Dear Editor,

Thanks for your letter. We highly appreciate the comments raised by the reviewers and revised our manuscript according to the comments you provided. The amendments were highlighted in red in the revised manuscript. Point to point responses were listed below. We would like to resubmit our manuscript for your consideration.

Thanks again.

Yours sincerely,

Ke Li
E-mail: likefggf@163.com

Reviewer #1:

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

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, 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?

1 Why were hiPSCs-KC used instead of mouse iPSCs-KC?

Response: Response: Thank you very much for your reminder! Previous studies have shown that induced pluripotent stem cells are pluripotent stem cells prepared from adult stem cells in recent years, with similar pluripotent differentiation potential to embryonic stem cells, capable of differentiating into any type of cell, and without immunogenicity or ethical controversies. There have been many studies on the transplantation of human induced pluripotent stem cells in mice, and the results are significant. Ishida et al.[1] showed that myocardial cell transplantation derived from human induced pluripotent stem cells is superior to myocardial cells derived from adult stem cells in restoring cardiac function and oxygen consumption in the treatment of porcine myocardial infarction. Templin et al.[2] used computer tomography to track human induced pluripotent stem cells transplanted into a rat myocardial infarction model, and the results showed that the survival, implantation, and distribution of cells achieved the expected results within 15 weeks after transplantation. Chen et al.[3] confirmed the ability of iPSC derived supraauricular dermal progenitor cells to differentiate into hair cell like cells with hair cell characteristics. The transplanted supraauricular dermal progenitor cells can migrate to the organ of Corti in mice, and the differentiated cells can form synaptic connections with natural SGN. In addition, the use of induced pluripotent stem cells derived from humans rather than mice is a trend in future research, which can provide possibilities for future clinical research. However, as mentioned by the reviewer, rejection reactions are highly likely to occur. Therefore, in order to prevent immune rejection, we used immunosuppressive agent FTY720 (3 mg/Kg/d) to suppress the immune response of mice, in order to reduce the immune rejection of mice to human induced pluripotent stem cells.

[1] ISHIDA M, MIYAGAWA S, SAITO A, et al. Transplantation of Human-induced Pluripotent Stem Cell-derived Cardiomyocytes Is Superior to Somatic Stem Cell Therapy for Restoring Cardiac Function and Oxygen Consumption in a Porcine Model of Myocardial Infarction [J]. Transplantation, 2019, 103(2): 291-298.

[2] TEMPLIN C, VOLKMANN J, EMMERT M Y, et al. Increased Proangiogenic Activity of Mobilized CD34+ Progenitor Cells of Patients With Acute ST-Segment-Elevation Myocardial Infarction: Role of Differential MicroRNA-378 Expression [J]. Arterioscler Thromb Vasc Biol, 2017, 37(2): 341-349.

[3] CHEN J, HONG F, ZHANG C, et al. Differentiation and transplantation of human induced pluripotent stem cell-derived otic epithelial progenitors in mouse cochlea [J]. Stem Cell Res Ther, 2018, 9(1): 230.

3.4 However, 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.
Response: We are very grateful to the suggestions raised by the reviewers. We have marked with arrows in Figure 4B (modified to Figure 4E) and Supplementary 1.

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?
Response: Thank you very much for your question! Figure 5B shows the statistical analysis of the percentage of EdU fluorescent cells. Cells that are positive for EdU fluorescence are proliferative and active. EdU is a thymidine nucleoside analogue that can replace thymine (T) to infiltrate replicating DNA molecules during cell proliferation, and then detect cell proliferation using immunofluorescence technology. This is a novel non-radioactive isotope cell proliferation detection method that has been widely used in cell culture, solid tissue, and clinical research. This technology can be used for cell imaging, flow cytometry, cell tracing, DNA damage repair detection, mitochondrial activity detection, and virus proliferation activity detection. Compared with traditional immunofluorescence staining (BrdU) detection methods, EdU detection method is simpler, faster, and more accurate, and does not require strict sample denaturation (acid hydrolysis, pyrolysis, enzyme hydrolysis) treatment. It effectively avoids sample damage and helps to observe the true situation of cell proliferation at the overall level of tissues and organs, with higher sensitivity and faster detection speed.

