Detailed response to Reviewers' comments

Reviewer 1 comments

This paper reveals some important research advances in orthopedics at the level of basic science. It is worth emphasizing that recently there has been a fundamental change in the perception of the orthopaedic condition, which is reflected in clinical practice. The authors describe the fundamental aspects of successful laboratory experiments on in vitro infections and expand on the latest evidence related to molecular biology, in vitro research, and orthopaedic artificial intelligence. The article is clear, readable and enlightening.

Response: Thank you for your comments.

Reviewer 2 comments

Comment 1: Several sentences are gray. Please fixt it

Response: Thank you for noticing this technical problem and we have now addressed this issue.

Comment 2: Several subsections, composed of few sentences should be merged to each other for a better readability.

Response: Thank you for your feedback and we have now re-organised the paper as per your request. To be more exact, we have merged the following sub-headings: 'Infection outcomes and administration/training requirements', 'Guidelines, statistical considerations, pictorial presentations and extrapolation of results', and 'Pictorial and graphical presentation of the results is essential'.

Comment 3: The aim of the work should be detailed at the end of the introduction.

Response: Thank you for raising that concern. Please note that the organisation of this paper deviates from the standard/traditional structure of peer reviewed articles due to the fact that it is classified to mini-reviews and therefore there is no introduction on that occasion.

Comment 4. Concerning Molecular biology techniques, I strongly suggest including several general notions of the described techniques and including these reviews as supporting reference which describe with high detail all these techniques PMID:35744711 and https://www.mdpi.com/2073-4441/13/24/3551

Response: We agree, and we have now considered both suggested references.

We added to the text: 'Molecular-biology-based techniques have rapidly evolved in the last years not only in orthopaedic infections but also in other fields such as in diagnosing SARS-CoV-2 and water surveillance industry. In particular, more sophisticated molecular methods including but not limited to clustered regularly interspaced short palindromic repeats, next-generation sequencing and droplet-digital PCR are currently being investigated to contribute to the detection of the above virus. What is more, rapid detection of harmful bacteria in wastewater is achievable when it comes to utilising DNA microarray and sequencing-based methods.'

Comment 5: Concerning AI and in vitro models, which is a very interesting topic, I suggest including more notions on the topic. I also suggest introducing these couple of interesting publications PMID: 37060240 and PMID: 37011281

Response: Thank you for your suggestion, and we have now added the requested information to enhance the AI section.

We added to the text: 'Another example would be application of AI to tumor models to enable monitoring the progression of tumorigenesis in addition to real-time modelling. Furthermore, AI could also aid in controlling the quality of organoids in the field of organogenesis and bioprinting technology.'

Reviewer 3 comments

Comment 1: It is recommended that the authors highlight the "context" of this review in the Introduction Section, i.e., "why" an in vitro laboratory infection research in orthopaedics should be established. Then, they should explain "when" it is appropriate to establish an in vitro laboratory for orthopedic infections. And finally, they should explain "how" to establish an in vitro laboratory for orthopedic infections, and what are the issues to be noted. To form a higher quality review, at least each

section has a combination of major headings and subheadings so that it appears logical and clear, rather than just multiple subheadings throughout.

Response: Thank you for your feedback on our work. Please note that we have now subdivided the whole of the paper into main headings which is in line with the title of this article. In particular, we added the following headings:

'Why and when to proceed with basic science orthopaedic research?', 'How to properly conduct infection orthopeadic research at a basic science level?', and 'Recently introduced techniques – Do they merit consideration?'

Comment 2: The use of picture and chart in the article seem far-fetched.

Response: Please note that the rationale behind presenting a picture and chart was to provide the readers with a visual representation of how biofilms can be successfully depicted and how the work should be presented in a table in an organized manner. Therefore, we have retained the study Figure and Chart.

Comment 3: References do not conform to journal guidelines and the references 4,5,6 are identical.

Response: Thank you for raising that issue and we have now addressed this reference problem.

Revision reviewer comments

This paper reveals some important research advances in orthopedics at the level of basic science. It is worth emphasizing that recently there has been a fundamental change in the perception of the orthopaedic condition, which is reflected in clinical practice. The authors describe the fundamental aspects of successful laboratory experiments on in vitro infections and expand on the latest evidence related to molecular biology, in vitro research, and orthopaedic artificial intelligence. The article is clear, readable and enlightening.

Response: Thank you for your comments.