

August 4, 2014

Dear Michelle Kinahan,

Please find our revised submission to **JoVE**, manuscript# 52375 entitled: "A surgical procedure for resecting the mouse rib: a model for large-scale long bone repair" uploaded to your online system.

We would first like to thank the reviewers and editor for suggestions to improve the paper. Overall the reviewers were very positive. Major suggestions involved clarifying the usefulness of the model while minor suggestions were aimed to help describe the protocol in more detail. We have addressed all major and minor concerns and outlined the changes in the following pages. We hope the concerns have been addressed to everyone's satisfaction and look forward to working with your team to prepare the video portion of the project and thus bring this procedure to the scientific community.

Please don't hesitate to contact me if you need any further information.

Sincerely,

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RESPONSE TO REVIEWERS:

Editorial comments:

In step 1.1, what tools are needed? It would be good to make a table/list of these items.

A reference to the materials list is now included.

Please expand your discussion section to focus more on the details of the protocol and cover 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.

We have now included subheadings covering these suggested topics in the discussion with the exception of the comparison with existing models which is covered in the introduction. Please let us know if you would like us to move that section from the introduction to the discussion.

Please remove the main figure titles from the figure files. Figure titles and legends should not appear in the figure but only be included in the text file.

The titles have now been removed.

Please upload a signed copy of the Author License Agreement with an original signature.

A scanned version is now uploaded.

Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammar issues. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

We have proofread the manuscript.

Reviewer #1:

Manuscript Summary:

This work describes a method for creating defects in a mouse rib to study bone regeneration. This model is novel and will be of interest to the broad community of researchers studying bone repair. The description of the protocol is excellent.

Major Concerns:

None.

Minor Concerns:

None.

Reviewer 1 had no suggested changes.

Reviewer #2:

Major Concerns:

My major concern with this model is the clinical relevance. In treatment of large bone defects the additional provided biomechanical environment by any kind of stabilisation method is a major influencing factor for the reconstruction of the missing bone. Even the presented method is well described and the point was made that the other ribs are helping to stabilise the defect, its use is limited to address questions to overcome large bone defects.

Indeed the exact reason for using this model is to provide a context within which to study large bone defects in mammals as no such model currently exists. To address the concerns of this reviewer we have made this point more obviously in the introduction.

If the authors could provide a reliable stabilisation technique or could provide a defined understanding of the biomechanical environment, when leaving the defect untreated, the model would gain higher

clinical/research value.

As requested, in the discussion section on limitations, we now elaborate on the biomechanical environment and how future studies could adapt this model to address this very interesting variable in repair.

Minor Concerns:

FIG.2 B - Please explain the defect on the left of the rib (not the defect itself). It seems that there was an additional fracture?

The gap at the left is the chondrocostal joint. This is now clarified in the figure and the accompanying legend.

Reviewer #3:

Manuscript Summary:

This article describes a technique to resect a portion of rib that could be used to study bone healing. The development in a mouse, using a controlled surgical technique, is an advance to the field as it 1) overcomes the high variability of long bone fracture healing and 2) works in mice and thus can be used to study genetic models. This is a nice article.

Major Concerns:

It would be nice to see images of what the healing looks like. Either CT or histology. In the same way, the text discusses good and bad surgeries as based on x-ray. Sample images would be nice.

A full description of the stages of healing will be included in another publication. For the moment we have included both a whole mount alizarin red preparation and also histology of the repair when completed (Figure 2B') which shows the repair at higher resolution than an Xray or CT image.

Good and bad surgeries can be assessed post-operatively by Xray or microCT on live animals. However this typically needs to be part of the animal use protocol. We do not currently have this approved. This caveat is now explained in the text.

Minor Concerns:

Does the mouse rib have a marrow cavity? Does it have trabecular bone? These details would be nice to know to help understand the model.

Figure 1B' shows the marrow cavity and the low presence of trabecular bone and this is now explained in the figure/figure legend.

Do all mice have 13 ribs? Do you recommend counting backwards from the bottom (13) or if there is variation in the mouse counting from 1?

There is reported variation caudally although we have not encountered this in CD-1 C57BL/6 strains. Counting should start from the top as #1. This is now explained in the protocol.

Step 2.2 says to alternate beta dine and alcohol. How many times each?

This depends on your animal use protocol. At USC, it needs to be done 3X. We have now included this repetition in the protocol and will adjust based on JoVE's recommendations.

Step 3.3 has a note about a pneumothorax. A sentence or two telling the investigator how they would know they have induced one would be helpful.

This is now included.

Step 3.4 What constitutes 'significant' blood loss.

Detailed specifics on blood loss is now included.

Step 4.4 What does 'secure the edges with large forceps mean in the context of gluing the incision.

This will be demonstrated in the video but essentially involves pinching the edge of the incision together. This is now more explicitly described.

Step 5.1 Is there something special about oral buprenorphine? If not, maybe be less specific and just stay to administer buprenorphine.

Oral buprenorphine is easier to administer as it does not require restraining the animal. This is now explained.

Step 5.2 So males can never be group housed?

Yes, this is basic knowledge for anyone who works with mice and is now explained.

Step 5.3 It would be helpful to know how the animals recover. Are they moving and ambulating normally and if so, when post-surgery?

The animals should show no signs of any problems at any time after recovery from anesthesia. This is now indicated in step 4.6.

Figures: Consider labeling something other than B' and C'. This was confusing to see B and B'.

The letters with prime are the same type of sample as without. We are happy to take recommendations from JoVE copy editors in this regard (Would B1, B2; C1, C2 be preferred?)

Reviewer #4:

Major Concerns:

Is this really a bone repair model? It seems that it is more a segmental defect that fills in with new bone formation. Although these two mechanisms might be somewhat similar, the title is not covering the text.

We have now changed the title to clarify this as a 'large-scale' repair model.

Minor Concerns:

In the protocol under 1.5 they should state what side the mouse is positioned. Lateral with right or left side up?

Either side is fine and dependent on preference and handedness of the surgeon. This point is now included.

In the protocol under 3.1 what is meant by proximal? Do they mean lateral?

The location is lateral with reference to the surgeon looking down on the animal but proximal with reference to the animal. We hope the video will clarify this.

Under 3.2: describe more specific how to get the periosteum of the rib.
Again we hope the video will clarify this.

Under 3.3: Speed (swift) does not necessarily mean "bad" in surgery. Reword.

We have reworded this sentence.

Under 4.1: do you need to close the periosteum (tubularize)?

It is not feasible to do this since the periosteum is so thin.