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.
Response: Thank you for your question! Firstly, we apologize for the error in our description. The results of this study indicate that hiPSCs-KC transplantation can migrate and rapidly proliferate to the wound site around mouse skin wounds, thereby accelerating the epithelial formation process and wound healing. In fact, after we transplanted hiPSCs-KC around the wound, hiPSCs-KC was able to migrate to the wound and rapidly proliferate, while also promoting the original keratinocytes around the mouse wound. After labeling hiPSCs-KC with CFSE, the migration process of hiPSCs-KC after transplantation into mice can be observed, indicating that hiPSCs KC can migrate to mouse skin wounds and participate in the epithelial process after transplantation. Although hiPSCs-KC has high proliferative capacity after transplantation, we only need to observe whether hiPSCs-KC migrates to the wound site during transplantation to assist in wound healing.

Figure 2 Was the K14 expression less? The K14-positive cells appear to be much less than the involucin and/or loricrin-positive cells.

Response: Thank you for your friendly question! In fact, the number of K14 positive cells is consistent with the strong positive expression of insulin and loricin, as can be seen from nuclear staining. However, this misunderstanding was caused by the different multiples of the immunofluorescence images we displayed. I'm very sorry! We have revised the immunofluorescence image multiples in the revised manuscript for comparison purposes.

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?

Response: Thank you for your friendly reminder! The burn wound tissue in Figure 3C is the whole epidermis and deep dermis structure, and the two panels are different magnification images of the same site. The normal tissue of Fig. 3D was the normal skin around the burn wound. We adapted the image of day 14 of Figure 4 to make it clearer. The black portion of the picture is the black fur of the mice, and since we used C57BL/6 Black mice. We knocked out hair on the back of the mice before the experiment started, however the mice re grew black hair by the time the experiment progressed to 14 days so that it resulted in an overall darkening of the background of the picture. We strongly agree with the reviewer's viewpoint that supplementing the HE stained normal control tissue in Figure 3C can serve as a reference for HE staining of the burn area, and staining at the transplant site to display human biomarkers (HLA, etc.) may better reflect the distribution of transplanted hiPSCs-KC in mice. However, the mice and tissues used in the experiment have been uniformly treated. Therefore, if additional experiments are to be conducted, the experimental process must be restarted, which is limited by time and funding. Nevertheless, we are still very willing to supplement the above experimental content with sufficient time in the future. If you believe that the supplementary experiment is indispensable, please give us sufficient time by the reviewer to complete it.

We would like to thank the referee again for taking the time to review our manuscript.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

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 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 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.

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.

Response: Thank you very much for your reminder. This study screened differentially expressed genes (DEGs) from the GSE140926 (containing 3 normal skin tissue samples and 3 burn eschar tissue samples) and GSE27186 (2 hiPSC samples and 2 hiPSC-KCs samples) datasets. By screening the GSE140926 dataset, differential genes before and after burns can be identified in cells including epidermal keratinocytes, dermal fibroblasts, and other skin cells. Indeed, as reminded by the reviewer, the expression changes in normal and burned skin tissues do not imply the existence of differentially expressed genes in keratinocytes. However, keratinocytes account for over 80% of epidermal cells and are the main constituent cells of the epidermis. Therefore, we speculate that the selected DEGs have a high probability of being present in keratinocytes. The first part of bioinformatics screening only played a predictive role, and later we conducted cell and animal experiments to further validate the bioinformatics results.

The authors downloaded the 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.

Response: Thank you for your comment. We strongly agree with your statement that the differentially expressed genes in hiPSC and hiPSC-KCs (KCs derived from hiPSC differentiation) obtained through screening the GSE140926 dataset only indicate that the gene expression in the differentiated keratinocyte nucleus is different compared to the initial stage of stem cells. In subsequent experiments, we induced human induced pluripotent stem cells to differentiate into keratinocytes using inducers, which is consistent with the pattern analyzed by bioinformatics. Bioinformatics analysis only played a predictive role and provided us with ideas for future research. Subsequently, we demonstrated that COL7A1 was downregulated in human deep second degree burn tissue and strongly expressed in hiPSCs KCs cells.

3 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.

