Detailed Response to Reviewer's and Editorial office's Comments

Manuscript ID:90031
Manuscript Title: Advances in the differentiation of pluripotent stem cells into vascular cells

Dear editors and reviewers,

Thank you for arranging a timely review for our manuscript. We have carefully evaluated the reviewer's critical comments and thoughtful suggestions. Our manuscript, "*Advances in the differentiation of pluripotent stem cells into vascular cells"*, was revised according to the comments from the reviewers. The itemized response to each reviewer's comments is attached. All changes made to the manuscript are highlighted with yellow color. Thank you very much for your suggestions.

We hope that the revised manuscript has addressed all the criticisms raised by editors and the reviewers, and if there are any other modifications we could make, we would be pleasure to do .

Thank you again for your positive comments and valuable suggestions to improve the quality of our manuscript.

Yours sincerely,

Fuchen Liu, MD, PhD

The reply of Reviewer #1's comments

Reviewer #1' s comments	Authors' reply
In transplantation, organ damage is one of the great challenges for graft survival. Using stem cell for organ repair or remodeling become an attractive approach in transplantation. This review summarizes the latest progresses in the field of the induction and differentiation of iPSCs into vascular cells. More specifically, the authors reviewed how to obtain two-dimensional vascular cells such as endothelial cells, smooth muscle cells or pericytes, as well as three-dimensional vascular organoid or tissue engineered vascular graft. This is a very good review covered the most important part of iPSCs induction. The review focused on progresses and detail technologies in induction of endothelial cells from iPSCs. Furthermore, the authors also discussed the progress in co-differentiation and co-culture of endothelial cells and myocytes, a key step for tissue engineering. Progresses in co-transplantation of endothelial cells and pericyte are very interesting. The review will certainly provide useful knowledge for transplantation researchers and other disease researchers.	We feel great thanks for your professional review work on our article. As you are concerned, our manuscript aims to provide a comprehensive summary of the research progress from the establishment of iPSCs to the in vivo transplantation of derived vascular cells. The differentiation of iPSCs into vascular cells is an undeniable cutting-edge technology that holds great promise for benefiting patients with vascular diseases. And the use of gene editing to correct such derivatives for the treatment of diseases shows promise as a personalized treatment. Coupled with the continuous advancement of cutting-edge technologies such as bioprinting and nanotechnology in biomaterial development, its combination with iPSC technology will bring new hope for the study of rare vascular diseases.

The reply of Reviewer #2's comments

Reviewer #2' s comments	Authors' reply
This review introduced the different approaches to differentiate iPSCs into vascular cells and the applications. This topic is interesting. Some minor issues are: 1. Please provide the scope of this review at the end of the first part. 2. Please explain the differences and significance of this review with existing reviews.	Thank you for your valuable comments on our article. We have made some modifications in our revised manuscript based on your suggestions. 1. We have provided the coverage of our manuscript at the end of abstract (**page 3**). 2. Most of the present reviews focus on the introduction of a certain type of vascular models derived from iPSCs, such as the introduction of iPSC-derived vascular cells, three-dimensional vascular organoids or vessels-on-chips. Our review made a systematic and comprehensive summary of various types of differentiation strategies to get vascular derivatives, showed the development process of this technology, and compared the advantages and disadvantages of these methods. Additionally, there is a lack of summary of application advances using iPSCs-derived vascular models. This review fills this gap and can reflect the significance of vascular models based on iPSC technology for exploring vascular diseases.
3. Fig. 1, please explain more about this figure in the main text, not in the figure legends. 4. In the main text, it is not that necessary to mention about the time of the studies.	3. We have given a more detailed description for Figure 1 in the main text (**page 6**). 4. Thank you for your advice, we have deleted the redundant record of the time in the main text. We did not list the changes here but marked in the revised paper.

The reply of Reviewer #3's comments

Reviewer #3' s comments	Authors' reply
I have thoroughly reviewed the manuscript titled "Advances in the differentiation of pluripotent stem cells into vascular cells." The manuscript offers a comprehensive overview of the current state of research in the differentiation of induced pluripotent stem cells (iPSCs) into various vascular cell types. Here are my detailed suggestions for revision:	Thank you very much for your careful review. We have carefully revised the manuscript according to your comments.
1. Clarity and Organization: The manuscript is generally well-organized, but it would benefit from a clearer delineation between sections. Consider adding subheadings within the larger sections to guide the reader through the various aspects of iPSC differentiation. The introduction could be more impactful by briefly highlighting the main challenges in the field and how this review addresses them.	1. We feel great thanks for your professional review work on our article. Based on your suggestion, we have added the following subheadings, **"Application" and "Conclusions and Future Perspectives**". We have also given a comprehensive discussion of the main challenges and coping strategies in the final section(**page 21,22**).
2. Depth and Coverage: While the manuscript does a good job of summarizing various methods of iPSC differentiation, it could benefit from more in-depth discussion of the challenges and limitations associated with these methods. For instance, issues of scalability, cost, and replicability in different lab settings could be addressed. The applications section is informative but could be expanded to include more recent advancements in iPSC technology in vascular disease modeling and regenerative medicine.	2. We wholeheartedly appreciate your professional suggestion. We have added more contents about recent advancements in iPSC technology in vascular disease modeling and regenerative medicine (**page 16,17,18,19,20**). More in-depth discussion of the challenges and limitations have also been added in the conclusion part of our revised manuscript (**page 21**).
3. Technical Accuracy and Updates: Ensure that all the cited studies are accurately represented. In a few instances, the description of the studies seemed overly simplified. Expanding on these descriptions would help the reader appreciate the nuances of the research.	3. Based on your suggestion, we have added more recent references to make our manuscript up-to date. We have also added more details of the references in the main text to help readers to understand these studies better (**page13,14**).

