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PEER-REVIEW REPORT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 84470

Title: Transplantation of human induced pluripotent stem cell derived keratinocytes accelerates deep degree II burn wound healing in mice by COL7 mediated pro-migratory effects

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03471268
Position: Editorial Board
Academic degree: PhD
Professional title: Associate Professor
Reviewer's Country/Territory: Japan
Author's Country/Territory: China

Manuscript submission date: 2023-03-17

Reviewer chosen by: AI Technique

Reviewer accepted review: 2023-03-18 09:31

Reviewer performed review: 2023-03-29 16:44

Review time: 11 Days and 7 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No novelty

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Creativity or innovation of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No creativity or innovation
Scientific significance of the conclusion in this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No scientific significance
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The authors reported that transplantation of human induced pluripotent stem cell derived keratinocytes accelerates deep degree II burn wound healing in mice and found that COL7A1 can play a role in accelerating wound healing by inhibiting the inflammatory response and promoting keratinocyte proliferation and migration. However, there are some problems with the current version of the manuscript. Major points: 1) Based on bioinformatics database analysis, the authors finally selected COL7A1 for further verification. A. Comparing 3 burn eschar tissue samples and 3 normal skin tissue samples, we all know that COL7A1 is also expressed in dermal fibroblasts, and the selected skin tissue samples include epidermal keratinocytes, dermal fibroblasts and other skin cells, then the expression changes in normal and burned skin tissue do not mean the possible expression differences of keratinocytes, please reconsider the scientific nature of its conclusions. B. The authors downloaded the

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differential expression gene of hiPSCs and hiPSCs derived keratinocytes (hiPSCs-KCs), which can only show that compared with the initial stem cell stage, the gene expression of differentiated keratinocytes is different and whether there is a differential expression analysis between primary keratinocyte versus hiPSCs-KCs, to show that high expression of COL7A1 in hiPSCs-KCs. 2) In this study, only some marker proteins of KC were expressed shown by immunostaining, but no further comparison with primary keratinocytes and functional characterization and identification of hiPSCs-KCs were performed, and the protocol used to induce differentiation of iPSC-derived keratinocytes was too simple, without stating the source of hiPSC and how to evaluate whether the induction was successful. 3) Using C57BL/6 mice as a burn model and transplanting hiPSCs-KC, is immunosuppression used? How to resolve the experimental interference caused by this immune rejection reaction? 4) The authors compared the effect on wound healing at days 3, 7, and 14 after transplantation of hiPSCs-KCs with inhibition of COL7A1, whereas the transient transfection of shRNA expression vector using Lipo3000 in this study could only have a short-term effect, please confirm and show us the efficiency and timeliness of knockdown COL7A1 and suggest additional overexpression experiments. 5) In the mouse burn model section, the authors mention “25 mL of hiPSCs-KCs was injected around the postoperative wounds” only in terms of the same transplantation volume, but do not quantify the specific number of cells transplanted between the different groups. 6) In Figure 4, statistical analysis of the relative size of burn wounds for each group of mice on day 3 is missing. Minor points: 1) Authors need to correct some language corrections, such as spelling mistakes and grammatical errors. For example, in the discussion section, “we used RA and BMP-4 to induce the differentiation of hips into keratinocytes.” “hips” might be hiPSCs. In 2.1 Bioinformatics analysis, “The expression profile datasets of mRNAs related to cerebral ischemia were screened in the GEO database”, what is the connection with cerebral ischemia? 2) The

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authors should show immunofluorescence images at the same magnification for easy comparison, e.g. K14 and the two sets of images below do not match in magnification in Figure 2. 3) In Figure 4, the authors forgot to add charts B and D, and the figure legend" HE staining was performed after burn tissues were paraffin sectioned at 7 and 14 days after hiPSCs-KCs transplantation" contradicts the 5 and 14 days shown in the bar chart.

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PEER-REVIEW REPORT

Name of journal: *World Journal of Stem Cells*

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Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03475330
Position: Peer Reviewer
Academic degree: MD, PhD
Professional title: Professor, Surgeon
Reviewer's Country/Territory: Japan
Author's Country/Territory: China

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Reviewer chosen by: Geng-Long Liu

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Reviewer performed review: 2023-04-10 04:20