Response: Thank you very much for your question! In recent years, researchers have focused on finding effective means to induce the differentiation of iPS cells into a single keratinocyte population. Through literature review, we found that Howard Green injected human embryonic stem cells into SCID mice for 2 months in 2003 to form a mass containing epithelial cells. The colonies formed after digestion and culture have the morphological characteristics of keratinocytes and express p63, basic nuclear protein and K14. Continued culture eventually leads to terminal differentiation and the expression of its marker, invitricin. The expression sequence of marker genes during the differentiation

of embryonic stem cells into keratinocytes has been revealed for the first time. Since then, studies that successfully and efficiently differentiated ESCs into keratinocytes have successively been published. Based on embryonic stem cell research, bilousova et al.[1] used hanging drop culture to culture mouse iPS cells to form embryoid bodies, which were induced by adding retinoic acid and BMP4 during differentiation, and used the characteristic that keratinocytes can rapidly attach to collagen IV to finally obtain 80-90% K14 + keratinocytes. The potent differentiation of human iPS cells into K14 + / p63 + keratinocytes under the induction of RA alone was proposed in 2013 by Joshua et al. [2], and has taken iPS cells a big step forward in the application progress of skin regeneration and repair, but also expanded the use of keratinocytes in tissue engineering. Based on the above reports, this study references numerous literatures using RA and BMP-4 to induce iPS differentiation into keratinocytes. Subsequently, immunofluorescence staining was used to identify marker proteins of keratinocytes, including K14, involucrin, and loricrin. K14, involucrin and loricrin are specific marker proteins of keratinocytes, and all three showed strong positivity indicating that we induced differentiated keratinocytes successfully. However, as the reviewers mentioned, the protocol used to induce the differentiation of iPSC derived keratinocytes is perhaps too simple. Our later studies will focus on optimizing the induction protocol of hiPSC in depth to improve precision and efficiency. In addition, we strongly agree with the reviewers that further comparison with primary keratinocytes may allow a better characterization of hiPSC-KCs. Human induced pluripotent stem cells used in this study were purchased from Beijing saibe Biotechnology Co., Ltd. However, primary keratinocytes were not purchased or cultured in our laboratory. Therefore, this is limited by time and funding if additional experiments are to be performed. Nevertheless, we are still very willing to have enough time to supplement the above experimental content in the future. If you believe that the supplementary experiment is indispensable, please give us sufficient time by the reviewer to complete it.

[1] Bilousova G, Roop D R. Generation of functional multipotent keratinocytes from mouse induced pluripotent stem cells[J]. Methods Mol Biol, 2013, 961: 337-50.

[2] Selekman J A, Grundl N J, Kolz J M, et al. Efficient generation of functional epithelial and epidermal cells from human pluripotent stem cells under defined conditions[J]. Tissue Eng Part C Methods, 2013, 19(12): 949-60.

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?

Response: Thank you very much for your reminder! Previous studies have shown that induced pluripotent stem cells are pluripotent stem cells prepared from adult stem cells in recent years, with similar pluripotent differentiation potential to embryonic stem cells, capable of differentiating into any type of cell, and without immunogenicity or ethical controversies. There have been many studies on the transplantation of human induced pluripotent stem cells in mice, and the results are significant. Ishida et al.[1] showed that myocardial cell transplantation derived from human induced pluripotent stem cells is superior to myocardial cells derived from adult stem cells in restoring cardiac function and

oxygen consumption in the treatment of porcine myocardial infarction. Templin et al.[2] used computer tomography to track human induced pluripotent stem cells transplanted into a rat myocardial infarction model, and the results showed that the survival, implantation, and distribution of cells achieved the expected results within 15 weeks after transplantation. Chen et al.[3] confirmed the ability of iPSC derived supraauricular dermal progenitor cells to differentiate into hair cell like cells with hair cell characteristics. The transplanted supraauricular dermal progenitor cells can migrate to the organ of Corti in mice, and the differentiated cells can form synaptic connections with natural SGN. In addition, the use of induced pluripotent stem cells derived from humans rather than mice is a trend in future research, which can provide possibilities for future clinical research. However, as mentioned by the reviewer, rejection reactions are highly likely to occur. Therefore, in order to prevent immune rejection, we used immunosuppressive agent FTY720 (3 mg/Kg/d) to suppress the immune response of mice, in order to reduce the immune rejection of mice to human induced pluripotent stem cells.

