

# TRANSLATIONAL LUNG CANCER RESEARCH

## Peer Review File

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### Reviewer A

We read with great interest the article by Xu and colleagues from Shanghai Lung Tumor Clinical Medical Center, Shanghai Chest Hospital - Shanghai Jiao Tong University School entitled “Feasibility of Bioengineered Carina Reconstruction Using In-Vivo Bioreactor Technique in Human”. The authors proposed to “establish the feasibility of carina bioengineering use In-Vivo Bioreactor technique” using an “uncontrolled single-center cohort study including three patients with long-segment airway lesions invading carina”. They concluded as follows: “In this uncontrolled study, In-Vivo Bioreactor technique demonstrated feasible for long-segment trachea, carina and bronchi reconstruction. Further research is needed to assess efficacy and safety”.

This group faced one of the most challenging operations, especially when the carina is involved: tracheobronchial replacement.

The article is very interesting. Nevertheless, we have some remarks and suggestions:

#### **Comment 1:**

-Title: In our opinion, the term “feasibility” should be used with caution as the study included only 3 patients. On the other hand, the title suggests that the authors observed in vivo tissue engineering after implantation of the stented substitute. To our knowledge, no evidence has been provided to this effect. We think the title should be changed.

**Reply 1:** We agree to change the feasibility to proof of concept study.

**Changes in the text:** The title has been changed as followed: “Bioengineered Carina Reconstruction using In-Vivo Bioreactor Technique in Human: proof of concept study”

#### **Comment 2:**

-Abstract: In the same way, the conclusion “In this uncontrolled study, In-Vivo Bioreactor technique demonstrated feasible for long-segment trachea, carina and bronchi reconstruction” should be mitigated as there was only 3 patients included. the conclusion should be mitigated.

**Reply 2:** We mitigated our conclusion as advised.

**Changes in the text:** In this “proof of concept study, In-Vivo Bioreactor technique demonstrated potential to be applied for long-segment trachea, carina and bronchi reconstruction. Further research is needed to assess efficacy and safety. (see Page 3, line 56-57)

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## **Comment 3:**

-Introduction: Why did the Shanghai Chest Hospital Ethics Board give permission for only 3 patients? This should be explained in the “introduction” or “methods” (better) section.

**Reply 3:** As a proof of concept study the investigator initially planned to enroll 3 patients into this study for the funding application. So according to the project protocol, Shanghai Chest Hospital Ethics Board give permission for only 3 patients.

**Changes in the text:** We add the explanation. (See Page.4-5, line 106-111)

## **Comment 4**

-Methods: The authors should precise how was ventilation maintained during the operation. On the other hand, the choice of total nucleated cells (for injection into the implanted substitute via the embedded Port-a-Cath system) should be explained. What was the rationale for the injections, especially regarding the number of cells and the frequency?

### **Reply 4:**

1. Ventilation: We describe how we maintain ventilation during the operation.

**Changes in the text:** See Page 8, Line 167-170 and Page 8, Line 176-179.

2. rationale for injections

We explain the rationale for cell injections by add one paragraph “This fact brought into question the necessity of cell pre-seeding and gave birth to the in situ tissue engineering theory, which neglected cells and implanted only the shaped biodegradable scaffold together with growth factor(s) to induce in situ stem cell growth and differentiation. This modification was not very successful and demonstrated the critical function of cells in the regenerative process. Considering the fact that we age because of a decreased capacity for stem cell proliferation, it should not be surprising that we need cells for truly functional reconstruction. Delayed revascularization kills most cells within implanted constructs and prevents the clinical application of large TE prostheses. So far, successful clinical application of TE products has only been accomplished in cartilage tissue (which is avascular) and skin (which is placed on a well-vascularized-wound surface)”

**Changes in the text:** We added the paragraph above. See Page.3-4, line 76-87.

Regarding the number of cells and the frequency:

We added “The choices of the number of cells harvested and the frequency of cell injection are mainly due to clinic convenience.” In line 154

## **Comment 5:**



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-Results: The first paragraph “Patients. The ethical board approved pilot clinic trial with maximum three cases who were enrolled from December 2017 till July 2018. The patient characteristics, type of operation, and indications for inclusion summarize in Table 1. All the cases required carina reconstruction” should be moved to the “methods” section. In the same way, the next paragraph entitled “procedures” is more about methods than results. Page 8, we read the following: “The airway defect was repaired with bioengineered substitute with In-Vivo Bioreactor design. The whole bioengineered substitute, including anastomoses, was buttressed with omentum”. At time of implantation, the term “bioengineered substitute” is not appropriate because in vivo bioengineering is based on post-implantation regeneration.

