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# PEER-REVIEW REPORT

**Name of journal:** *World Journal of Stem Cells*

**Manuscript NO:** 89107

**Title:** High quality repair of osteochondral defects in rats using the extracellular matrix of antler stem cells

**Provenance and peer review**: Invited manuscript; Externally peer reviewed

**Peer-review model:** Single blind

**Reviewer's code:** 05536533
**Position:** Peer Reviewer
**Academic degree:** MS, PhD
**Professional title:** Academic Research, Assistant Professor, Research Associate
**Reviewer's Country/Territory:** India
**Author's Country/Territory:** China

**Manuscript submission date:** 2023-10-20

**Reviewer chosen by:** AI Technique

**Reviewer accepted review:** 2023-10-23 04:15

**Reviewer performed review:** 2023-10-23 08:46

**Review time:** 4 Hours

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|---|---|
| **Scientific quality** | [ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good<br>[ ] Grade D: Fair [Y] Grade E: Do not publish |
| **Novelty of this manuscript** | [ ] Grade A: Excellent [ ] Grade B: Good [ ] Grade C: Fair<br>[Y] Grade D: No novelty |
| **Creativity or innovation of this manuscript** | [ ] Grade A: Excellent [ ] Grade B: Good [ ] Grade C: Fair<br>[Y] Grade D: No creativity or innovation |

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|---|---|
| **Scientific significance of the conclusion in this manuscript** | [ ] Grade A: Excellent [ ] Grade B: Good [ ] Grade C: Fair [ Y] Grade D: No scientific significance |
| **Language quality** | [ ] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ Y] Grade C: A great deal of language polishing [ ] Grade D: Rejection |
| **Conclusion** | [ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [ Y] Major revision [ ] Rejection |
| **Re-review** | [ Y] Yes [ ] No |
| **Peer-reviewer statements** | Peer-Review: [ Y] Anonymous [ ] Onymous |
| | Conflicts-of-Interest: [ ] Yes [ Y] No |

**SPECIFIC COMMENTS TO AUTHORS**

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**Peer-review model:** Single blind

**Reviewer's code:** 02444834
**Position:** Peer Reviewer
**Academic degree:** PhD

**Professional title:** Professor

**Reviewer's Country/Territory:** United States

**Author's Country/Territory:** China

**Manuscript submission date:** 2023-10-20

**Reviewer chosen by:** Yu-Lu Chen

**Reviewer accepted review:** 2023-11-17 19:12

**Reviewer performed review:** 2023-11-27 22:53

**Review time:** 10 Days and 3 Hours

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| **Scientific quality** | [ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good<br>[ ] Grade D: Fair [ ] Grade E: Do not publish |
| **Novelty of this manuscript** | [ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair<br>[ ] Grade D: No novelty |
| **Creativity or innovation of this manuscript** | [ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair<br>[ ] Grade D: No creativity or innovation |

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| **Language quality** | [ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection |
| **Conclusion** | [ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection |
| **Re-review** | [Y] Yes [ ] No |
| **Peer-reviewer statements** | Peer-Review: [Y] Anonymous [ ] Onymous |
| | Conflicts-of-Interest: [ ] Yes [Y] No |

## SPECIFIC COMMENTS TO AUTHORS

This manuscript describes a study to determine whether decellularized sheets of ECM generated by deer antler stem cells can facilitate osteochondral defect repair by serving as a cell-free scaffold. The significance lies in the introduction of a new xenogeneic ECM cell scaffold. Experimental designs, sample sizes, and methods generally appear to have been sound. Sheets of ECM were grown from rat adipose-derived stem cells (aMSC, allogeneic), deer antler periosteal cells (APC, xenogeneic), and deer antler reserve mesenchymal cells (RMC, xenogeneic). They were characterized with respect to the support of rat bone marrow stromal cell attachment and proliferation and also with respect to their capacity to promote osteochondral chondral defect healing in rats. RMC supported the highest cell attachment and proliferation. The sheets were decellularized and residual DNA, GAG, and total collagen quantified. Collagen and GAG were substantially retained. The decellularized sheets were then packed into osteochondral defects surgically created in the distal femurs of rats. A limitation of the study that should be mentioned is the size of the defect. While 1.4mm is considered to be the critical size of a rat OCD, defects of 2.0mm are commonly used to avoid obfuscation of

