**Peer Review File**

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**Reviewer A:**

1. Comment 1: There is lacking in evidences that describe the location and the amount of infused ADSCs that works in this system and explanation of the mechanism.

   Reply: Thank you again for your kind advice, and we have concerned about the distribution of ADSCs in this model. Actually, in the previous study of Soares et. al. , they found that labeled mesenchymal stem cells perfused through the vasculature localized to the perivascular space, which has previously been shown to be a stem cell niche and a site of immunologic importance, where mesenchymal stem cells are able to modulate inflammatory cells extravasating from the vasculature. Further, the mesenchymal stem cells persist in tissue up to 48 hours after transplantation(1). However, long-term residence of infused mesenchymal stem cells is unclear. These facts suggests that ex vivo perfused stem cells engraft within allograft tissue and may form long-term niches. In our study, we emphasized on the improved origin of mesenchymal stem cells, which is adipose tissue, and the potential mechanism of ADSC induced prolonged survival of allografts in transplantation. The amount of donor-derived ADSCs retained in allografts may be studied in our further study. As for the referee's concern, we have made modifications in the revised manuscript (Page 16).

   Reference: (1) Soares MA, Massie JP, Rifkin WJ, et al. Ex vivo allotransplantation engineering: Delivery of mesenchymal stem cells prolongs rejection-free allograft survival. Am J Transplant 2018;18:1657-67.

   Changes in the text：In addition, previous work suggests that ex vivo infused mesenchymal stem cells engraft within allografts, implying that infused MSCs may form long-term niches (24). However, the length of time and the amount of ADSCs remain active in allograft tissue remains to be solved in our further studies.

2. Comment2: there is lacking in discussion about the inhibitory effects of ADSC infusion on the decrease of CD4+ and CD8+ T cells, along with total T cells, and lacking sham-operative comparison or non-operative comparison.

   Reply: Thank you for your suggestion. It's very helpful for the clarification of the results. T cell proliferation and infiltration plays a vital role in

allograft rejection, which increases along with the severity of rejection in VCA. T cells in ADSCs groups didn't decrease compared to the same group at different time points. However, the proliferation of T cells which may be caused by rejection was, to some extent, inhibited by ADSC treatment. Indeed, it will be more clarified if we get a comparative assessment on non-operative or sham-operative group. Yet, in the previous study of Gao et.al and Larocca et.al. they used operative control group in the analysis of T lymphocytes (2,3). For the concern of the limitation and ethics of experimental animals and previous studies, we designed this study. However, concerning to the non-operative group may better clarify the results. We may explore this effect in the further study. Therefore, for the referee's concerns, we have modified our text as advised (see Page 12).

References: (2)Gao W, Zhang L, Zhang Y, et al. Adipose-derived mesenchymal stem cells promote liver regeneration and suppress rejection in small-for-size liver allograft. Transpl Immunol 2017;45:1-7.

(3). Larocca RA, Moraes-Vieira PM, Bassi EJ, et al. Adipose tissue-derived mesenchymal stem cells increase skin allograft survival and inhibit Th-17 immune response. PLoS One 2013;8:e76396.

Changes in the text：The results show that the percentage of CD3+ and CD4+ T cells decrease in both ADSC and hypoxia primed ADSC treated groups on postoperative day 10 and day 14 compared with the control group(Figure 3).

3. Comment3: Foxp3 is widely expressed and is not the specific marker of Tregs. The study of Tregs should be redesigned and tested.

   Reply: Indeed, it will be more convincing if we get a more comprehensive assessment on Treg cells. However, the regulatory T cells are a complicated subset of helper T cells. Active Treg cells can suppress alloimmune responses by contact-dependent mechanisms and can also ac in a contact-independent manner by elaborating soluble immunosuppressive factors, such as TGF-β and IL-10. Additionally, Treg cells has different types including CD4+,CD25+CD127-,FoxP3+ ;CD4-,CD8+;CD4-,CD8- andCD4+,FoxP3- type 1 Treg(4). However, Regulatory T cells deficient in the transcription factor Foxp3 lack suppressor function(5). Thus, in our study we tested the immunoregulatory cytokines, such as IL-10 and TGF-βand Foxp3 in the animal models to testify the possible involvement of active Treg cells in the effect of ex vivo infused ADSCs. For the the referee's concerns, we have modified our text as advised (see Page13).

   References: (4). Ligocki AJ, Niederkorn JY. Advances on Non-CD4 + Foxp3+ T Regulatory Cells: CD8+, Type 1, and Double Negative T Regulatory Cells in Organ Transplantation. Transplantation 2015;99:1553-9.

(5). Charbonnier LM, Cui Y, Stephen-Victor E, et al. Functional reprogramming of regulatory T cells in the absence of Foxp3. Nat Immunol 2019;20:1208-19.

Changes in the text: The CD4+CD25+CD127-FoxP3+ Regulatory T (Treg) cells are a unique subset of helper T-cells, which regulate immune response and establish self-tolerance through the secretion of immunoregulatory cytokines, such as TGF-β and IL-10, and Treg can arise spontaneously or can be induced in situ. Treg inhibition of acute rejection and immune response modulation during transplantation was confirmed in a previous study(15).

