Reviewer’s report

Title: In vitro antibacterial activity of Bioactive Glass S53P4 on multiresistant pathogens causing osteomyelitis and prosthetic joint infection

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Reviewer: Marjan Wouthuyzen-Bakker

Reviewer’s report:

Trinconi Cunha et al performed an in vitro study evaluating the efficacy of bioactive glass on bacterial growth. There is few data in literature about the usefulness and efficacy of bioactive glass in the treatment of osteomyelitis, so in my opinion it provides a nice addition to literature. The paper is clear and easy to read, although I would suggest a native speaker to review and improve the grammar. I do have several questions that I think need to be addressed prior to publication (especially the limitations concerning the study design should be more thoroughly discussed), and suggestions to improve the paper:

1. Gentamicin should be written with an i instead of y throughout the entire manuscript.
2. The abstract: there is double info in the material and method section and result section on the type of bacterial strains used.
3. The abstract: It is a bit confusing speaking about inert glass and glass beads, I guess these are the same? It is better to use the same words.
4. The abstract: I would prefer to delete the sentence about statistical analysis and add a sentence about the methods used (culture in broth, and subsequently study the growth on agar plates).
5. The abstract: the 0.5-0.8 mm is the length of the inert glass? Because now it is written in parenthesis behind the bioactive glass.
6. In general: it is not clear for me why to speak of oxacillin resistant strains instead of methicillin resistant. Now both terms are used throughout the paper.
7. Background: just a general comment; in my knowledge very few osteomyelitis cases are described due to CoNS without any osteosynthetic material present.
8. Background: in my experience bioactive glass is still present in the bone months or even years after insertion, so theoretically this can act as foreign material as well. Do the authors have any information, or are they aware of studies suggesting otherwise? Can the authors comment on this in the discussion section? In addition, can the authors comment on how long the release of antibiotics from the beads is expected compared to the high pH from the glass?
9. Material and method section: where did the authors obtain the 'inert glass?' And how was it processed?

10. Material and method section: 'BAG was blended' ; do the authors mean that it was mixed with the broth? Or was the glass crushed so it dissolved with the broth? If the latter is the case, the results can probably not be extrapolated to clinical practice.

11. Material and method section: only if the pH was above 10 this was considered as suitable for the experiment. How often then did the authors measured a pH that was below this point? This crucial information, and in addition; what pH can be expected to occur in vivo situations? I think this is an important limitation that needs to be discussed in the discussion section.

12. Material and method section: in addition to the previous point, I have the same concern with 'testing 3 different concentrations of BAG'. If these optimal conditions are needed to show the benefit of the glass, how can this be expected to occur in patients? This needs to be explained and discussed.

13. Material and method section: please explain on what clinical ground the 3 to 4 CFU are chosen and if this is comparable to the inoculum we can expect in patients with OM that are debrided.

14. A lot of GN were gentamicin resistant according to Table S2. This probably explains the reduced efficacy of beads in GN-infections. What do the authors think / propose is the explanation of the reduced efficacy of bioactive glass in these GN cases?

15. Maybe I don't fully understand the concept, but please explain why, in the Figures, the CFU in the inert glass group is constant, and does not increase in time.

16. Based on the questions above, I think the discussion section should be improved, addressing several limitations of the study design (in relation to clinical practice). It would also be nice to add one section about the results that have been published about the bioactive glass in clinical studies.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.
No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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Needs some language corrections before being published

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