[1] ISHIDA M, MIYAGAWA S, SAITO A, et al. Transplantation of Human-induced Pluripotent Stem Cell-derived Cardiomyocytes Is Superior to Somatic Stem Cell Therapy for Restoring Cardiac Function and Oxygen Consumption in a Porcine Model of Myocardial Infarction [J]. Transplantation, 2019, 103(2): 291-298.

[2] TEMPLIN C, VOLKMANN J, EMMERT M Y, et al. Increased Proangiogenic Activity of Mobilized CD34+ Progenitor Cells of Patients With Acute ST-Segment-Elevation Myocardial Infarction: Role of Differential MicroRNA-378 Expression [J]. Arterioscler Thromb Vasc Biol, 2017, 37(2): 341-349.

[3] CHEN J, HONG F, ZHANG C, et al. Differentiation and transplantation of human induced pluripotent stem cell-derived otic epithelial progenitors in mouse cochlea [J]. Stem Cell Res Ther, 2018, 9(1): 230.

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.

Response: Thank you for your friendly suggestion! We stably transfected the COL7A1 shRNA vector into hiPSCs KCs using Lipo3000. In addition, in order to obtain stable transfected hiPSCs KCs. In the experiment, a suitable concentration of purinomycin was added for stable transfection cell screening, and finally, the screening of all cell death markers in the blank well was completed. Based on your suggestion, we have supplemented the experiment to demonstrate the efficiency of knockdown and overexpression of COL7A1. Firstly, we evaluated the transfection efficiency of COL7A1 shRNA before and after hiPSCs-KCs transplantation. Figure 4A showed that the mRNA level of COL7A1 was decreased nearly 5-fold after transfection of hiPSCs-KCs with COL7A1 shRNA. Interestingly, we also constructed the COL7A1 overexpression vector to show the overexpression efficiency of COL7A1 to provide a reference for our experimental manipulation. The results suggested that the overexpression efficiency of COL7A1 was

equally good. In addition, significant upregulation of COL7A1 expression was observed after transplantation of hiPSCs-KCs around mouse wounds, while transplantation of hiPSCs-KCs with COL7A1 knockdown resulted in a decrease in COL7A1 expression. The above prompts that the efficiency of knocking down COL7A1 is good and can be used for subsequent 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.

Response: Very nice questions, thank you for the reminder. We supplemented the specific number of cells transplanted in the revised manuscript. 25 mL (2.0×10^7 cells) of hiPSCs-KCs was injected around the postoperative wounds.

6) In Figure 4, statistical analysis of the relative size of burn wounds for each group of mice on day 3 is missing.

Response: Thank you for your friendly reminder. We supplemented the statistical analysis of the relative size of the burn wound in each group of mice on day 3 in Fig. 4B.

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?

Response: Sorry for the silly mistakes and thanks for the kindly reminding. We review all spelling and grammar throughout the manuscript. We corrected the above errors in our revised manuscript.

2) The 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.

Response: We are so sorry for carelessness. We modified the magnification of immunofluorescence images to be consistent according to your suggestion.

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.

Response: Sorry for the silly mistakes and thanks for the kindly reminding. We added the annotations of Figures 4B and 4D and corrected the 5 days shown in the Figure 4D (Figure 4F) bar graph to 7 days.

We would like to thank the referee again for taking the time to review our manuscript.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

Round 2

Dear Editor,

Thanks for your letter. We highly appreciate the comments raised by the reviewers and revised our manuscript according to the comments you provided. The amendments were highlighted in red in the revised manuscript. Point to point responses were listed below. We would like to resubmit our manuscript for your consideration.

Thanks again.

Yours sincerely,

Ke Li

E-mail: likefggf@163.com

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?

Response: Thank you for your friendly question! Although the skin samples were mainly keratinocytes in the epidermis and fibroblasts in the dermis, the differentially expressed genes (DEGs) in the skin tissue (but not the epidermis) did not imply a difference in keratinocytes, the proportion of keratinocytes was much higher than that of fibroblasts. To reassure the reviewer, we measured the expression level of COL7A1 using immunofluorescence staining in hiPSCs and fibroblasts, and the results are presented in Figure 3E. The results showed that COL7A1 was highly expressed only in hiPSCs KCs, but hardly expressed in hiPSCs and fibroblasts. Our findings further confirmed the results obtained by bioinformatics analysis. We would like to thank the referee again for taking the time to review our manuscript.

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.

Response: Thank you for your friendly question! In fact, the main reason why we used human hiPSC-KCs instead of mice was to understand the mechanism of human hiPSC-KCs transplantation on tissue repair for the subsequent generalization to humans. However, there are a number of study limitations, as mentioned by the reviewer. Therefore, double immunofluorescence staining with EdU and the human marker HLA was performed to determine the type of proliferating cells, and the results are shown in Figure 5E. The results showed that the number of proliferation cells were increased significantly after transplantation of hiPSCs-KCs, and most of them overlapped with the human marker HLA, suggesting that most of the proliferating cells were hiPSCs-KCs. In addition, there were still a small number of active proliferating cells that did not coincide with HLA, indicating that these cells were natural mouse cells involved in tissue repair. Therefore, our results suggested that hiPSCs-KCs proliferates actively after human hiPSCs-KCs transplantation to the burn site of mice and play a major role in tissue repair, while a small number of mouse natural fibroblasts are activated after hiPSCs-KCs transplantation and play an assisting role in tissue repair. We used FTY720 injection alone to suppress immune rejection in mice, based on experiments performed with reference to the literature. FTY720 is a newly developed immunosuppressant in recent years. FTY720 selectively reduces the number of peripheral circulating lymphocytes and significantly prolongs the survival of transplanted organs in experimental animals. It does not damage the immune response and immune memory function of the body, and has low side effects. The immunosuppressive effect of FTY720 has been demonstrated in various animals, including rat skin transplantation, heart transplantation, liver transplantation, small intestine transplantation, limb transplantation, pancreas transplantation, kidney transplantation, canine liver transplantation, canine kidney transplantation and monkey kidney transplantation. Jaeyoon Ryu et al.[1] found an increase in the number of T lymphocytes in mesenteric lymph nodes and a decrease in the number of T lymphocytes in spleen cells in FTY720-treated mice. In addition, tissue analysis showed that FTY720 reduced markers of skin and intestinal inflammation and fibrosis. As mentioned in a review of FTY720 studies, the regenerative effects of FTY720 were characterized in three animal models, with different delivery mechanisms emerging over the past 20 years. In these studies, local delivery of FTY720 was found to increase pro-regenerative immune cell phenotypes (neutrophils, macrophages, monocytes), vascularization, cell proliferation and collagen deposition. Delivery of FTY720 to a localized wound environment demonstrated increased bone, muscle, and mucosal regeneration through changes in gene and cytokine production primarily by controlling the local immune cell phenotypes. These changes in gene and cytokine production reduced the inflammatory component of wound healing and increased the migration of pro-regenerative cells (neutrophils and macrophages) to the wound site. The application of FTY720 delivery using a biomaterial has demonstrated the ability of local delivery of FTY720 to promote local wound healing leveraging an immunomodulatory mechanism[2]. Taken together, we hypothesized that FTY720 injection alone could suppress immune rejection in mice, and the ability of local delivery of FTY720 to promote local wound healing by immunomodulatory mechanisms may also avoid delayed tissue repair. Unfortunately, a control group without FTY720 injection was not included in our experiment, so it was not possible to observe whether FTY720 caused a significant delay in tissue repair. However, according to our experimental results, there was no immune rejection in each group of mice, and the wound healing rate was within the normal range. We strongly agree with the reviewer's suggestion that the effect of FTY720 on tissue repair delay should be included in the experiment, which will be the focus of our research group's subsequent research content.

[1] RYU J, JHUN J, PARK M J, et al. FTY720 ameliorates GvHD by blocking T lymphocyte migration to target organs and by skin fibrosis inhibition [J]. J Transl Med, 2020, 18(1): 225.

[2] BEHARA M, GOUDY S. FTY720 in immuno-regenerative and wound healing technologies for muscle, epithelial and bone regeneration [J]. Front Physiol, 2023, 14: 1148932.

We would like to thank the referee again for taking the time to review our manuscript.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.