**Reviewer B:**

1. Comment 1: A few typing mistakes exists in the manuscript.

   Reply: We are very sorry for the mistakes in this manuscript and inconvenience they caused in your reading. The manuscript has been thoroughly revised and edited by a native speaker, so we hope it can meet the journal's standard. Thanks so much for your useful comments.

   Changes in the text: Solutions to minimize the adverse effects of immunosuppressants have been a research focus during recent years and so on.

2. Comment 2: There is lacking in evidences that describe the location and the distribution of infused ADSCs that works in this system.

   Reply: Thank you again for your kind advice, and we have concerned about the distribution of ADSCs in this model. Actually, in the previous study of Soares et. al. , they found that labeled mesenchymal stem cells perfused through the vasculature localized to the perivascular space, which has previously been shown to be a stem cell niche and a site of immunologic importance, where mesenchymal stem cells are able to modulate inflammatory cells extravasating from the vasculature. Further, the mesenchymal stem cells persist in tissue up to 48 hours after transplantation(1). However, long-term residence of infused mesenchymal stem cells is unclear. These facts suggests that ex vivo perfused stem cells engraft within allograft tissue and may form long-term niches. In our study, we emphasized on the improved origin of mesenchymal stem cells, which is adipose tissue, and the potential mechanism of ADSC induced prolonged survival of allografts in transplantation. The amount of donor-derived ADSCs retained in allografts may be studied in our further study. As for the referee's concern, we have made modifications in the revised manuscript (Page 16).

   Reference: (1) Soares MA, Massie JP, Rifkin WJ, et al. Ex vivo allotransplantation engineering: Delivery of mesenchymal stem cells prolongs rejection-free allograft survival. Am J Transplant 2018;18:1657-67.

Changes in the text：In addition, previous work suggests that ex vivo infused mesenchymal stem cells engraft within allografts, implying that infused MSCs may form long-term niches (24). However, the length of time and the amount of ADSCs remain active in allograft tissue remains to be solved in our further studies.

3. Comment3: there is lacking in semi-quantitative data for HE analysis(Fig 2).

   Reply: Thank you for your advice. The semi-quantitative analysis of lymphocytes infiltration in allotransplantation are included in the criteria of identifying acute allograft rejection which was divided into 4 grades, including: grade 0, no or rare inflammatory infiltrates (less than 5%); grade I, mild perivascular infiltration, less than 20% perivascular infiltration; grade II, moderate-to-severe perivascular inflammation (21-50% perivascular infiltration)with or without mild epidermal and/or adnexal involvement (limited to spongiosis and exocytosis); grade III, dense inflammation (over 50% perivascular infiltration ) and epidermal involvement with epithelial apoptosis, dyskeratosis and/or keratinolysis and grade IV, necrotizing acute rejection. We applied this criteria and analyzed the HE results of different groups which shows that the allografts in medium control group presented with obvious signs of grade III rejection within 16 days. The samples we took at day 7 and day 14 showed that in the control group one specimen showed grade I rejection and the rest should grade 0 rejection at day 7. One biopsy specimen displayed grade III rejection in control group, and 3 specimens showed grade II at day 14. All samples from ADSC and Hypoxic ADSC treated group showed grade 0 at day 7, and 2 specimens showed grade I rejection in ADSC treated group ,while the rest showed grade 0 rejection on postoperative day 14. For the the referee's concerns, we have modified our text as advised (see Page 11).

   Changes in the text: As shown in Figure 2, no significant differences were found 7 days post-transplantation, between the control and ADSC treated groups, while signs of grade III rejection, such as histological desquamation, dense inflammation, epidermolysis and gross exudation and necrosis were obvious on postoperative day 14 post-transplantation in control group, but scarcely observed in the ADSC-treated groups that 2 specimens showed grade I rejection in ADSC treated group and the rest showed grade 0 rejection on postoperative day 14.

4. Response to comment: The results did not reveal whether the beneficial effect of pretreating allografts is due to ADSCs or its paracrine effect, which should be discussed. In addition, the discussion part should include more about new findings of this study.

   Reply: Indeed, the results didn't reveal whether the effect of ex vivo infusion of ADSCs is due to contact or paracrine effect, and the fact should be discussed in the article. As for the referee's concern, we have made modifications in the revised manuscript.

   Changes in the text: Although the results didn't reveal the beneficial effect is due to ADSCs contact or its paracrine effect, pretreatment of ADSCs is proved to be an efficient way to improve the immunomodulatory effect of ADSCs in VCA and so on.

5. Response to comment: Chondrogenesis of the ADSCs should be done.

   Reply: We agree with the reviewer, and have added the result of chondrogenesis in the appendix.

   Changes in the text: Adipogenesis, osteogenesis and chondrogenesis were induced using a differentiation medium (Cyagen Bioscience, Inc., Guangzhou, China), and the differentiated cells were identified by staining using oil red, alizarin red and alcian blue, respectively.

   E. Chondrogenesis of adipose derived stem cell, picture shows section after stained with alcian blue (scale bar=50μm).