

Dear authors,

Your manuscript has been reviewed, but prior acceptance there are some revisions that were suggested by the reviewers.

Reviewer's comments:

**Reviewer 1:**

The article entitled "Different conditioning agents provide TGF- $\beta$ 1 release from root dentin: an in vitro study" aimed to evaluate, through ELISA assay, the effectiveness of different conditioning agents in promoting the release of TGF- $\beta$ 1 from root dentin. The authors compared 10% and 17% EDTA, citric acid and the combination of citric acid and ferric chloride.

The manuscript is well written, well structured, and the conclusion is justified by the results. However, several methodological aspects require clarification or correction, as detailed below:

1. Figure 1:

"OUTCOMES ASSESSED, INCLUDE DEPENDENT VARIABLES AND TYPE - Quantification of TGF- $\beta$ 1 in relation to conditioning agents and time"

The authors included "time" as a variable. However, in the manuscript the authors did not mention any time variation. I suggest clarifying this information or correcting the figure.

1. The Discussion section does not address the methodological limitations of the study nor the future research directions, both of which are essential for contextualizing the findings.

The authors state that they used CHX as the irrigant, followed by the immersion of specimens in 220  $\mu$ L of conditioning agents (EDTA, CA, and 10-3 Solution).

- Was the irrigant volume sufficient to immerse the specimen in the solution?
- What is the rationale for using CHX, considering that NaOCl is the gold-standard irrigant for RET according to AAE and ESE guidelines?

- Other limitations included the exposure time and the volume of the conditioning agents used.

The authors should cite future directions, as evaluating stem cell viability after exposure to the conditioning agents and examining the root dentin surface, suggesting the need for new investigations.

1. In the Methods section - Radicular dentin treatment:

The authors justified the methodology by references 11 and 27. Reference 27 refers to the PRILE checklist. I suggest reviewing this information and correcting it if necessary.

**Reviewer 2:**

The aim of this study was to evaluate the release of TGF $\beta$ 1 after the use of different conditioning agents.

Abstract: The result for the group with just CA is missing.

Introduction: It is reported here some systematic reviews on the release of TGF $\beta$ 1 with EDTA and CA. Therefore, the gap in the knowledge is not clear. Is it the use of ferric chloride? If so, this should be made clear to the readers. Also, the authors should better describe what is ferric chloride, what it does and previous research, similarly to what was done for EDTA and CA.

Materials and methods: Well described.

Results: The way that the statistics is presented is confusing. Authors should include here the quantity of TGF $\beta$ 1 for each group in pg/ml. Standard deviation for the 10<sup>-3</sup> group is much higher than the others, any possible explanation?

Discussion: Methodology used should be better discussed. For example, why were the samples immersed and shaken? Why was ferric chloride not used alone? Was a control group (no treatment) evaluated? Why not?