Consider including the latest developments in the field, especially those from 2023 and early 2024, to ensure the review is up-to-date.	**Those references have been cited in this manuscript.** **[69]Al-Thani M, Goodwin-Trotman M, Bell S, et al. A novel human iPSC model of COL4A1/A2 small vessel disease unveils a key pathogenic role of matrix metalloproteinases. Stem Cell Reports. 2023 Dec 12;18(12):2386-2399.** **[70]Liu G, Li J, Ming Y, et al. A hiPSC-derived lineage-specific vascular smooth muscle cell-on-a-chip identifies aortic heterogeneity across segments. Lab Chip. 2023 Mar 28;23(7):1835-1851.** **[72]Wang J, Zhang L, Wu G, et al. Correction of a CADASIL point mutation using adenine base editors in hiPSCs and blood vessel organoids. J Genet Genomics. 2023 May 8:S1673-8527(23)00110-8.** **[73]Kawakami E, Saiki N, Yoneyama Y, et al. Complement factor D targeting protects endotheliopathy in organoid and monkey models of COVID-19. Cell Stem Cell. 2023 Oct 5;30(10):1315-1330.e10.** **[80]Guo Z, Gong A, Liu S, Liang H. Two novel compound heterozygous variants of the GCDH gene in two Chinese families with glutaric acidaemia type I identified by high-throughput sequencing and a**

	literature review. Mol Genet Genomics. 2023 May;298(3):603-614.
4. Figures and Tables: The manuscript would benefit from the addition of more figures and tables that summarize key points, such as a table comparing different differentiation protocols or a figure illustrating the stepwise process of differentiation. Ensure that all figures have clear, descriptive legends.	4. Thank you for your suggestion. We added a summary figure of the existing methods for constructing human vascular model based on iPSC technology (**Figure 3**). More detailed explanations have been given in figure legends (**Figure 1,2,3**).
5. References: Double-check all references for accuracy and completeness. Ensure that all cited works are relevant and current. Consider adding more recent references to support statements, especially in rapidly evolving areas of the field. Where possible, include recent studies to demonstrate the manuscript's alignment with current research trends. In particular, consider including additional references to support the discussion and to provide context to the study's findings. I suggest adding data related to recent bulk transcriptomics studies which could represent a strong substrate to enforce the role of described molecular mechanisms, such as the recent PMID: 36490268, PMID: 27737651, PMID: 26115622 and PMID: 32184807.	5. We sincerely appreciate your valuable comments. We have checked our manuscript carefully to make sure our cited studies are relevant and current. We also added several references related to the application of cut-edge technology such as gene editing, high-throughput sequencing, proteomics in the field of medical area based on iPSCs. The numbers of these newly added reference are **53,72,73,75,79,80.** **[53]Nikolova MT, He Z, Wimmer RA,et al. Fate and state transitions during human blood vessel organoid development. bioRxiv. 2022;2022.03.23.485329.** **[72]Wang J, Zhang L, Wu G, et al. Correction of a CADASIL point mutation using adenine base editors in hiPSCs and blood vessel organoids. J Genet Genomics. 2023 May8:S1673-8527(23)00110-8.** **[73]Kawakami E, Saiki N, Yoneyama Y, et al. Complement factor D targeting protects endotheliopathy in organoid and monkey models of COVID-19. Cell Stem Cell. 2023 Oct 5;30(10):1315-1330.e10.**

	[75]Song HY, Yang YP, Chien Y, et al. Reversal of the Inflammatory Responses in Fabry Patient iPSC-Derived Cardiovascular Endothelial Cells by CRISPR/Cas9-Corrected Mutation. Int J Mol Sci. 2021 Feb 27;22(5):2381. **[79]Scimone C, Donato L, Alafaci C,et al. High-Throughput Sequencing to Detect Novel Likely Gene-Disrupting Variants in Pathogenesis of Sporadic Brain Arteriovenous Malformations.** **Front Genet. 2020 Feb 28;11:146.** **[80]Guo Z, Gong A, Liu S, Liang H. Two novel compound heterozygous variants of the GCDH gene in two Chinese families with glutaric acidaemia type I identified by high-throughput sequencing and a literature review. Mol Genet Genomics. 2023 May;298(3):603-614.**
6. Language and Style: The manuscript is generally well-written but could benefit from proofreading to correct minor grammatical errors and improve sentence structure for better readability. Use consistent terminology throughout the manuscript to avoid confusion (e.g., consistently use either "iPSCs" or "induced pluripotent stem cells"). **7. Ethical Considerations:** While the manuscript mentions the ethical advantages of using iPSCs over embryonic stem cells, it could further discuss the ethical considerations in more detail, particularly	6. Thanks for your careful checks. Based on your comments, we have made the corrections to make the terminology harmonized within the whole manuscript and we have tried our best to polish our language and eliminate minor grammatical errors to improve the readability of our manuscript. We did not list the changes here because they will not influence the content and framework of the article. 7. We think this is an excellent suggestion. We have added more discussions on the disputes existing in the field of iPSC technology, especially the controversy that may arise from the chimeric research. The

concerning patient consent and the use of genetic material. **8. Conclusion and Future Directions:** Strengthen the conclusion by summarizing the key findings more concisely and offering insights into future research directions.Highlight any potential breakthroughs or innovative methods that could significantly impact the field.	revisions can be found in **page14 and 15,"Moral and Regulatory Issues"**. 8. We have rewritten the conclusion according to your suggestion (**page 20,21,22**). We make a summary from the aspects of ethical and regulatory issue, challenges and limitations,, prospects and opportunities, and strive to conduct a comprehensive discussion on the research status and future potential of iPSCs and its vascular derivatives in the medical field.