Dear Dr. Wu,

We appreciate your time in reviewing our manuscript titled “Seeding and implantation of a biosynthetic tissue-engineered tracheal graft in a mouse model”. We have adjusted the manuscript to address the unit suggestions, removed trademarked words and have included more information regarding the safety and care of the animals as well as a step-by-step protocol for conducting the immunofluorescence. Additionally, we have selected the appropriate sections for filming. We look forward to your decision on the manuscript and are very grateful for your consideration for publishing in the Journal of Visualized Experiments.

Best regards,

Matthew Wiet

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Manuscript was proofread for grammar and spelling.

2. Please use standard SI unit symbols and prefixes such as µL, mL, L, g, m, etc. Please use mL instead of cc.

Changes were made to remove “cc” and replaced it with “mL” (Lines 114, 132)

3. Please use h, min, s for time units.

Time units were changed in the appropriate places Protocol 2.3, 2.5, 2.6, 2.9, 3.2, 3.3, 4.1

4. JoVE cannot publish manuscripts containing commercial language. This includes company names of an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. Examples of commercial language in your manuscript include Ficoll, Rotina 420R, Histopaque, Fisherbrand, etc.

Commercial language has been removed line 137, 141, 156-157, 183. 191-192, 215

5. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) in yellow that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

We feel that the important parts of this protocol are in the graft manufacturing (electrospinning and cell seeding) and the implantation procedure. We have highlighted these sections excluding the anesthesia/euthanasia portions.

6. Step 1.3: Please ensure that all text is written in imperative tense.

Thank you for noticing this, it has been adjusted

7. Step 5: Please rewrite the note as protocol steps. Please ensure that all text is written in imperative tense.

Thank you for the suggestion, Step 5 has been rewritten in protocol format. (Lines 239-263) Additionally, materials for this were added to the materials list.

8. For survival strategies, discuss post-surgical treatment of animal, including recovery conditions and treatment for post-surgical pain.

Recovery conditions have been added to 4.14. Pain treatment consists of close observation by the veterinary staff. If prolonged, euthanasia is indicated. Both of these points have been added as a note (Lines 233-235)

Discuss maintenance of sterile conditions during survival surgery.

A note has been added to the beginning of step 4.

Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.

Protocol 4.14 has been added to help clarify this.

Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.

Language has been added to protocol 4.13 to reflect this.

9. Please do not highlight any steps describing euthanasia or anesthesia