Author's response to reviews

Title: The effect of cartilage and bone density of mushroom-shaped, photooxidized, osteochondral transplants: an experimental study on graft performance in sheep using transplants originating from different species

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Author's response to reviews: see over
Re-submission of manuscript 1646147307062463

Dear Sirs

Herewith we re-submit our manuscript for publication with the title:

The effect of cartilage and bone density of mushroom-shaped, photooxidized, osteochondral transplants: an experimental study on graft performance in sheep using transplants originating from different species

by Anja Waselau et al.

We have found the comments of the reviewer to be very helpful and have tried to accommodate most of his comments as will be outlined in a separate report. For those, that we could not comply with (only few), we kindly ask you to consider our view and arguments.

We sincerely hope that the manuscript can now be considered for publication in your electronic Journal.

In any case, we thank you for your efforts and remain with

Kind regards, sincerely yours
Brigitte von Rechenberg, PD Dr.med.vet., Dipl. ECVS
Head MSRU
Report of changes to meet the reviewers report:

We thank the reviewer for his overall (benevolent) review and also for his comments to improve the manuscript. We tried to adapt most of the changes as outlined below:

**Major compulsory Revisions:**

Page 19/20: The conclusion has been changed and worded differently:

"Differences were found in the performance of photooxidized, mushroom-shaped osteochondral grafts of bovine, ovine, human and equine origin, if grafts were implanted in the medial and lateral femoral condyles of sheep. The superior performance of the equine grafts was attributed to the higher cartilage and subchondral bone density of the grafts compared to the other species, although in the current study differences in density and mechanical properties were not proven by scientific means such as biomechanical testing or density measurements. The slower resorption of the equine grafts during the process of osteointegration and photooxidized cartilage matrix repopulation seemed to be favorable to overall graft survival and performance, at least after 6 months. The newly applied, more rigorous cleansing process with alcohol was not beneficial for graft survival".

In this study, we did not test the mechanical superior characteristics of the equine grafts or the increased density. However, from other studies we know (histology and microCT) that differences exist. In addition, the empirical approach in surgery, where equine cartilage and bone can hardly be penetrated using the same instrumentation (Draenert instruments) compared to all other species, supports our hypothesis. We agree with the reviewer that it should be proven on a more scientific basis to allow the statements, and, therefore, have changed the text accordingly:

Abstract: page 3: Conclusion: Performance of grafts from equine origin was better compared to bovine, ovine and human grafts. The exact reason for this difference was not proven in the current study, but could be related to dif-
ferences in density of cartilage and sub-
chondral bone between species.

Introduction: page 6: The goal of this experi-
ment was i) to study the effect on species dif-
ferences by comparing photooxidized, mushroom-
shaped bovine, human and ovine grafts, in addi-
tion to ii) investigating the effect of a new
cleansing procedure of the photooxidized
grafts.

Page 16: The slower resorption and different
cartilage and bone density of the equine grafts
was held responsible for their improved overall
performance.

Page 19: The integrity of the cartilage matrix
may be due to the relative high density and
strong mechanical properties of equine car-
tilage [35-37]. Although these features were not
measured in the current study, the denser ap-
pearance of the equine bone in histology sec-
tions and the fact that harvesting the plugs
with the same instrumentation resulted in a
much higher resistance to the hollow drill bit
support this assumption on an empirical level.
It could be speculated that equine grafts pre-
pared with the original photooxidation process
would have resulted in even better performance
compared to this study. In the current study,
the main interest was focused on the effect of
the species differences and graft resistance
after implantation, where equine grafts per-
formed best although only grafts with the more
vigorous cleansing procedure were tested.

Statistics:
All tests were run parallel for ANOVA and Kruskall
Wallis test as we routinely do at the time of
original evaluation, but no differences in signifi-
cances were found between the tests. We then pre-
ferred to report the ANOVA results. The Friedman-
test proposed by the reviewer is foreseen for de-
pendent variables, which in our study is not the
case. The values always represent data of one ani-
mal that was killed for the evaluation, and thus,
dependency between the 6 and 12 months results is
not given. If the same animals could have been used
for the 6 and 12 months evaluation, then a test for
repeated measurements would have been appropriate.
Whether parametric or non-parametric test have to
be used can be matter of debate, however, since no
differences were found we kindly ask the editors to leave the statistical evaluation as originally proposed by our group.

Histology:
Bone of the graft/vs. bone of the host: A passus was introduced in the section of the results on page 14:

Creeping substitution of the graft bone by new bone formation from the host was visible in all sections. The new bone could be easily distinguished from the original (dead) graft bone through its woven structure and darker blue color. The original graft exhibited still its original lamellar structure and its lighter blue coloration. Even if the original graft was not replaced, infiltration with osteoblast progenitor cells and deposition of new osteoid at the surface of the trabecula was noticed also in the center of the grafts.

Repopulation of photooxidized cartilage matrix (no cells) with new cells from the subchondral area has been reported in 3 of our earlier studies published in BMC (Akens et al) and Osteoarthritis and Cartilage (BvR et al). With the new digital technology it is sometimes difficult to obtain good histology pictures if color differences are high. The blue matrix and the light blue coloring of the cell contents could not be viewed more clearly, although we tried with several techniques among them toluidine blue, a classic stain for cartilage matrix. Since we normally work with undecalcified bone sections, hematoxylin is not a good staining in contrast to paraffin sections and is therefore rarely used in our laboratory. Nevertheless, the dark blue seam of the surroundings can be seen, which is indicative for new matrix synthesis. The text was adapted accordingly in the legend to Fig.7.

Minor Essential Revisions:

Page 6: The goal of the study was clarified:

The goal of this experiment was i) to study the effect on species differences by comparing photooxidized, mushroom-shaped bovine, human and ovine grafts, in addition to ii) investigating the effect of a new cleansing procedure of the photooxidized grafts.
Page 13: The mistake has been corrected and matched with table 4:

All grafts were still in their original location, but positioning differed between species. Overall, 24 of the total 64 grafts were in excellent position and 40 grafts were sunk slightly into the original defect (tab.4).

Table 5 has been corrected

Labeling of Fig 3 and 5 has been changed as proposed by the reviewer.

The legend for Fig.7 has been adapted (see above).

The amendment in the conclusion has been made according to the reviewers proposal:

The slower resorption of the equine grafts during the process of osteointegration and photooxidized cartilage matrix repopulation seemed to be favorable to overall graft survival and performance, at least after 6 months. The newly applied, more rigorous cleansing process with alcohol was not beneficial for graft survival.

**Discretionary Revisions:**

Cleansing procedure was clarified in the abstract:

... more rigorous cleansing procedure using alcohol during preparation.

Figures in the result section of the abstract were removed.

The scores were given as earlier published. We felt that should not change the scoring system, since then results of the current study could not truly be compared to our earlier publications. We kindly ask to leave as proposed.

The order of table 1, 4 and 5 was adapted.

The legend of table 4 was amended.