**Reply 5:** We agree that part of the results section should move to methods section. We also change term “bioengineered substitute” with “trachea substitute” during surgery.

**Changes in the text:** We move the regarding paragraph and procedures to methods section. See Page 6, Line 126-129 and 157-181.

We also change the term “bioengineered substitute” as “tracheal substitute” Page 8, line 176-179.

## **Comment 6:**

-Discussion: We read page 10 line 249-252: ”Following the stented aortic matrix airway reconstruction method, a temporary membrane stent was inserted to prevent biodegraded ADM fragments depositing caudally into the lung and causing lethal pneumonia”. To our knowledge, a silicone stent was used in the “stented aortic matrix” method in order to avoid collapse and stenosis because aortic grafts are elastic conduits. In some patients, it has been removed after de novo cartilage generation. Did the investigators find a regenerated tissue within the dermis matrix they implanted?

**Reply 6:** We have not removed the stent to avoid the collapse and we have not performed biopsy for regenerated tissue.

## **Changes in the text:**

We described the regeneration tissue within the dermis matrix by adding “ The bronchoscopy follow-up shew nicely the gradually biodegradation process of ADM together with the regeneration process of granulation tissue. The last bronchoscopy follow-up of all the three patients found no ADM remain the substitutes are all replaced by the recipient’s granulation tissue.” In the long-term follow-up of the result part. (Page.10, line 216-219)

## **Comment 7:**

We read page 11 line 228-9 the following: ”Stented aortic matrix is the most popular choice in the field of bioprosthetic reconstruction”. As the first author has been cited

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for other technique (Dealere et al. ; Fabre et al.), the authors should add:” Stented aortic matrix as reported by Martinod et al. is the most popular choice in the field of bioprosthetic reconstruction. [Martinod E, Chouahnia K, Radu DM et al. Feasibility of bioengineered tracheal and bronchial reconstruction using stented aortic matrices. JAMA 2018;319:2212-2222.].

**Reply 7:** We agree to add the sentence as advised.

**Changes in the text:** See Page 12, line 252-253.

## **Comment 8:**

We read page 12 line 261-2 the following: ”The antibiotics inside the perfusate prevented local bacterial colonization that might lead to lethal pneumonia”. Should the authors provide a reference demonstrating that tracheal perfusion of antibiotics can prevent lethal pneumonia?

**Reply 8:** This the first time we apply tracheal perfusion of antibiotics. The 3 patients all survived without lethal pneumonia.

## **Changes in the text:**

We changed the sentence “The antibiotics inside the perfusate prevented local bacterial colonization that might lead to lethal pneumonia” to

“Based on the experience of chest lavage for empyema treatment in BPF (bronchopulmonary fistula) patients the antibiotics inside the perfusate play some role in the prevention of local bacterial colonization that might lead to lethal pneumonia”. See Page 13, line 285-288.

## **Comment 9:**

Page 12 line 266 -271, we read the following: “According to the stented aortic matrix airway reconstruction method, the implanted membrane stent was withdrawn after a two-year follow-up. Although theoretically the membrane stent can be removed once the revascularization process is complete, it was decided to keep it until two-year follow-up as a precautionary measure. No severe stent-related complications, such as bleeding, bronchitis, or pneumonia, were recorded until the one-year follow-up”. We do not understand whether this is a prospect or that the stent has been removed. This should be clarified.

**Reply 9:** We do not remove the stent

**Changes in the text:** See Page 13-14, Line 293-297. We clarified that we do not removed the stent and reason.