Brazilian Dental Science

Subject: [BDS] Editor Decision

Dear Matheus Kury, Marcelo Totti, Thaís Feldens, Marcelo Goulart, Vanessa Cavalli, Fábio Coelho-de-Souza, Maria Carolina Erhardt:

Your submission Longitudinal dentin bond strength evaluation using different application modes of a metalloproteinase inhibitor to Brazilian Dental Science, has been revised and according to reviewers' comments, there are questions to be addressed and/or points to be clarified/corrected.
Please answer the reviewers considerations point-by-point in a separate document and also please make all the corrections in the text highlighted in yellow.

Deadline: 30 days

Thank you for considering Brazilian Dental Science for publishing your research.
We are looking forward the revised version of you manuscript.

Sincerely,

Sergio Eduardo de Paiva Gonçalves EDITOR-IN-CHIEF São Paulo State University (Unesp), Institute of Science and Technology, São José dos Campos, SP

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Reviewer A:
Recommendation: Revisions Required

Questionnaire

Does the manuscript contain new and significant information to justify publication?*

Yes

Does the Abstract (Summary) clearly and accurately describe the content of the article?

Yes

Is the problem significant and concisely stated?

Yes

Are the methods or research design described comprehensively? Is the statistical analysis adequate?

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Yes

Are the interpretations and conclusions justified by the results?

Yes

Is adequate reference made to other work in the field?

Yes

Is the language acceptable?

Yes

Manuscript Structure

Length of article is:*

Adequate

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Number of tables is:

Adequate

Number of figures is:

Adequate

Please state any conflict(s) of interest that you have in relation to the review of this paper (state “none” if this is not applicable).

None.

Rating

Interest*

Good

Quality

Good

Originality

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Good

Overall

Good

Recommendation

Minor Revision

Would you be willing to review a revision of this manuscript?

Yes

Comments

Comments to the Author

The manuscript is well written and addresses a relevant topic for restorative dentistry, particularly concerning strategies for preserving the longevity of adhesive restorations. Although the theme of chlorhexidine (CHX) and its effects on the dentin–resin interface has

been previously explored, the present study proposes a novel approach to its application, which adds originality to the work. However, before the manuscript can be considered for publication, some aspects should be refined and clarified as detailed below.

Comments about the Abstract

The abstract is well written and clearly structured. However, it would be important to specify whether CHX was added to the solution in mass% or vol%.

Comments about the Introduction
General: Well-structured and goes straight to the main point of the study. However, I am leaving some recommendations to enhance the text.
• In the first paragraph, it is recommended to specify which matrix metalloproteinases (MMPs) are most commonly found in dentin, as this is a large family of proteolytic enzymes. Additionally, the introduction should acknowledge the role of cysteine cathepsins, which are also responsible for the degradation of the dentin organic matrix.
• In the second paragraph, it would add depth to mention that one of the strategies investigated in adhesive dentistry to prolong the stability of the hybrid layer is the inhibition of proteolytic enzymes. Moreover, it is important to clarify that chlorhexidine can play two roles: as a protease inhibitor and as a collagen cross-linking agent (concepts that are further explained in the following paragraph).
• Still in the second paragraph (line 8), it would be appropriate to indicate the range of CHX concentrations commonly reported in the literature, as most studies demonstrate effective activity between 0.5 and 5 wt%, whereas concentrations below 0.1% tend to be ineffective.
• In the fourth paragraph, please verify whether the correct terminology is “dental unit waterlines” or “dental unit water lines”, ensuring consistency with the abstract.
• In the fifth paragraph, there is a double space between “the present study”; this should be corrected.

Comments about the Materials and Methods
• Specimen restoration: Please include the approval number of the

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Local Ethics Committee.
o Provide detailed information about the manufacturer of both phosphoric acid and CHX. Indicate whether the CHX solutions were prepared in vol% or wt%, and specify if they were diluted in deionized water.
o What was the rationale for selecting 2% and 5% CHX concentrations?
o Include the irradiance value of the light-curing unit used.
o The description “the roots were sectioned and the pulp chambers were restored with Filtek Z350XT composite resin and Adper Single Bond 2 adhesive resin” could be reordered to reflect the logical procedural sequence, i.e., adhesive application prior to composite resin placement.
• Microtensile bond strength test:
o Explain why only seven specimens per group were tested. Was a power analysis conducted?
o Indicate the number of beams obtained and tested per tooth.
o Correct “1 mm2” to “1 mm².”
o Justify the use of distilled water instead of simulated body fluid as the storage medium.
o Ensure that the statistical analysis is described in the appropriate “Statistical Analysis” section rather than embedded within the test description.
o Include the manufacturer details of the stereomicroscope used.

Scanning Electron Microscopy (SEM):
Add information about the manufacturers of the silicon carbide sandpapers, sodium hypochlorite, and the oven used for specimen drying.

