generally well written and the data presented clearly.
The only issue I have is that the number of samples used in some experiments are rather small and so difficult to draw any concrete conclusions.
Answer: We acknowledge that the rather small sample size does not mandate strong conclusions and have added this in a new paragraph in the Discussion section page 12 line 8.

The material and methods are well described but the statistical section (lines 10 -14 in page 9) is inadequate. Please explain more fully the statistical methods used in the study.
Answer: We agree and have expanded the Statistics section and highlighted the statistics in the figure legends.

The discussion section is focused but rather concise! Please expand.
Answer: Thank you for the suggestion to expand the Discussion section. We have added an additional paragraph with a discussion of the limitations of this study (page 12 line 8).

Ming Feng Hsueh (Reviewer 2):
The first main concern is that cartilage sample has been mentioned several times in abstract and methods but none of the results were related to cartilage. Please clarify.
Answer: Thanks for identifying these two mistakes. The reason for the mistakes was that cartilage samples were included in a previous version of the manuscript. We thought we had rigorously proof-read our paper for this submission. We apologize for the confusion. We have removed “cartilage” in line 8 of Abstract and in the subheading in the M&M Section.

Last part of the method of immunofluorescence of OA synovium membranes (Page 7 line 24) is duplicated from monolayer culture methods and is incorrect. There are several other similar mistakes.
Answer: We agree. We made appropriate corrections. Thank you.

The authors claimed the tissue was snap frozen but the section was conducted using microtome which is for paraffin embedded tissue. Please clarify.
Answer: Indeed, a cryostat was used. Thank you.

Please clarify the function of polymyxin B for the reader.
Answer: We agree. Polymyxin B is a polypeptide that blocks LPS binding to TLR4. We added this in the M&M, Results and Discussion sections.

TGFβ, TNFα, LPS, and IL-6 were used to treat OA FLS. Why these four molecules? Why these concentrations?
Answer: The three cytokines were chosen because they are well-known cytokines with effector mechanisms implicated in OA pathogenesis. We could have chosen to include more but had to limit our selection because of practical reasons. LPS was used because it is a TLR4 agonist and therefore mimics
TLR signaling also implicated in the OA pathogenesis. The concentrations were chosen based on previous in vitro studies using these molecules. We have included a sentence about the limitations caused by only studying TGFβ, TNFα, LPS, and IL-6 in a new paragraph in the Discussion section page 12 line 8.

Why only TNFα was tested in OA synovium?
Answer: Again, the focus on TNF is because it is a major cytokine in inflammatory diseases and OA and we could have chosen more. We have included a sentence about the limitations caused by only studying TNFα as downstream molecule in a new paragraph in the Discussion section page 12 line 8.

Since TNFα and ED-A fibronectin was stained on different sections, it is hard to say if they are co-localized.
Answer: We agree. We only want to state that ED-A FN and TNF are found in the same areas (co-distributed). We take great care not to conclude that they are co-expressed. The sentence in the Results section is: “The staining of TNFα was mostly located to cells in close proximity to the ED-A fibronectin positive cells but not specifically to the ED-A fibronectin positive cells.”

RAW264.7 macrophages were treated with 100ng/ml LPS as a positive control in all comparisons. Why this concentration?
Answer: The concentration was chosen based on previous in vitro studies with LPS. This concentration will generate a maximum response. It was therefore used as a positive control. We have included a sentence about the limitations caused by only studying one concentration of LPS and ED-A FN in the new paragraph in the Discussion section page 12 line 8.

In figure 5 legend, LPS concentration was, however, 1ng/ml.
Answer: This was a mistake. Thank you very much for identifying this error. It has now been corrected.

Also, the result of positive control, LPS group, varied group by group. Why is that? It seems this model generated unstable results.
Answer: We agree that the results vary in the four experiments. The experiments were done by experienced lab technicians but small variations in the freeze/thaw procedures, cell counting etc might have occurred.

This sentence is vague. Please rephrase. (Page 3, line 9)
Answer: We agree that the sentence was not unambiguous. We have rephrased.

Enzymatic digestion has been mentioned twice. (Page 4 line 21), (Page 5 line 25) (Page 11, line 16). What is the enzyme used?
Answer: In page 4 line 21 and page 11 line 16 we now mention some examples of enzymes capable of digesting fibronectin (plasmin and thermolysin). In page 5 line 25 we have highlighted the enzyme used for degrading the synovial membrane to isolate fibroblasts (collagenase grade II). Thank you for
specifying this.

Please clearly state the goal of the study in abstract and introduction.
Answer: We agree. We have tried to state the aim more clearly at the end of the Introduction section.