

February 28, 2019

Dr. Phillip Steindel
Editor, **Journal of Visualized Experiments**

Dear Dr. Steindel,

We are submitting the revised original manuscript entitled “Adult mouse digit amputation and regeneration; A simple model to investigate mammalian blastema formation and intramembranous ossification” by Lindsay A. Dawson, Regina Brunauer, Katherine N. Zimmel, Osama Qureshi, Alyssa R. Falck, Patrick Kim, Connor P. Dolan, Ling Yu, Yu-Lieh Lin, Benjamin Daniel, Mingquan Yan, and Ken Muneoka to the **Journal of Visualized Experiments**. We are submitting a protocol of adult mouse distal terminal phalanx amputation, which is a procedurally simple and reproducible model of epimorphic regeneration characterized by blastema formation and bone regeneration via intramembranous ossification. Our protocol demonstrates the standardized terminal phalanx amputation plane, the processing of amputated digits for immunofluorescent analysis, and a detailed description of fluorescent immunohistochemistry to investigate blastema formation, revascularization in the context of regeneration, and bone regeneration via intramembranous ossification. Moreover, we demonstrate the use of in-vivo microcomputed tomography to create high resolution images and for the quantification of bone changes in the same digit over the course of regeneration. We believe this protocol will add to the somewhat limited database for mammalian epimorphic regeneration and the manuscript is of interest to the **Journal of Visualized Experiments** readership.

In this resubmission, we have edited the document to address all of your and the reviewers comments, as well added an additional figure and a second panel to Figure 3 to further address the reviewer comments.

We have no conflicts of interest and this work has not been previously published nor is it under review at another journal. All authors meet the qualifications for authorship and have had opportunity to read and comment upon the manuscript.

All experimental animal studies were performed in accordance with approval from the Institutional Animal Care and Use Committee at the College of Veterinary Medicine and Biomedical Sciences at Texas A&M University.

Sincerely,

A handwritten signature in cursive script that reads "Lindsay Dawson".

Lindsay Dawson