Comments about the Results
The results are well described and clearly presented. However, consider including information regarding possible pre-test failures.

Comments about the Discussion
The overall premise of the study was to evaluate the effects of CHX application methods during the adhesive procedure, based on its proposed ability to inhibit MMPs and thus enhance hybrid layer durability. However, enzymatic activity itself was not assessed, representing an important limitation that should be discussed. The

study could also benefit from additional interface characterization tests, such as nanoleakage, micropermeability and mechanical properties analysis (elastic modulus, nanoindentation of the interface).

First paragraph:
o The fourth line contains a typographical issue with two consecutive dots after “12 months.”
o The statement “These results suggest that CHX, regardless of concentration or application method, does not undermine resin–dentin bond strength over time, corroborating previous findings [11,18] of MMP-inhibitory effects” is not fully supported, as MMP inhibition was not directly evaluated in this study. It would be valuable to discuss whether the 2% CHX concentration might have been too low to exert a measurable effect. Moreover, if CHX was expected to promote enzyme inhibition and collagen cross-linking, higher bond strength values might be anticipated in the CHX-treated groups. If this did not occur, as presented, what would be the justification for its use?

Second paragraph:
o Correct the phrase “aging - a finding” to ensure proper punctuation and spacing.

Third paragraph:
o The statement “In our study, while this protocol reduced immediate bond strength, it also exhibited minimal μTBS reduction over time” could be reconsidered, as low baseline μTBS values might have masked further decreases.

Fourth paragraph:
o Although the SEM observations are valid, the μTBS data do not appear to reflect the described hybrid layer degradation. If degradation of the hybrid layer occurred wouldn’t a reduction in bond strength be expected? Please discuss this apparent discrepancy.

Fifth paragraph:
o Define EDC in full at first mention. Furthermore, it would enrich the discussion to mention other known protease inhibitors and collagen cross-linking agents tested in the literature, such as metallic ions, polyphenols, antibiotics, and flavonoids, and their effects comparing to the study findings.

o Finally, it is important to note that, although the study's design allows for meaningful conclusions regarding bond strength, the lack of direct assessment of enzymatic activity or hybrid layer degradation limits the mechanistic interpretation of the results. Additionally, the absence of degradation over time in both control and CHX-treated groups, despite prolonged immersion, should be addressed. The authors are encouraged to include a clear limitations paragraph, highlighting that the study relied on only one quantitative (μTBS) and one qualitative (SEM) analysis.

In summary, the manuscript presents an interesting and well-conducted study with potential relevance to adhesive dentistry. With the clarifications and adjustments suggested above, the article would be considerably strengthened and more suitable for publication.

Reviewer C:
Recommendation: Decline Submission

Questionnaire

Does the manuscript contain new and significant information to justify publication?*

No

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Yes

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Adequate

Please state any conflict(s) of interest that you have in relation to the review of this paper (state “none” if this is not applicable).

NONE

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Rating

Interest*

Below Average

Quality

Below Average

Originality

Below Average

Overall

Below Average

Recommendation

Reject

Would you be willing to review a revision of this manuscript?

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Yes

Comments

Comments to the Author

Introduction:

Page 2: Correct English verb conjugation:

The null hypothesis tested was

Material and Methods:

Was the study approved by the ethics committee? Please add it to the text.

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Why were the teeth extracted? Please add it to the text.

What is the commercial brand of the 37% phosphoric acid?

What volume of fluid is needed to perform a complete 30-second flush 2% and 5% chlorhexidine from DUWL for 30 s?

Was the volume of chlorhexidine solution applied to the dentin standardized (2%CHX_primer AND 5%CHX_primer GROUPS)? For example, using a micropipette? one application or more?

The protocol for drying etched dentin is to use absorbent paper. Why was the dentin air-dried? Was the dentin kept slightly moist after air-drying?

The adhesive application protocol followed the manufacturer's instructions. However, it's helpful to describe the step-by-step application and photoactivation protocol (time, distance, power density, brand of the photoactivator device).

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What load cell was used for the microtensile test? Add the trademark for the microtensile testing device and the stereomicroscope.

Were there any pre-test failures? Were the pre-test failures added to the statistics?

Discussion

Correct the text: the null hypothesis was partially accepted, because 5% CHX as a primer resulted in lower bond strength at 24 hours when compared to the control group.

What volume of fluid is needed to perform a complete 30-second flush 2% and 5% chlorhexidine from DUWL for 30 s? It's important to consider the disadvantage of using a 30-second 2% chlorhexidine from DUWL flush compared to the control group using a conventional triple syringe flush.

Dear authors

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As we have one analysis in favour and one against the acceptance of your manuscript, the editor decided to allow you to discuss with the reviewers, offering an answer to their comments.

Pay attention to the rules for the rebuttal letter and for the highlighted text.

We are looking forward to hearing from you.

Good luck.
