Reviewer's report

Title: Effect of hyperbaric oxygen on mesenchymal stem cells for lumbar fusion in vivo

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Reviewer: francesca gori

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In this manuscript the Authors study the effect of hyperbaric oxygen on the osteogenic potential of mesenchymal cells for spine fusion and show that although preconditioned mesenchymal cells survive and give rise to bone, hyperbaric therapy does not affect the osteogenic potential of these cells.

This manuscript implicates a potential role for the effect of hyperbaric oxygen treatment on bone formation.

Clarification of the following points would strengthen the manuscript:

Major Compulsory Revisions

1) The effect of hyperbaric oxygen on the differentiation of alginate-mesenchymal cells in vitro should be shown to further evaluate the timing of osteoblast differentiation.

2) The Authors report that histological analyses of the specimens from alginate-mesenchymal cells plus (not shown) or minus HBO (shown) treatment indicate that new bone formation is present in these grafts. However, to strengthen these findings, mRNA expression or protein levels for type I collagen and osteocalcin should be evaluated as well, on adjacent sections of control and alginate-mesenchymal cells plus or minus treatment grafts.

4) Images from control and alginate-mesenchymal cells plus and minus HBO treatment grafts should be shown for all experiments performed to confirm the Authors’ findings and to allow the readers to compare treatment effects.

Minor Essential Revisions

1) Figures are not very clear. They should be labeled as A, B, C….and described in the Figure Legends.

Discretionary Revisions

1) The Authors show the survivor of the transplanted mesenchymal cells, as assessed by evaluating the PKH67-labeled mesenchymal cells in the grafts. To demonstrate that these cells are alive in the grafts, TUNEL staining could be employed as well to demonstrate lack of dead cells. In addition, cell proliferation could be evaluated as well using the PCNA staining.
**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests