Submission ID #: 61722
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18825258

Title: A Mouse Model of Lumbar Spine Instability

Authors and Affiliations: Shufen Liu[1], Yueli Sun[1], Jingcheng Dong[2], and Qin Bian[2]

[1]Spine Research Institute, Longhua Hospital, Shanghai University of Traditional Chinese Medicine
[2]Department of Integrative Medicine, Huashan Hospital, Fudan University

Corresponding Author:
Qin Bian
bianqin213@126.com

Co-Authors:
xiaoliufenfen@163.com
yueli_sun@foxmail.com
jcdong2004@126.com

Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **Y, Olympus SZ61**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **20**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Shufen Liu**: This surgical-induced mouse model offers an alternative for lumbar intervertebral disc degeneration-related studies **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Yueli Sun**: This robust LSI model requires no special equipment, is reproducible, and can be used to induce intervertebral disc degeneration in a relatively short period of time **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Shufen Liu**: Controlling the incision depth and hemorrhage and performing a full resection of each entire spinous process are two key aspects for the implantation of a successful experiment **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Shanghai University of Traditional Chinese Medicine.

Protocol

2. Surgical Preparation

2.1. After confirming a lack of response to pedal reflex **[1-TXT]**, place an anesthetized 8-week-old, make, C57BL/6J (C-fifty-seven-black-six-J) mouse onto a surgical pad in the supine position **[2]** and apply ointment to the animal's eyes **[3]**.

2.1.1. WIDE: Talent pinching toe Videographer: More Talent than mouse in shot
TEXT: Anesthesia: 4% isoflurane

2.1.2. ECU: Ointment being applied

2.2. Use a small animal trimmer to shave the surgical area from the lower thoracic region to the top of the sacral region **[1]** and remove the shaved fur with tissue wipes **[2]**.

2.2.1. Fur being trimmed
2.2.2. Fur being wiped

2.3. Apply depilatory cream to the shaved area **[1]**, using gauze to remove the cream after no more than 3 minutes **[2]**, and flush the exposed skin with 2 milliliters of 0.9% sterile saline **[3]**.

2.3.1. Cream being applied
2.3.2. Cream being wiped
2.3.3. Skin being flushed

2.4. Then place a custom-made surgical cylindrical pad under the abdomen to raise the lumbar spine **[1]**.

2.4.1. Pad being placed *Videographer: Important step*

3. Lumbar Third to Lumbar Fifth (L_3-L_5) Spinous Process and Ligament Resection

3.1. To expose the L-three to L-five vertebrae, use the index finger to touch the subcutaneous spinous processes of the lumbar vertebrae **[1]** and palpate to compare the processes with those of the thoracic vertebrae and sacral vertebrae to identify the lumbar region **[2]**.

3.1.1. WIDE: Talent touching processes *Videographer: More Talent than mouse in shot*

3.1.2. Mouse spine being palpated

3.2. Using a dissecting microscope **[1]**, Rinse the skin with 75% alcohol **[1]** and use a scalpel to make a 3-4-centimeter midline skin incision over the lumber region from the mid-thoracic region to the hip **[2]**.

3.2.1. Skin being rinsed
3.2.2. Incision being made

3.3. identify the lumbar spine by the "V" shape of the posterior fascia inserted onto the tips of the spinous processes **[2]** and use the scalpel to make shallow posterior paraspinous muscle incisions along the spinous processes from L-three to L-five laterally on both sides of the spine **[3-TXT]**.

3.3.1. Talent moving mouse under microscope/moving microscope into place over mouse *Videographer: More Talent than mouse in shot*
3.3.2. SCOPE: Shot of processes *Videographer: Important step; Video Editor: please overlay "V's" and L3-S1 texts over processes as illustrated in Figure 2B*
3.3.3. SCOPE: Incision(s) being made **TEXT: Caution: Control incision depth toward facets to reduce hemorrhage**

3.4. Then use two pairs of ophthalmic forceps to separate the muscle layers to expose the L-three to L-five spinous processes and supraspinous ligaments **[1]**.

3.4.1. SCOPE: Muscles being dissected/processes being exposed *Videographer: Important step*

3.5. To separate individual spinous processes, use Venus shears to cut off interspinous ligaments **[1]** and to resect the L-three to L-five spinous processes and interspinous ligaments **[2]**.

3.5.1. SCOPE: Ligaments being cut *Videographer: Important/difficult step*
3.5.2. SCOPE: Processes and ligaments being resected *Videographer: Important/difficult step*

3.6. Then use sterile number 5 silk braided sutures to close the skin incision without reattaching the paravertebral muscles **[1]** and apply chlortetracycline hydrochloride ointment to the surgical site **[2]**.

3.6.1. Suture being placed
3.6.2. Ointment being applied

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.4., 3.3., 3.4., 3.5.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.5.

Results

4. Results: Representative Morphologic and Histologic Effects of Lumbar Spine Instability (LSI)

4.1. 3D histomorphometric analysis allows intervertebral disc measurement **[1]**, while 3D structural analysis allows quantification of the total tissue volume **[2]**.

4.1.1. LAB MEDIA: Figures 4A and 4B *Video Editor: please emphasize red color in Figure 4A*

4.1.2. LAB MEDIA: Figures 4A and 4B *Video Editor: please emphasize TV text in Figure 4B*

4.2. The intervertebral disc volume significantly increases 1 week after surgery **[1]** before decreasing 2-16 weeks after the procedure **[2]**, consistent with a decrease in the intervertebral disc height measured over the same time period **[3]**.

4.2.1. LAB MEDIA: Figure 4C *Video Editor: please emphasize black 1 w data bar*

4.2.2. LAB MEDIA: Figure 4C *Video Editor: please emphasize black 2 w to 16 w data bars*

4.2.3. LAB MEDIA: Figures 4C, 4F, and 4G *Video Editor: please emphasize LSI images from 2 w to 16 w*

4.3. Increased cavities within the cranial endplates are also observed in LSI (L-S-eye) mice **[1]**, as quantified by an increased percentage of trabecular separation values greater or equal to 0.089 **[2]** and a significant increase in the endplate volume at 16 weeks post-surgery **[3]**.

4.3.1. LAB MEDIA: Figures 5D and 5E *Video Editor: please emphasize LSI images in Figure 5D*

4.3.2. LAB MEDIA: Figures 5D and 5E *Video Editor: please emphasize LSI pie graphs in Figure 5E*

4.3.3. LAB MEDIA: Figure 5F *Video Editor: please emphasize black 16 w data bar*

4.4. Caudal endplates exhibit a similar phenotype **[1]**, indicating that LSI leads to endplate hypertrophy and an increase in cavity number **[2]**.

4.4.1. LAB MEDIA: Figures 5G and 5H *Video Editor: please emphasize LSI pie graphs and black data bars*

4.4.2. LAB MEDIA: Figures 5G and 5H

4.5. L-five vertebra volumes slightly increase post-surgery **[1]** with statistical differences observed at 16-weeks **[2]**.

4.5.1. LAB MEDIA: Figure 6B *Video Editor: please emphasize black data bars*
4.5.2. LAB MEDIA: Figure 6B *Video Editor: please add/emphasize asterisk at 16 w*

4.6. A significant decrease in the bone to total tissue volume ratio is also present 16 weeks after surgery **[1]**, indicating that LSI causes vertebral bone loss at a later stage **[2]**.

4.6.1. LAB MEDIA: Figures 6A and 6C *Video Editor: please emphasize LSI image and black data bars*
4.6.2. LAB MEDIA: Figures 6A and 6C

4.7. A reduction in intracellular vacuoles of nucleus pulposus cells is also accelerated in LSI animals **[1]**, with increased numbers of osteoclasts observed in LSI endplates **[2]**.

4.7.1. LAB MEDIA: Figures 7A and 7B *Video Editor: please emphasize LSI images in Figure 7B*
4.7.2. LAB MEDIA: Figures 7E and 7F *Video Editor: please emphasize LSI images in Figure 7E and black data bars in Figure 7F*

Conclusion

5. Conclusion Interview Statements

5.1. **Shufen Liu**: The model can be modified by targeting different lumbar vertebrae, such as L5 only or from L1 to L5 **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera