Reviewer's report

Title: Effects of concentration and structure on synergistic proteoglycan 4 + hyaluronan cartilage boundary lubrication

Version: 2  Date: 8 January 2015

Reviewer: Eiki Koyama

Reviewer's report:

In this report, Ludwig et al investigated the effect of wild type and chemically modified PRG4 (proteoglycan 4) and HA (hyaluronan) on cartilage boundary lubrication. Osteochondral samples were isolated from the patellofemoral groove of bovine stifle joints and stored at -80°C in PBS until testing. Static and kinetic friction coefficients were measured using a cartilage-cartilage boundary mode friction test. The authors found that the concentration of PRG4 and high MW HA affected their ability to reduce friction similar to that of whole synovial fluid (SF). The authors also found that chemically modified (R/A) PRG4 plus HA was unable to significantly reduce friction. Lastly, the authors reported that fractionated HA (hylan G-F20) + PRG4 failed to provide boundary lubricating ability equivalent to SF. Based on these data, the authors conclude that both PRG4 and HA are necessary for effective friction reduction, comparable to that of whole SH and that a deficiency of PRG4 and/or HA, or alteration(s) in PRG4 structure may be detrimental to SF cartilage boundary lubricating function.

This study has several positive aspects. PRG4 and HA play critical and synergistic roles in synovial joint lubrication. However it is not clear how the concentration and/or structure of both components effects their lubricating function. Therefore, this study is both novel and timely. The findings in this manuscript suggest the cartilage boundary lubricating function of PRG4 and HA are dependent on the concentration of each component and PRG4 structure. The manuscript, however, also has several weaknesses.

(1) Figures. As presented, all figures are nearly unreadable, thus it is very difficult for reviewers and readers to evaluate the data.

(2) Figure 1- μstatistic, Neq for Prg4 at 150 mg/ml appears to be equivalent to that at the concentration of 45 and 450 mg/ml. This is not reflected on the <μkenetic, Neq>. This data needs to be justified/clarified.

(3) The authors tested whether PRG4 protein structure is important for its synergistic action with HA, to reduce friction. This reviewer wonders why the effect of wild type and R/A-treated PRG4 alone on cartilage boundary lubrication ability was not analyzed, to validate the synergism between these two lubricants in this experimental system.

(4) Similarly, the static friction coefficient of Prg4 alone needs to be included to support the authors argument regarding the additive action of PRG4 on hylan
G-F20 lubrication.

(5) Protein purity. The purity of PRG4 from bovine cartilage culture media and (R/A) PRG4 was not presented. A protein gel stained with Commassie blue and/or Western blot should be included in the revised manuscript.

Minor point
(1) In Table 1, the amount of the lubricant sequence of 1A:PRG4 low dose needs to be corrected.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests' below