**Author’s response to reviews**

**Title:** Chitosan in viscosupplementation: in vivo effect on rabbit subchondral bone

**Authors:**

Romain Rieger (romain.rieger@ec-lyon.fr)

Caroline Boulocher (caroline.boulocher@vetagro-sup.fr)

Sema Kaderli (sema.kaderli@gmail.com)

Hoc Thierry (thierry.hoc@ec-lyon.fr)

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**Author’s response to reviews:**

Reviewer reports:

1. Stephan Zeiter (Reviewer #1):

Most of the concerns raised by the reviewers were addressed. Especially the inclusion of the saline only group strengthens the paper.

1.1. The only point which is not yet solved is the contralateral controls. In their answer the authors first basically rise the valid concerns that the outcome/ changes observed in the contralateral group is also dependent on the efficacy of the treatment applied. Wouldn’t with this rationale the "right" comparison be the one to the knee of an unoperated animal (same age/ weight/ gender)? On the other hand the authors "solve" the raised issue themselves by stating that there was no difference between the contralateral knees among the groups suggesting that all knee were analysed. Based on these results they decided to randomly choose legs from all groups to avoid group`s size discrepancies. In the reviewer`s opinion this is appropriate if the "random" group is not different to the contralateral legs of each group since by random selection a bias could have been introduced. Further, these information on the analysis of the contralateral knee among groups and the rationale to choose a random selection out of them has to be added to the manuscript prior to publication.
Authors’ response:

The authors agree that a non-operated group is different from a contralateral group and that inclusion of healthy non-operated rabbits might have provided additional insight about structural changes and strengthen the study. However, the inclusion of a non-operated group (ie. healthy animals) has not been approved for ethical reasons since the objective of the study was to compare treatment effects. Thus, non-operated legs were used to build a non-operated group. This approach has been used in numerous studies [6,43-45].

Before random selection of the contralateral, all the contralateral legs have been assessed by X-ray for osteophyte scoring (Kaderli et al. [32]). This measurement demonstrated that all operated legs had OA induced and that all contralateral legs had no OA induction. In consequence, it has been decided to randomly select contralateral knees from each treated group in order to build the unoperated group.

This aspect of the protocol establishment was added in the discussion section lines 265-273 as following:

“In the present study, OA induction was verified by X-ray osteophyte scoring on all operated knees. The absence of such was also verified on all contralateral knees [32]. In order to avoid group’s size discrepancy, a “Contralateral-group” has been formed with randomly chosen contralateral legs from each treated group. This approach has been used in numerous studies [6,43-45]. Moreover, the same significant changes were found whether the medial or lateral condyles were investigated. As well, when lateral condyles were compared to medial condyles within each group, there were no significant differences within Contralateral-group, Saline-group, HA-group and Hybrid-group. Thus, equilibrated contact-loads on tibial condyles are suggested.”


1.2. The reference to the histological analysis/images should be added to the manuscript

According to the reviewer’s opinion the following sentence has been added in the discussion section line 229:

“The effect on cartilage and especially cartilage histology has also been evaluated and published by Kaderli et al. [32].”

1.3. While reading the revised manuscript another discussion point raised: it is recommended to give the Ostenil for three to five weeks so that it has its appropriate effect. One rationale of the Hybrid HA is to avoid this repeated injections. Therefore, the authors investigate the effect of a single administration of both HA and HA Hybrid in this study and demonstrate that the subchondral bone changes are more pronounced in the HA Hybrid group. The question is now what would be the effect of repeated injections of HA (as recommended) on the subchondral bone? Would the changes in the subchondral bone be more pronounced and similar to HA Hybrid - this may be an interesting point which might be considered for the discussion.

In the method section line 81 it is indicated that 5 intra-articular injection of either HA, Hybrid, or Saline solution were performed as recalled below:

“Intra-articular injections were performed at weeks 1, 2, 3, 4, and 5 post-ACLT after a short time of anaesthesia (40 mg/kg Ketamine 1000® and 80 μg/kg Domitor®) and careful disinfection (Vetedine® soap and solution).”

Consequently, the authors have no data to discuss the effect of a single injection on subchondral bone changes.

2. Teja Guda (Reviewer #2):

The authors have thoroughly addressed the comments raised in review and the manuscript is much enhanced in clarity in this submission.

Authors’ response:

Thank you.