21-Aug-2020

Dear Editors,

We would like to thank the reviewers for their thoughtful and constructive comments regarding our manuscript. We have carefully read through the comments from reviewers. To fully address reviewers' questions and comments, we have rewritten and reorganized our manuscript. Revised portions are highlighted in blue in the manuscript. The point-by-point responses to the reviewers' comments and related references are listed as follows.

Reviewer #1:
Specific Comments to Authors
The authors Gan et al. in the following review article entitled: "New Insight into Dental Epithelial Stem Cells: Identification, Regulation, and Function in Tooth Homeostasis and Repair", clearly describe the current knowledge about the niche of dental epithelial stem cells (DESC) of the rodent incisor that generate enamel-producing ameloblasts and other supporting dental epithelial lineages in homeostasis and injury repair during rodent life span cycle. The authors also report a study about the rare formation of human ameloblasts, enamel and enamel-dentin tissue for a potential therapeutic use in human tooth regeneration and tissue engineering. Further progress is needed in this regard. The manuscript is well written and satisfy the all criteria for the publication on World Journal of Stem Cells, except for a minor revision in point 6.

Response: Thank you very much for your affirmation. The issues raised are addressed as follows.

6. Illustrations. The figures 1 and 2 are of good quality and appropriately illustrative of the paper contents, but figure 3 requires a better legend for understanding the differences of the mouse incisor during homeostasis and injury repair. Authors are requested to clarify it in the legend of figure 3.

Response: Thanks for raising this point. We have modified the legend of figure 3 and highlighted in blue at the last page of our manuscript, which will be more clarified for understanding the differences of the mouse incisor during homeostasis and injury repair.

Reviewer #2:
Specific Comments to Authors
The review presents a comprehensive discussion of the main aspect of incisors DESCs biology. The information is given in a somehow too synthetic way - the authors wished to discuss many topics in a brief text, and at times the review sounds like a list of results rather than an organic text. The text contains some imprecise or wrong statements. Overall, the fluidity and interconnection of the paragraphs could be significantly improved.

Response: We appreciate this comment to improve our manuscript. The issues raised are addressed

as follows.

“Tooth enamel, one of the most highly mineralized tissues in the human body,” - enamel is the highest mineralized tissue of the body.

Response: We feel sorry for this mistake. We have corrected as "Tooth enamel, the most highly mineralized tissues in the human body" in the 1st paragraph of "Introduction" section, which is highlighted in blue.

“Fully formed enamel has been detected on polyglycolic acid fiber mesh using dissociated porcine third molar tooth germ cells, suggesting tissue engineering as an alternative strategy to regenerate enamel [9].” - the reference for such a big statement is actually wrong. To date, no evidence of synthetic enamel equivalent to the natural one has been provided.

Response: We feel sorry for this mistake. We have corrected as " Enamel–dentin complex structure has been detected on polyglycolic acid fiber mesh using dissociated porcine third molar tooth germ cells, suggesting tissue engineering as an alternative strategy to regenerate enamel " in the 2st paragraph of "Introduction" section and highlighted it in blue. We have also replaced the reference [9] with the correct one.

"Klein lab has identified dental epithelial stem cells (DESCs) at the proximal end of mouse incisor, in a structure named as labial cervical loop (laCL)." The first report of the cervical loop as DESC was provided not by the Klein Lab, but by Harada et al. 1999 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2164976/).

Response: Thank you for this suggestion. We have modified as " Harada *et al.* have identified DESCs at the proximal end of mouse incisors, in a structure named the labial cervical loop (laCL) " at the 2nd paragraph of "Introduction" section and highlighted it in blue. The related reference (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2164976/) has also been added as [14] and the rest references are renumbered.

The language requires extensive editing; in some situations, it is not possible to understand the meaning of the sentence.
E.g. "General stem cell marker Lgr5 is knocked into a bicistronic message to detect actual stemness in recent years. The expression of Lgr5 is identified as actively cycling stem cells in the SR of cervical loop epithelium,".
The authors should clarify better the difference between the classical model and the newly proposed model of cervical loop cellular organization.
"While the differentiation of SI cells was inhibited when the Jagged1 antibody was neutralized," - Jagged1 was neutralized, not its antibody.
"Hox is another target gene of Bmi1,". Hox is not a single gene, it indicates a family of genes.
"Conditional overexpression of Lef-1 in dental epithelium increased cell proliferation and created a new stem cell compartment in the laCL." - is this activity of Lef-1 Wnt-independent? The authors stated before that Wnt is not active in the cervical loop.

