Author’s response to reviews

Title: Effects over time of two platelet gel supernatants on growth factor, cytokine and hyaluronan concentrations in normal synovial membrane explants challenged with lipopolysaccharide.

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Author’s response to reviews: see over
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NICHOLAS ROBERT FORSYTH
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Ref: Corrections for manuscript MS: 2866400441567957. “Effects over time of two platelet gel supernatants on growth factor, cytokine and hyaluronan concentrations in normal synovial membrane explants conditioned with lipopolysaccharide.”

Dear doctor Nicholas Robert:

On behalf of the authors, I am sending you the corrected manuscript of the reference. All the changes performed in the new manuscript appear highlighted. I wish to state that this manuscript was revised by Cambridge Proofreading LLC from UK for grammar and spelling.

I hope to have solved all the reviewers concerns and I am open to any additional suggestions that could to improve this manuscript.

Best regards,

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ANSWERS FOR REVIEWER 1.

1. Is the question posed by the authors well defined? YES. However, in contradiction to the 1st line of the Introduction, synovitis is a common manifestation of osteoarthritis.
   Answer (A): Sorry, this mistake was corrected. Please, see line 56.

3a) Data represent a phenomenon rather than a mechanistic response. That is some measurements go up, some go down and others remain the same.
   A: Our methodological approach starts from a mechanistic model point of view in which external environmental forces (i.e. LPS, PRP, etc.) act over the cells (i.e. synovial membrane explants) to produce a determined biological output. However, biologically, it is not always easy to produce a complete disruption between the adequate description of a phenomenon and a mechanistic response because the cells or tissues are always changing their environment in order to adapt to the external stimuli.

3b) There is no clear-cut rationale for why reaching or approaching synovial fluid levels for the response to LPS for the various factors that were measured was a chosen as an endpoint. This is most troublesome for why TNF-α levels in the equine synovial fluid was used as one of the barometers of response.
   A: We wished to investigate how PRP could induce the production/degradation of cytokine/growth factor/hyaluronan in a culture media and to know if the molecular profile of the culture media could be altered to resemble that of normal synovial fluid. However, we also knew that TNF-α is an important cytokine implicated in the environment of synovial fluid in horses with osteoarthritis (see: Kamm JL, Nixon AJ, Witte TH. Cytokine and catabolic enzyme expression in synovium, synovial fluid and articular cartilage of naturally osteoarthritic equine carpi. Equine Vet J 2010, 42(8):693-699.). Consequently, we expected that this cytokine might be affected by PRP in culture media from synovial membrane explants challenged with LPS. However, our results indicated that, at least in this experiment, TNF-α acted more like a regulatory cytokine than a catabolic cytokine. (see lines 309-314 and 375-377).

3c) Why was LPS used as the activation step? The use of LPS as a model activator PRP would appear to have little relevance to osteoarthritis.
However, we used an *in vitro* system of LPS-induced synovitis because our budget precluded the purchase of equine recombinant IL-1.

6) Discussion and Conclusions (NO) Much of the Discussion recapitulates the Results section. The authors should focus on addressing what the significance of these results mean in mechanistic terms.

A: Thank you for your input, several paragraphs of in the discussion section have since been deleted or modified to improve the manuscript and to avoid redundancy. Please, see lines 302-314, 315-320, 321-330, 337-340, etc.

10) Writing. It was difficult to follow the Results section where the data in the Figures were simply recapped in the text.

A: Sorry, the manuscript was revised once more to improve the readability of the manuscript. Particularly attention has been pair to the readability of the results section.

Major Compulsory Revisions: The author’s must more clearly provide a clear-cut rationale for choosing the response indicators PDGF-BB, TGF-β1, TNF-α, IL-4, IL-1ra, HA.

A: We completely agree with you suggestion. Two paragraphs were added to explain the rationale for choosing the molecules evaluated in this study. Please, see lines 302-320.

The points raised in the Discussion regarding IL-1ra should be re-thought.

Anti-IL-1 therapy in several osteoarthritis clinical trials had little success, probably because there was sufficient IL-1ra already in the OA synovial fluid or that IL-1ra just doesn’t work well in osteoarthritis in the context of an inflammatory milieu.

