

Dear Reviewer,

We thank you for your comments and suggestions regarding the manuscript entitled “Comparison of niobium to other radiopacifiers agents in endodontic cements: A systematic review of physicochemical properties”.

We have revised the manuscript according to the reviewers’ concerns.

Yours sincerely,

Corresponding author.

Author’s point-by-point response to Reviewer 2

1. Concern of reviewer: Antimicrobial Analysis (Section 3.7–3.8). Only two studies were included. Could the authors increase the number of cited publications on this topic?

Our response: We thank the reviewer for the valuable corrections and comments. We acknowledge that few included studies directly evaluated antimicrobial activity. As suggested, we have added further discussion in the Discussion section, incorporating relevant literature to better contextualize our findings.

Revised Text – Discussion, lines 560 - 578: “Antimicrobial outcomes were evaluated in only two studies, and activity was more pronounced in functionalized biomaterials (nHA/NAR or BDMDAC), whereas base formulations showed limited effects [9,43]. This limited evidence highlights a critical gap in current research, particularly considering that persistent infection remains one of the main causes of failure in regenerative endodontic procedures.

The variability in antimicrobial performance across studies may be attributed to differences in scaffold composition, degree of functionalization, and testing methodologies, including the microbial strains used and experimental conditions [57-59]. Notably, bioactive and ion-releasing materials alone may not be sufficient to achieve predictable antimicrobial effects, reinforcing the need for targeted functionalization strategies [60].

Furthermore, the lack of standardized microbiological models limits the comparability of results and hinders the establishment of clear conclusions regarding antimicrobial efficacy. From a clinical perspective, scaffold development should not focus solely on regenerative potential but also incorporate antimicrobial properties to help control residual infection within the root canal system [60,61]. Equally important,

however, is prior canal disinfection through appropriate intracanal medication, which remains a critical step for reducing the microbial load before scaffold placement. This combined approach should be emphasized when translating these strategies into clinical practice.”

2. Concern of reviewer: After carefully examining the bibliographic references provided by the authors, it is my professional opinion that the number of references (59) for a review falls short of expectations. A cursory search of the manuscript's title keywords from the past decade reveals a vast array of over 200 articles, medical reports, book chapters, and reviews that could be of relevance to the topic at hand.

Our response: Thank you for this important observation. We would like to clarify that the manuscript includes a total of 64 references, with substantial coverage in both the Introduction and Discussion sections. However, we understand that the perception of bibliographic depth may be influenced by how references are distributed throughout the Discussion. In response, we have carefully revised this section to improve the integration of additional relevant literature, particularly in key areas such as cell behavior and bioactivity. Importantly, these additions were made to enhance contextual support and critical interpretation, while preserving the methodological rigor and avoiding redundancy. We believe these revisions strengthen the scientific depth and overall balance of the manuscript.

Revised Text – Discussion: Please see the changes that have been made throughout the Discussion section.

3. Concern of reviewer: The discussion summarizes multiple materials (MTA, Biodentine, nHA, NAR, PCL-based composites) but lacks clear comparative evaluation. Could the authors show which material systems are most promising overall? What is the best strategy overall?

Our response: We appreciate the reviewer’s comments. We revised the Discussion to provide a clearer comparative evaluation of the included material systems.

Revised Text – Discussion, line 537 - 545: “These physicochemical findings help explain why composite systems, particularly those combining calcium silicate-based bioactive phases with printable polymeric matrices, have emerged as the most promising approaches, as they promote a balance between structural properties, bioactivity, and biological performance [2,11,12,23,33,42]. In contrast, isolated material modifications or

acellular systems, although useful for elucidating specific mechanisms, do not yet demonstrate the same level of integrated regenerative performance [11,35,43]. Thus, the most promising strategy involves the development of multifunctional composite scaffolds that integrate structural support, bioactive ion release, and, when necessary, targeted biological or antimicrobial functionalization.”

4. Concern of reviewer: Can the authors describe the ideal scaffold?

Our response: We appreciate the reviewer’s comments. We have addressed the suggestions and expanded the Discussion by describing the key characteristics of an ideal scaffold for regenerative endodontics.

Revised Text – Discussion, lines 550 – 559: “From a translational perspective, these findings allow outlining the key characteristics of an ideal scaffold for regenerative endodontics. Based on the available evidence, the ideal scaffold for regenerative endodontics should combine an interconnected porous architecture with high biocompatibility, supporting cell infiltration, viability, and differentiation [22,44-47]. It should incorporate bioactive components capable of releasing therapeutic ions to promote mineralization [48-51], while maintaining adequate physicochemical stability and controlled degradation [52-56]. Importantly, the scaffold should also allow functionalization to introduce antimicrobial or bioactive agents [9,43,57–60]. Overall, current evidence supports multifunctional composite scaffolds that integrate structural support, bioactivity, and therapeutic functionality [2,11,22,33,42].”

5. Concern of reviewer: Several minor typographical errors should be corrected throughout.

Line 316: “MTT assays [9,43] (indicated overall biocompatibility...” → missing closing parenthesis.

Line 496 ([2,9,12,23,42,) remove extra comma

Our response: We appreciate the reviewer’s comments. We have implemented the suggestions as requested.

Revised Text – Results, line 289 - 290: “MTT assays [9,43] (indicated overall biocompatibility) of PLA-based scaffolds and naringin-containing formulations, as well as a synergistic effect when naringin was combined with nHA.”

Revised Text – Discussion, lines 484 - 486: “Despite variability in biomaterials and experimental goals, the predominance of hDPSCs aligns with their widespread use as a reference model for pulp regeneration investigations [2,9,12,23,42].”

6. Concern of reviewer: Comments on the Quality of English Language. Regarding the English language, I don't have observed inaccuracies. But I recommended that authors conduct a thorough review to identify and rectify any mistakes prior to final submission.

Our response: Thank you for your comment. The manuscript has been carefully reviewed to ensure clarity, consistency, and accuracy of the English language. Minor adjustments were made to improve readability and maintain a high standard of scientific writing.