Peer review file
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Reviewer A

Comment 1: The material-method and volume balance of the results feels a bit bad to me because the results section is too short without details. Detailed addition of pathological findings such as description of the degree of immune cell infiltration and stenosis of the lumen will be required.

Reply 1: Thank you for your suggestion. About the stenosis of the lumen have described in the article, and about the description of the degree of immune cell infiltration, we have published related research reports in the earlier stage. (Cui P, Liu P, Li S, Ma R. De-epithelialized heterotopic tracheal allografts without immunosuppressants in dogs: long-term results for cartilage viability and structural integrity. *Ann Otol Rhinol Laryngo*l. 2020 Sep 10:3489420957357. doi: 10.1177/0003489420957357. Online ahead of print)

Changes in the text: see Page 12, line 207-211.

Comment 2: For orthotropic transplants, you may need as many recipients as there are allografts. However, due to the adoption of the heterotopic transplant model, this experiment does not require as many recipient dogs. There are many benefits to using a single or minimal donor dog to reduce the number of animals and the bias that can be avoided among individual dogs. How would you describe this very important issue?

Reply 2: Thank you for your advice. In this experiment, we implanted a trachea with a length of 6 cm, so that only one trachea can be implanted in the latissimus dorsum of the Beagle. Secondly, in order to better observe the reaction after implantation and avoid confusion. If multiple tracheas are implanted and the recipient beagle has an adverse event after implantation, we will not be able to distinguish which trachea is the cause.

Comment 3: Lines 207-8: "1 and 6 months after transplantation, microscopic examination filled the epithelial layer with fibroblasts."
Do you think that the fact that the surface of the tracheal lumen is covered with fibroblasts instead of epithelial cells can lead to subsequent stenosis of the graft?
Were no epithelial cells found?

Reply 3: That's a good question. The key points of this experiment are to study whether the trachea will be rejected and whether its mechanical properties will change after de-epithelialization by 1% SDS and ectopic implantation. So, after a long period of implantation, there are fibroblasts filling in the epithelium of the trachea. In our later experiments, we consider covering the buccal mucosa of the receptor in the inner layer of the trachea, which would reduce the filling of fibroblasts in the epithelial

layer of the trachea.

Comment 4: In Discussion, you mentioned xenograft “We also tried to determine whether de-epithelialized trachea could be applied for allografting and xenotransplantation.”
Where are your goals in the clinical setting? If you do not logically link the results of this study, your claim will not be clear.

Reply 4: We think your proposal is very well. This sentence does not accurately express our goals in the clinical setting, so we modified it appropriately.

Changes in the text: see Page 14, line 257.

Minor comments

Comments 1. Lines 104-109: Why separate group names? It is better to unify the group name and the other.

Reply 1: We have unified the group name according to your suggestion and divided it into Group A, Group B, Group C and Group D.

Changes in the text: see Page 7-8, line 118-121.

Comments 2. Lines 128: Since the group description is a mixture of uppercase and lowercase letters, it may be better to unify it.

Reply 2: Thank you for your careful review. This is a clerical error and I have made a change.

Changes in the text: see Page 8, line 140.

Comments 3. Table is quoted in the discussion section. The description here should move to the Result section.

Reply 3: We have described the table in the results section and modified the discussion section.

Changes in the text: see Page 14, line 263.

Comments 4. Lines 303, 307: I feel that quoting literature in the conclusion section is not a general academic paper.

Reply 4: This may be our statement that caused your misunderstanding. We mainly want to summarize the two points of the article, not to cite references.We have already modified it in the manuscript.

Changes in the text: see Page 16, line 301, 305.

Reviewer B

Comments 1: I can understand that the strength was weak at one month and it became strong enough in six months, but I wonder if the allograft at one month can be

work well if it is implanted as orthotopic graft. Authors should state how big the influence of this significant change is from the clinical point of view.

Reply 1: That's a good question. In the experiment, we compared the mechanical values of each group when the trachea was stretched to 50% of its original length. In this case, the tensile strength of trachea implanted for 1 month is different from that of fresh trachea, which does not mean that the trachea implanted for 1 month has no tensile strength. If applied in clinical practice, patients will be advised to avoid excessive extension during the 1-month transplantation. Just as we do after trachea amputation anastomosis, we advise the patient to avoid excessive extension. Furthermore, stretching has little effect on the transplanted trachea clinically since it does not affect the lumen diameter. Whether or not the lumen collapses under pressure is important because it blocks the trachea.

Comments 2: Another concern is about the denuded epithelium. The epithelium layer has been filled with fibroblasts instead of ciliated pseudostratified columnar epithelium. Although this model is a heterotopic implantation and authors were focusing on the strength of the trachea, authors should discuss about this epithelial change. If the graft was implanted as orthotopic transplantation, re-epithelialization would be expected like in another similar previous report [2]?
To answer these questions, I would like know the results of studies on orthotopic transplantation to assess the feasibility of de-epithelialized tracheal allografts.

Reply 2: As noted by the reviewers, epithelialization is important for tracheal transplantation. If there is no epithelialization, cicatricial stenosis could occur later. We are currently conducting an experimental study of orthotopic tracheal transplantation in rabbits with the receptor's buccal mucosa covering the superior cortex of the tracheal after de-epithelialized. At present, the experimental results have not come out completely, we cannot give you an accurate answer.