May, 12, 2018

Dear Alisha DSouza, Ph.D.

JoVE Scientific Review Editor

JoVE

Dear Editor:

I wish to re-submit the manuscript titled “Ethanol-induced cervical sympathetic ganglion block applications for promoting canine inferior alveolar nerve regeneration using an artificial nerve.” The manuscript ID is JoVE58039.

We thank you and the reviewers for your thoughtful suggestions and insights. The manuscript has benefited from these insightful suggestions. I look forward to working with you and the reviewers to move this manuscript closer to publication in the *JoVE*.

The manuscript has been rechecked and the necessary changes have been made in accordance with yours and the reviewers’ suggestions. The responses to all comments have been prepared and are given below.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,

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Responses to Reviewers

Ethanol-induced cervical sympathetic ganglion block applications for promoting canine inferior alveolar nerve regeneration using an artificial nerve

*Response to JoVE Scientific Review Editor: We wish to express our appreciation for your insightful comments, which have helped us to significantly improve our manuscript.*

**Changes recommended by the JoVE Scientific Review Editor:**

**• Textual Overlap:** Significant portions show significant overlap with previously published work. Please re-write the text indicated in red in the attached document to avoid this overlap.

*Response: We agree with your comment. In order to avoid duplication with previously published works, we have re-written the text as much as possible.*

**• Protocol Detail:**

Comment 1): 1.1.1-1.1.6 should not be substeps of 1.1.

*Response: We agree with your comment and have revised lines 105–128 as follows.*

*“1.1.1-1.1.6” → “ 1.2.-1.5.”*

Comment 2): 1.1: Please reference Fig 1 here.

*Response: We have revised line 103, according to your comment.*

Comment 3): 1.1.2: Needs a reference for extraction. What is the atelocollagen concentration?

*Response: We have added more information on atelocollagen concentration and preparation (paragraph 1.3, lines 107-113) and added reference 7.*

Comment 4): Lines 116-117: Need a reference.

*Response: We have added reference 7 and revised this information as follows (lines 109-113):*

*“Note: Atelocollagen is extracted from porcine skin via enzyme treatment and subjected to a virus check. It mainly consists of type I (70–80 %) and type III collagen, the ratio of which is described in detail in reference 7. Prepare the collagen solution by dissolving 1 g collagen in 100 mL hydrochloride solution (pH = 3.0). Since the density of the hydrochloride solution is approximately 1.0, the w/w collagen concentration is almost 1 %.”*

Comment 5): 1.1.3: what it the collagen hyodrochloride concentration in M or mg/ml? Please specify how many times should you dip and for how long should the tube be submerged during each dip? Should you dry the tube between dippings? Is temperature important?

*Response: We agree with your comment and have revised lines 115–121 as follows:*

*“1.4. Coat the tube with the collagen layers by repeatedly dipping it into the 1 % collagen hydrochloride solution for 5 seconds each time.*

*1.4.1. After dipping, dry the tube on a clean bench at room temperature. Perform next dipping after ensuring the tube is completely dry (about 6 hours for air-drying).*

*1.4.2. Repeat the coating process 10 times.”*

Comment 6): 1.1.4: Air dry? How is the tube handled?

*Response: The tube was air-dried on a clean bench at room temperature. We have added this information in paragraph 1.4.1. All processes were carefully performed under aseptic conditions.* *We have revised lines 124-125 as follows:*

*“Perform the entire process under aseptic conditions.”*

Comment 7): unclear what is meant by alcoholic solution. Do you mean ethanol or isopropyl alcohol? Mention %.

*Response: We agree with your comment and have revised paragraph 2.4. (lines 143–144) as follows:*

*“2.4. Wear sterilized gloves to disinfect all surfaces of the operating setting with an 80 % ethanol solution. Discard the used gloves.”*

Comment 8): 3.2: Please describe the surgical steps in detail, Mention all tools used, incision site, depth and size. What is the tube diameter and length?

*Response: We agree with your comment and have revised paragraph 3.2. (line 155) as follows:*

*“3.2. Intubate by a tracheal tube with a diameter of 7.5 mm and length of 25 cm.”*

Comment 9): 3.3., 4.10.1: Mention sevoflurane dosage, and oxygen flow rate.

*Response: We have revised this according to your comment.*

*Lines 157-158: “3.3. Place the dog on the right lateral position and maintain general anesthesia with 1.5 % sevoflurane in oxygen (4 L/min) and air (6 L/min). ”*

*Lines 201-203: “4.10.1 Perform computed tomography (CT) imaging of the facial bone under anesthesia, achieved with a mixture of 5 mg/kg ketamine hydrochloride and 1 mg/kg xylazine”*

Comment 10): 3.6: Please specify the surgical site.