Review time: 1 Day

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No novelty

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Creativity or innovation of this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No creativity or innovation
Scientific significance of the conclusion in this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No scientific significance
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Comments to the author The manuscript entitled "Transplantation of human induced pluripotent stem cell derived keratinocytes accelerates deep degree II burn wound healing in mice by COL7A1 mediated pro-migratory effects" was reviewed. The work was carefully carried out. A good aspect of this study is that the authors have demonstrated the importance of COL7A1 and its role in burn wound healing. A weakness of this paper is the lack of objective evaluation. There was no statistical analysis in some parts. We know how difficult this can be, but the authors do not seem to draw a clear role for hiPSCs-KCs. As I spelled out in my review, there are a number of issues that need to be raised and addressed. Why were hiPSCs-KC used instead of mouse iPSCs-KC? In the case of hiPSCs-KC, it is easy to predict stronger rejection compared to mouse cells. Why did the authors choose human cells instead of mouse cells? Where did the authors get the iPS cells from? Results section ・3.4 However,

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skin injury improvement was prevented after knockdown of COL7A1 in hiPSCs-KCs, and the re-epithelialization capacity of the wound was diminished (Figure 4B and Supplemental 1). →Can the authors show these results in Figure 4B and Supplementary 1? Indicate by star or arrow etc. ・3.5 Furthermore, cell experiments also confirmed this result, and COL7A1 knockdown significantly inhibited the EdU fluorescence intensity in hiPSCs-KCs (Figure 5B). → How did we evaluate them? I recommend the authors to prepare the result of the statistical analysis. Also, which cells were positive for EdU fluorescence? ・3.6 In short, the above results confirmed that hiPSCs-KC transplantation promoted the proliferation and migration of keratinocytes toward the wound site around skin wounds in mice, which in turn accelerated the epithelialization process and wound healing. → I think it has to be spiculation. Which cells, hiPSCs-KC or mouse native KC, promoted the proliferation and/or migration of keratinocytes? What happened to the hiPSCs-KC after transplantation? Did they disappear? CFSE should be difficult to drace if the cells have a high proliferative capacity. Figures ・Figure 2 Was the K14 expression less? The K14-positive cells appear to be much less than the involucin and/or loricrin-positive cells. Figure 3 Which part is the burn wound tissue in the sections in Fig. 3C? Please prepare the normal tissue in Fig.3C. Which is the normal tissue in Fig.3D? Figure 4 The day 14 panels should be replaced. I cannot see properly because of the darkness. Which is the epidermis in the picture? Please be consistent in all photographs. The same in Fig. 1. Figure 5 Can the authors show human markers (HLA, etc.) by staining at the graft site?

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 84470

Title: Transplantation of human induced pluripotent stem cell derived keratinocytes accelerates deep degree II burn wound healing in mice by COL7 mediated pro-migratory effects

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03475330
Position: Peer Reviewer
Academic degree: MD, PhD
Professional title: Professor, Surgeon
Reviewer's Country/Territory: Japan
Author's Country/Territory: China

Manuscript submission date: 2023-03-17
Reviewer chosen by: Li Li
Reviewer accepted review: 2023-05-16 07:58
Reviewer performed review: 2023-05-16 09:11
Review time: 1 Hour

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The manuscript entitled " Transplantation of human induced pluripotent stem cell derived keratinocytes accelerates deep degree II burn wound healing in mice by COL7A1 mediated pro-migratory effects" was re-reviewed. Unfortunately, the responses in the manuscript are not adequate. It is not clear why human hiPSC-KCs rather than mice were used in this study. We, the reviewers, are aware of what the authors describe as an answer. Was the rejection suppressed by FTY720 alone? The use of immunosuppressive drugs also significantly delays tissue repair. Is tissue repair not delayed? Similar to answer 2, we know that EdU-positive cells are proliferating cells. Which of these cells, i.e. which cell type, was EdU-positive? Mouse fibroblasts? hiPSC-KCs or native mouse cells involved in tissue repair? Differentiated from hiPSC-KCs? Without a clear understanding of this mechanism, it may be difficult to extrapolate to humans. As humans do not have the same proliferative capacity as mice, it would be difficult to extrapolate to humans if only hiPSC-KCs are involved in tissue repair.

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

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Reviewer's code: 03471268
Position: Editorial Board
Academic degree: PhD
Professional title: Associate Professor
Reviewer's Country/Territory: Japan
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Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The author has mostly revised the corresponding questions, which has been greatly improved compared with the previous version, but by screening the GSE140926 data set, the skin samples are mainly keratinocytes of the epidermis and fibroblasts of the dermis, differentially expressed genes (DEGs) in skin tissue (not the epidermis) do not mean differences in keratinocytes, whether this bioinformatics prediction is scientific; in addition, the author replied: "COL7A1 was downregulated in human deep second degree burn tissue and strongly expressed in hiPSCs KCs cells." Does this so-called strong expression have a corresponding reference cell to compare, so that the comparison can reflect the corresponding scientific significance, is it with normal physiological keratinocytes or other cells?