Reviewer’s report

Title: Cyclic compression emerged dual effects on the osteogenic and osteoclastic status of LPS-induced inflammatory human periodontal ligament cells according to loading force

Version: 0 Date: 18 Aug 2019

Reviewer: Tadashi Yamamoto

Reviewer's report:

Mechanosignaling is a powerful mechanism to regulate gene expression and cell differentiation. PDL is frequently subjected to mechanical forces acting on the teeth. PDL cells have a capacity to differentiate into cells that produced mineralized tissue. However, the mechanisms involved are not well understood. The present study utilizes LPS challenge and cyclic stress to clarify appropriate stimulation for osteogenic / osteoblastic differentiation of PDL cells in vitro. The 0-90 kPa cyclic stress would exert critical role in PDL cells for the differentiation, although remains unconfirmed. The data in Fig. 1 and Fig. 2 is not highly original.

In addition, I have the following specific comments:

Title:

Bone homeostasis is solely in vivo phenomenon. The author should replace the term, appropriately represent their in vitro data.

Abstract:

In the conclusion, excess speculations should be downplayed; this manuscript did not demonstrate any data about bone homeostasis.

Background:

1st paragraph: Disorders of bone homeostasis cause bone loss during periodontal inflammation. The author should describe appropriately. Again, this manuscript demonstrated nothing about bone homeostasis, only examined expression patterns of osteogenic and osteoclastic markers.

Abbreviations, such as TNF-, IL-, MMP, should be defined at their first mention.

Methods:
In all experiments, the author showed data from six independent experiments (N=6), however, N is not specified in the methods and figure legends. It is unclear that the author used PDL cells from 6 different patients for each analysis and how many total patients are enrolled in this study. The author needs at least 12 patients for each figure (Fig.1 for MTT and mRNA, Fig.2, 3, 4 for mRNA and protein analysis). This is important to disclose and provide appropriate data/information for each experiment given that there may be considerable heterogeneity among cells from different individuals.

It is not clear how the author defined the time period (1h/ day, 5-days) of cyclic stress. Moreover, there was no useful information about cyclic stress in reference 13. Moreover, reference 14 and 15 were not found in PubMed database. They should describe the information of the pressure booster and add appropriate references.

The author needs to indicate the company and catalogue number of the protein marker, since it is difficult to trim the membrane between 45 kDa and 57 kDa.

Abbreviations, such as COL-1, RUNX2, MMP, should be defined at their first mention.

Results:

The subheading, "Establishment of periodontitis induction model in vitro" is not appropriate. This manuscript only demonstrated LPS-induced inflammation in PDL cells in vitro.

Results included many assumptions (last part of Fig. 2A, Fig. 3B, 3C, and Fig.4A). The indications should be included in discussion.

Abbreviations, such as MCP-1, ALP, should be defined at their first mention.

No description about CTSK and PTHLH protein was found.

Last part of result: Bone resorption was never found in vitro.

Figure-1A:

In the absence of LPS, there should be significant difference of A490 in MTT between 24h, 48h, and 72h. This may cause unclear data of LPS toxicity in PDL cells. 100 μg/ml-LPS should have more toxicity after 72 h of culture.

Figure-3, 4:

In PDL cells with or without 90 kPa cyclic stress and LPS, double-immunocytochemistry of either ALP, RUNX-2, RANKL and F-actin are required to understand the osteoblastic/osteoclastic mechanotransduction in PDL cells.
Discussion and Conclusion:

The author needs to be careful to use the in vivo term; periodontitis and bone homeostasis (bone formation and resorption) thoroughly in the discussion and conclusion, because this manuscript demonstrated nothing about in vivo phenomenon.

**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.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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