"MiRNAs have a specific function in a distinct compartment of CL because their expression of miRNAs in the liCL, the laCL and the ameloblasts are different through microarray analysis" This sentence is not clear.

"It is thought that stem cells resided in OEE generate TA cells and SI cells of IEE, which include actively proliferative cells and these cells migrate toward the incisal tip, differentiate to pre-ameloblasts, then to functional ameloblasts, which are responsible for enamel formation [18,39]. A recent study reported a new renewal model in which active-cycling IEE cells give rise to both the functional ameloblasts and the surrounding non-ameloblast epithelial cell populations. There seemed only one cell population, the dividing cells of the IEE, which go through self-renewal during homeostasis [17,18]." This section in paragraph 3.1 is really a repetition of what said at the beginning, when discussing the models.

"It has been reported that human tooth germ stem cells could differentiate into epithelial cell types, but not functional cells" Do the authors mean "functional dental epithelial cells"?

Response: We feel sorry for the confusion made in our manuscript. We have carefully checked and rewritten the sentences in our manuscript according to your suggestions. The manuscript has been revised by a native English speaker. The certificate document has also been provided. We wish the revised manuscript could be up to the standard.

1) "General stem cell marker Lgr5 is knocked into a bicistronic message to detect actual stemness in recent years. The expression of Lgr5 is identified as actively cycling stem cells in the SR of cervical loop epithelium," has been changed into " *Lgr5*, a Wnt signaling target gene and stem cell marker, is expressed in the putative stem cell region, the SR region underlying the OEE. These *Lgr5*$^{+}$ cells are identified as slow-cycling stem cells and a subpopulation of Sox2^{+} DESCs. " in 3rd paragraph of section "REGULTORY NETWORK OF DESCs IN MOUSE INCISORS-Genetic regulation".
2) To better address the difference between the classical model and the newly proposed model of cervical loop cellular organization, we have added the section "Difference between the classical and updated models" accordingly and highlighted it in blue in our manuscript.
3) In 2nd paragraph of section "REGULTORY NETWORK OF DESCs IN MOUSE INCISORS-Genetic regulation", "While the differentiation of SI cells was inhibited when the Jagged1 antibody was neutralized," has been changed into " when *Jagged1* was neutralized with specific antibody ".
4) In 4th paragraph of section "REGULTORY NETWORK OF DESCs IN MOUSE INCISORS-Genetic regulation", "Hox is another target gene of Bmi1," has been changed into " *Bmi1* also targets *Hox* genes ".
5) Regarding 4th paragraph of section "REGULTORY NETWORK OF DESCs IN MOUSE INCISORS-Genetic regulation", "Conditional overexpression of *Lef-1* in dental epithelium increased cell proliferation and created a new stem cell compartment in the laCL." The original work only discussed the *Pitx2* and *Sox2* as transcriptional regulator of *Lef-1*, so the involvement of Wnt signaling remains unresolved.
6) In 2nd paragraph of section "REGULTORY NETWORK OF DESCs IN MOUSE INCISORS-Epigenetic regulation", "MiRNAs have a specific function in a distinct

compartment of CL because their expression of miRNAs in the liCL, the laCL and the ameloblasts are different through microarray analysis" has been changed into "Furthermore, microarray analysis unraveled that the distinct expression pattern of miRNAs in different compartments of dental epithelium, including laCL, lingual CL and ameloblasts, suggests the potential role of miRNAs in the self-renewal and differentiation of DESCs ".

7) The repetition part at 2nd paragraph of section "FUNCTION OF DESCs-Role of DESCs during homeostasis" has been removed accordingly.
8) Regarding "It has been reported that human tooth germ stem cells could differentiate into epithelial cell types, but not functional cells" in 2nd paragraph of "FUNCTION OF DESCs- Role of DESCs during injury repair ", the "functional cells" refers to "functional ameloblasts". The manuscript has been modified accordingly and highlighted in blue.

The implications for enamel regeneration in humans - and the strong limitations - should be more precisely addressed.

Response: We appreciate this comment. We have included the implication for enamel regeneration in humans and the strong limitations in conclusion section, which is highlighted in blue. However, considering our emphasis on DESCs, they are not described in details.

Figure 2 is not really clear; the authors should propose an alternative visualization.

Response: Thanks for this suggestion. We have modified Figure 2 for better visualization.

We have carefully read through and addressed the reviewers' comments. We hope our responses could address reviewers' concern and our revised manuscript now is appropriate for publication in *World Journal of Stem Cells*.

Sincerely,

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