Author's response to reviews

Title: High resolution MRI imaging at 9.4 Tesla of the osteochondral unit in a translational model of articular cartilage repair

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Author's response to reviews: see over
We wish to thank the Reviewers, Dr. Marcos L Loreto Sampaio and Dr. Marco Demange, for their careful and thoughtful reviews. We believe that the manuscript has been significantly strengthened as a result of their recommendations.
Reviewer 1 (Marcos I. Loreto Sampaio, MD):

We thank Dr. Sampaio very much for his valuable comments and the helpful review of our manuscript.

Minor Essential Revisions:

I. **Reviewer comment:** Introd, last paragraph – “specific aim to detect recently describe alterations…” These could have been emphasized in the previous paragraphs.

   **Author response:** We thank Dr. Sampaio for this helpful comment. Recently described alterations of the subchondral bone include upward migration of the subchondral bone plate, formation of intralesional osteophytes, and appearance of subchondral bone cysts [1].

   **Author action:** The alterations of the subchondral bone are now described in detail as suggested (lines 73-78).

II. **Reviewer comment:** M&M, last paragraph – please inform who made the analysis, level of experience; please inform all variables to be measured and that will latter appear in “results”

   **Author response:** We also want to thank Dr. Sampaio for this valuable comment. The image analyses were performed by Henning Madry, a senior consultant orthopedic
surgeon and Lars Goebel, a registrar for orthopedic surgery. Images were acquired by Andreas Müller, a biologist with special expertise in high-field MRI, following an established algorithm for image analyses. The orthogonal reconstructions were compiled by Lars Goebel. Measured variables include measuring time, diameter of drill holes and cysts, and intrallesional osteophytes

**Author action:** The last paragraph of the Material and Methods section was adapted as suggested (lines 185-190).

### III. Reviewer comment: Discussion last paragraph – line 365 “bone marrow edema relevant cause of prolonged pain”… please, add reference.

**Author response:** We thank the Reviewer for this important remark. Bone marrow edemas, as diagnosed by magnetic resonance imaging, are a cause of prolonged pain. They are associated with different conditions, e.g. posttraumatic, idiopathic, degenerative, inflammatory, metabolic or ischemic, while the pathogenesis remains unclear.

**Author action:** References by Roemer *et al.* [2], Xu *et al.* [3] and Anagnostakos *et al.* [4] were added to the manuscript (lines 388-389).

### IV. Reviewer comment: Introd paragr 3 line 103 – “here…” Please, what are you referring to? To one of the prior studies? Consider removing this whole paragraph. Some of the information would fit better in the discussion.
Author response: We thank Dr. Sampaio for this suggestion.

Author action: As suggested, we moved the whole paragraph to the Discussion and rephrased parts of it as suggested (lines 372-385).
Reviewer 2 (Marco Demange MD):

We wish to thank Dr. Demange for his overall very helpful and constructive review.

Minor Essential Revisions

I.  **Reviewer comment**: In the method section, it is important to state if the calcified cartilage was properly removed in all cases.


**Author response**: We thank Dr. Demange for this very important remark. In each case utmost care was taken to meticulously remove the calcified cartilage from the subchondral bone, thus allowing for a reproductive surgical procedure [5].

**Author action**: A sentence was added to *Material and methods* pointing out this important fact. Also, these two classical references [6, 7] have been included in the manuscript (lines 119-124).

II.  **Reviewer comment**: A figure demonstrating the bone drilling intervention would be useful as “figure 2” of the following paper Orth P, Goebel L, Wolfram U, et al. Effect

**Author response:** We also thank the Reviewer for this constructive remark. Adding a figure to the manuscript will ease the understanding of the manuscript.

**Author action:** To better illustrate the Pridie drilling procedure, a new figure was added to the manuscript (Figure 1).