A: Having considered your suggestion, we have added an additional reference. Please, see lines 337-340 and reference 44.

Page 18: 2nd paragraph, lines 1-2. With respect to reference 14, it is recommended that the conclusion drawn in this sentence be toned down.

A: Reference 14 has subsequently been deleted from this phrase and the tone of the phrase has also been changed. Please, see lines 382-383.

Minor: References #9 and #26 are incomplete. Reference #33 is missing the year of publication.

A: Thank you for pointing this out. These oversights have since been corrected.
The manuscript is interesting but unfortunately must undergo some revision before being deemed acceptable for publication. In general the quality of written English is not at an acceptable level for publication and the authors are encouraged to seek assistance with proof-reading in a bid to correct the multiple grammatical errors found throughout the manuscript.

Answer (A): Thank you for your suggestion. This manuscript has since been revised for grammar and clarity by a native English-speaking editor from Cambridge Proofreading LLC in the UK.

Abstract. The conclusion is relatively weak as presented and would benefit from the inclusion of a further rationale supporting the conclusion.

A: Your suggestion has been taken under consideration and the conclusion has subsequently been changed. Please, see lines 50-53 and 411-415.

Introduction. The introduction ends with a hypothesis. It would be valuable to have some insight into the reasoning behind this hypothesis.

A: The last paragraph of the introduction has been changed to clarify the hypotheses of the study; furthermore, an additional reference has been included in this paragraph. Please, see lines 88-95.

Methods. The rationale behind the inclusion of LPS, the role of LPS ‘conditioning’ (not convinced that ‘conditioning’ is the correct word, and the methodology of this are not included. This should be corrected.

A: Please accept my apologies for this methodological omission. We completely agree with your concerns. Bearing in mind this, we have dispensed with the erroneous term “conditioning” in favor of “challenging” or “challenge”. Moreover, we have included a new paragraph detailing the methodology of LPS challenging. The title has also been modified in accordance with these changes to the content of the manuscript. Please see lines 2, 29, 37, 139 and 138-140.

Complete media characteristics should be supplied. High glucose, low glucose, hepes etc etc? No mention is made of L-glutamine? Is this a purposeful omission?

A: Please accept my apologies. This is not a purposeful omission in the manuscript, just a simple oversight on our part. This revised manuscript provides a significantly more comprehensive account of the culture medium used in the study. Please see lines 134-137.

Results. The 2nd paragraph of results; Page 10, ln 189 – 200 should be rewritten with a focus on clustering results.
For instance “Synovial fluid demonstrated highest levels of….whereas low levels were observed in … etc.”

A: In following up on your suggestion, a number of changes have been made to this revised manuscript. Please see lines 193, 199-200, 202, 224-225, 237-238, 250-251, 267-268 and 276-277

A symbol error has crept in from line 266 onwards where p values are indicated.
The results section in general is presented as a statement of facts. The authors should attempt to draw some form of summary before progressing to the discussion. This would help remove a lot of the text from the discussion which is very inward looking and less focussed on the external view (which should be its focus).

A: Some redundant paragraphs have since been deleted from the discussion section. Some important results were recalled in the discussion section following your recommendations.

Figure footnotes. The figure titles should not contain the “mean standard errors [m.s.e]” text. This is repeated in all but plays no role in a title. This should be included in the figure legend. Figures 2, 4, 5, and 6.

A: Thank you for your suggestion. Amendment have been made in accordance with your suggestion. Please, see figure footnotes.

Can the authors provide a clarification in regards to these figures as to why the control values are inconsistent between panels a and b and why the 48hrs and 49hrs values are so different?

A: As you have correctly observed, there is discrepancy in the representative scale of figures A and B for IL-1ra, but not for the rest of the figures. The problem with the IL-1ra figure was due to the fact that IL-1ra was produced at such a high concentration in the culture media of the synovial membrane explant groups at 96 h. In fact, the concentration was so high that it became problematic trying to construct a graph with the same scale, as the difference in the magnitude of the concentration of this cytokine was so much higher between the time points of evaluation. On the other hand, the difference between the values obtained at 48 and 49 h was due to the fact that fresh culture media with the lower PRG supernatant concentrations were added at 49 h when the old culture media was replaced.