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results by spontaneous healing. ICRS scores and histology support the claim of superior defect filling and more seamless lateral integration of regenerated tissue with native cartilage in the RMC group. However, results do not support the claim of almost perfect restoration with articular hyaline cartilage. Saf-O staining suggests the repair tissue was far less rich in GAG than native cartilage in all experimental groups, and collagen immunostaining does not demonstrate an abundance of Col II positive staining in any experimental group. Although the study is interesting and the RMC may indeed hold promise as a cell scaffold for osteochondral tissue repair, the conclusions must be tempered to reflect the actual results. Additional concerns and comments are enumerated below. 1. In the Introduction, "the osteochondral interface" is referred to as a type of tissue. The type of tissue is calcified cartilage. 2. References 1 and 2, cited to support the opening statement, do not address the natural progression of osteochondral defects (see, for example, Knee . 2002 Feb;9(1):7-10. doi: 10.1016/s0968-0160(01)00133-8.). Please check that all cited references are appropriate. 3. While certain scaffolds may circumvent the need for exogenous cells, they do not undoubtedly do so ("scaffolds… can undoubtedly…"). The sentence in question should be revised. 4. The Introduction is a bit too long. It should focus on cell-free ECM scaffolds that support osteochondral tissue regeneration. It should also mention any limitations thereof that antler ECM may address. 5. The last sentence of the Introduction does not make sense. It seems to say that cell-free MSC-ECM will provide an unlimited source of cells. Please clarify. 6. Cells isolated from adipose tissue should not be referred to as MSCs unless they were demonstrated to be capable of differentiating into multiple phenotypes (e.g. bone, fat, cartilage). The same is true for bone marrow-derived cells, which are more accurately termed bone marrow stromal cells. 7. Ascorbate is very unstable and rapidly oxidizes in aqueous systems. Therefore, it is typically replenished daily. Was it? Ascorbic acid 2-phosphate, an oxidation resistant analog of ascorbate, can be used to avoid the need

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for constant replenishment. 8. It would be nice to see the cross-sectional images of the ECM sheets before and after decellularization. 9. Specify the "nucleic acid scavenging solution containing DNA enzyme." Were the sheets treated with RNase? 10. Report the density of bMSC cell seeding in cells/sq. cm. 11. Approximately what area of ECM sheet was pressed into each osteochondral defect? 12. Provide additional details of the in vivo experiment. How were the wounds closed? Were the rats administered any pain medication? Any restrictions on animal activity or food post surgery? If not, so state. 13. Please list the variables that were quantified for statistical analysis. 14. Regarding DNA, the result is either % DNA removed or % of original. As stated, the results should be 1.9%, 2.2%, and 2.8%, respectively, of the original level. DNA removal may have been efficient, but it should be determined whether residual DNA is less than the accepted upper limit of 50 nanograms per milligram dry weight. 15. Correct the sentence presenting residual collagen and GAG contents. Is Table 1 missing? I could not find it. 16. ICRS scores are presented in the Results, but the methodology is not (e.g., how many raters? Were they blinded to the experimental groups?). ICRS scores may have been significantly higher in the RMC group, but only by a slim margin. 17. The Col I and Col II immunostaining is not convincing. For example, there seems to be hardly any brownish staining in the bone to demonstrate Col I. And there is lack of positive Col II staining in the native articular cartilage on either side of the defect, and in the tissue which had filled the defect, regardless of group. Perhaps the antigen retrieval methods were ineffective. I don't see how collagen staining could be quantified from the representative images. And results are overstated with respect to Col II. There were clearly no differences in positive areas among the experimental groups. RMC may have facilitated defect filling, but safranin-O staining is lacking in regenerated tissue, with the exception of a slight amount in the RMC 12W group. There is inadequate evidence of tissue rich in proteoglycan and Col II to support a claim of hyaline-like cartilage

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regeneration. 18. The second sentence of the Discussion is not supported by results, as successfully repaired would require clear demonstration of hyaline-like cartilage regeneration. 19. Regenerated bone is characterized as well-vascularized. Was this vasculature observed? If not, then refrain from claims regarding vascularity. 20. The overall Conclusion is not supported by results and must be tempered. The RMC-ECM facilitated a degree of restoration that was far from "almost perfect." 21. What is meant by "barely detectable immune response?" Was any effort made to characterize or measure the immune response? This claim should only be made if the tissue was examined macroscopically (e.g. osteophyte) and microscopically for signs of an immune response. In particular, the aMSC and BMS (allografts) should be carefully compared to APC and RMC (xenografts).

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# RE-REVIEW REPORT OF REVISED MANUSCRIPT

**Name of journal:** *World Journal of Stem Cells*

**Manuscript NO:** 89107

**Title:** High quality repair of osteochondral defects in rats using the extracellular matrix of antler stem cells

**Provenance and peer review**: Invited manuscript; Externally peer reviewed

**Peer-review model:** Single blind

**Reviewer's code:** 05536533
**Position:** Peer Reviewer
**Academic degree:** MS, PhD
**Professional title:** Academic Research, Assistant Professor, Research Associate
**Reviewer's Country/Territory:** India
**Author's Country/Territory:** China

**Manuscript submission date:** 2023-10-20

**Reviewer chosen by:** Jing-Jie Wang

**Reviewer accepted review:** 2023-12-17 04:05

**Reviewer performed review:** 2023-12-17 04:07

**Review time:** 1 Hour

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| **Scientific quality** | [ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good<br>[ ] Grade D: Fair [ ] Grade E: Do not publish |
| **Language quality** | [Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing<br>[ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection |
| **Conclusion** | [ ] Accept (High priority) [Y] Accept (General priority)<br>[ ] Minor revision [ ] Major revision [ ] Rejection |
| **Peer-reviewer** | Peer-Review: [Y] Anonymous [ ] Onymous |

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| **statements** | Conflicts-of-Interest: [ ] Yes [ Y] No |

**SPECIFIC COMMENTS TO AUTHORS**

All the queries were well explained