III. **Reviewer comment:** In the publication “Orth P, Goebel L, Wolfram U, et al. Effect of subchondral drilling on the microarchitecture of subchondral bone: analysis in a large animal model at 6 months. Am J Sports Med. 2012;40(4):828-836.” The authors observed that Subchondral drilling led to the formation of subchondral bone cysts (63%) and intralesional osteophytes (26%). Please describe if it is the same rate by 9.4 T MRI observation or discuss these data.

**Author response:** We thank Dr. Demange for pointing out these important facts. Indeed, in the microcomputed tomography (µCT) analysis of the subchondral bone, as described by Orth et al. [5], in 12 out of 19 condyles (63 %) subchondral bone cysts were observed while in five defects (26 %) intralesional osteophytes were found. In contrast, by 9.4 T high-field MRI (µMRI) analysis, subchondral bone cysts were detected in 17 out of 38 (45%) condyles and intralesion osteophytes in five out of 38 (13 %) condyles. The different rates between both imaging techniques may in part be caused by different spatial resolution. While voxel size for the µMRI assessment was 120 µm edge length, samples in the µCT were scanned with a resolution of 15 µm.
Especially, the mainly small intralesional osteophytes are then easier to display. *Vice versa*, with µCT only osseous structures are visible depicting the real extend of a subchondral cyst while with µMRI analysis also the lining of the cyst is visible making it difficult to determine the real extent of the cyst.

**Author action:** The data of both µMRI and µCT assessment are now discussed in the manuscript (lines 320-330).

Discretionary Revision

I. **Reviewer comment:** Authors should consider and discuss that this animal model (as well as most animal models for cartilage repair) are acute lesions with very few chronic changes to osteochondral unit before the surgical intervention. Ideally, the cartilage defect model should be sub-acute, as it would be more similar to human problems. It means that having an acute defect, leave it for some weeks, and then performing the cartilage repair (drilling) procedure would be more similar to “real world problems”. Anyway, this is just a comment authors should consider in future researches.

**Author response:** We thank the reviewer for pointing out this very important fact. Certainly, sub-acute focal cartilage defects may be present in a large group of patients presenting at an orthopedic hospital with an onset of symptoms for, most of the time, several weeks or months prior to the initiation of reconstructive surgical treatments.
**Author action:** We have added the valuable suggestion of the reviewer that, in the future, similar studies should be conducted in animal models of subacute cartilage defects and added a supporting reference (lines 398-403) [8].

II. **Reviewer comment:** There is lack of control group, which means, only defect with no cartilage repair (drilling) procedure.

III. **Author response:** We want to thank the Reviewer for this comment. Reviewer II is correct that only defects with drilling procedure were shown. However, the scope of this study was solely laid on descriptive high resolution imaging of the osteochondral unit after Pridie drilling. Thus, we felt that such a control is not needed for a descriptive study. Future studies will quantitatively compare different cartilage repair procedures, as well as untreated full-thickness cartilage defects.

**Author action:** We have added this as a limitation of the study (lines 399-401).

IV. **Reviewer comment:** Microfracture is performed more frequently compared to drilling in a surgical perspective in humans. Authors should discuss the option for drilling. I imagine that reproducibility and standardization of the procedure may have driven this choice. This may also be explained in the main study protocol, as “The samples were part of a study on experimental osteochondral repair in a translational large”

**Author response:** We agree with Reviewer II that microfracturing is more frequently used in clinical routine to treat symptomatic cartilage defects than Pridie drilling during arthroscopic surgery [9]. Indeed, as standardization of the defects created by microfracture is more difficult to achieve than using a drill, we have selected this technique as kindly noted by the reviewer.

**Author action:** We have added a paragraph to the discussion (lines 392-393).

V. Histology evaluation would have been interesting, especially analyzing cartilage repair in comparison to imaging analyses.

**Author response:** We fully agree that histological evaluation of osteochondral repair is important. Of note, we have devoted an entire manuscript to the correlation of histological analysis with the 2D and 3D MOCART systems [10].

**Author action:** We now point out specifically that histological evaluation remains the gold standard for the evaluation of experimental cartilage repair (lines 398-399).
References