*Response: We have revised line 164 as follows.*

*“3.6. Carefully shave the surgical field (left side chest area) using surgical clippers.”*

Comment 11): 4.1, 5.4: Where exactly is lidocaine delivered? Mention needle gauge, and injection volume ie. dose?

*Response: We have revised this according to your comment.*

*Lines 171-172: “4.1. Inject 3 mL of 1 % lidocaine using a 27-gauge needle to the left mandibular gingiva as a local anesthetic and analgesic.”*

*Lines 217-218 “5.4. Inject 5 mL of 1 % lidocaine using a 21-gauge needle to the left side chest area as a local anesthetic and analgesic.”*

Comment 12): 4.3: What is the vibration frequency and amplitude (should be available in the manufacturer’s datasheet).

*Response: As the numerical value varies depending on various conditions, the amplitude was not described in the manufacturer's data sheet. We have revised line 180 as follows:*

*“Note: The vibration frequency was 28-32 kHz.”*

Comment 13): 4.4: How?

*Response: We have revised lines 182–183 as follows:*

*“4.4. Remove the frontal part of the mandibular bone plate (dimensions, 3 cm × 8 mm) to expose the left IAN (Figure 2A) 18. ”*

Comment 14): 4.7: mention magnification of the scope

*Response: We have revised lines 192–193 as follows:*

*“4.7. Use 8-0 nylon sutures and a surgical microscope at 8 x magnification to suture the tube to the proximal and distal nerve ends (Figure 2B) 18.”*

Comment 15): 4.10.2: Mention any CT parameters for imaging.

*Response: We have included this information in 4.10.1. (line 203) as follows:*

*“Set CT parameters as follows: 120 kVp, 200 mAs, 0.5 mm/s, 0.5-mm slice thickness.”*

Comment 16): 5.2: Mention anesthesia method.

*Response: We have revised lines 211–212 as follows:*

*“5.2. Anesthetize the animal with 1.5 % sevoflurane in oxygen (4 L/min) and air (6 L/min) and shave and clean the intended surgical field, as described in paragraph 3.”*

Comment 17) The above list is not exhaustive, please apply the ideas to all your steps. You are the best judge of the missing details in your protocol, and its reproducibility.

*Response: We wish to express our appreciation for your insightful comments. According to all your comments we have revised our manuscript.*

**• Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. This should be <2.75 pages long.

1) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

2) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.

3) Please do not highlight anesthesia and euthanasia as these will not be filmed.

*Response: Thank you for your comment. We have re-evaluated the length of our protocol section.*

**• Result:** Please mention the statistical tests performed and report sample sizes.

*Response: Thank you for your comment. We have described all statistical tests and sample sizes in the results (lines 378-382).*

**• Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

*Response: Thank you for your comment.* *Accordingly, we have revised the discussion to focus on the methods and protocol.*

**Comments from Peer-Reviewers:**

**Reviewer #1:**

Comment: Minor Concerns:

Conclusions of the manuscript have to be more clearly described

*Response: We thank you for this pertinent comment. We have revised the discussion for greater clarity, according to your comment.*

**Reviewer #2:**

Comment: Manuscript Summary:

This manuscript is well-organized and well written. The current status is almost ready for publication except the experimental procedures, especially the surgical part should accompany additional pictures/color photos/figures that would be beneficial for readers to understand this procedure.

*Response: Thank you for your comments. We are considering of supplementing the detailed procedure of the surgical operation with a video file, characteristic of JoVE.*

**Reviewer #3:**

Comment: Manuscript Summary:

The current paper is another vesrion of the already published paper entitled "Can nerve regeneration on an artificial nerve conduit be enhanced by ethanol-induced

cervical sympathetic ganglion block?" by the same authors, which exists as citation number 18 in the current review. I cannot find what new information adds the current paper in the already published data (the same material was used, same figures, similar citations). The authors should explain the reason of this submission. No mention is made about the same figures. Did the authors took a copy-editing from PLOS ONE? I suggest the paper's rejection.

*Response: Thank you for your comments. JoVE is a methods-based journal.　The reason of this submission is to introduce our new surgical method for patients with peripheral nerve injuries to the world. We do hope this method will be widely performed clinically and contribute to the recovery of several patients. Therefore, we introduce a detailed protocol for the procedure of cervical sympathetic ganglion block, as well as for nerve reconstruction using an atrial nerve.*

*With regard to the technique of cervical sympathetic ganglion block and inferior alveolar nerve reconstruction, our maneuvers are always improving. Therefore, the current manuscript differs from our previous report as it includes further descriptions and methodological details. Moreover, our protocol is supplemented with a video file so that it is visually easier for the reader to understand and reproduce the procedure.*

*We would like to add that we have already obtained permission from the editorial department of PLOS ONE to use our figures and table already published in PLOS ONE. In addition, we have also informed and received confirmation from JoVE's scientific editor about this in advance.*