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Title: A proinflammatory, degenerative organ culture model to simulate early-stage intervertebral disc disease

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

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 videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, 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? **No**

Current Protocol Length

Number of Steps: 13
Number of Shots: 30

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Babak Saravi:** This protocol aims to simulate in vivo conditions of intervertebral disc degeneration to investigate its pathophysiology without the need of animal study.

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

1.2. **Zhen Li:** Compared with in vitro cell culture, the bioreactor organ culture technique maintains the cells within their native biological and biomechanical microenvironment, in addition to the supply of controllable and reproducible culture conditions.

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

Protocol

2. Dissection of the bovine intervertebral disc

2.1. Start with rinsing the entire bovine tail thoroughly with tap water to remove dirt and hair on the surface **[1]**, then immerse the tail in 1% betadine solution for 10 minutes to disinfect the surface **[2]**.

2.1.1. WIDE: Establishing shot of talent rinsing the whole tail.

2.1.2. Talent putting the whole tail in 1% betadine solution.

2.2. Use a scalpel number 20 to remove the soft tissue from the caudal spine to facilitate the identification of the intervertebral discs, or IVDs **[1-TXT].** Remove the spinous and transverse processes of the vertebrae with a bone removal plier **[2].**

2.2.1. Talent removing soft tissue with scalpel. **TEXT: Desired diameter for IVDs: 15-20mm**

2.2.2. Talent removing processes of the vertebrae with plier.

2.3. Cut transversely with bone pliers through the middle of each vertebral body to obtain individual motion segments **[1]**, then put the collected motion segments in a Petri dish with a gauze wetted in Ringer's solution **[2].**

2.3.1. Talent cutting vertebral body with plier.

2.3.2. Talent placing motion segment in a petri dish.

2.4. Locate the IVD and vertebra by moving the motion segments gently **[1].** Then, identify the location of the growth plate by touching and finding the convex side of the bony endplate **[2].** *Videographer: This step is difficult and important!*

2.4.1. Talent moving motion segments to locate IVD.

2.4.2. Talent showing location of the growth plate.

2.5. Cool the blade of the band saw with Ringer's solution **[1]** and use it to make two parallel cuts in the growth plate of the IVDs, one on each side **[2].** Transfer the IVDs to a clean Petri dish with clean gauze wetted with Ringer's solution **[3].** *Videographer: This step is difficult and important!*

2.5.1. Talent wetting blade with Ringer's solution.

2.5.2. Talent making cuts in growth plate.

2.5.3. Talent placing IVDs in petri dish.

2.6. Scrape off the vertebral body and growth plate using the scalpel blade, leaving the endplate intact **[1].** Position the two surfaces flat and parallel for the loading procedure

[2] and transfer scraped IVDs to a fresh Petri dish with gauze wetted with Ringer's solution **[3].** *Videographer: This step is important!*

2.6.1. Talent scraping vertebral body.

2.6.2. IVDs laying flat in a petri dish.

2.6.3. Talent transferring IVDs to a fresh petri dish.

2.7. Measure the disc height and diameter with a caliper **[1]**, then clean the blood clots in the vertebrae bone with Ringer's solution using a jet lavage system **[2].** Disinfect IVDs in PBS and 10% Penicillin-Streptomycin with shaking for 15 minutes **[3].** Rinse off the high concentration antibiotics with PBS and 1% Penicillin-Streptomycin **[4].**

2.7.1. Talent measuring disc height with a caliper.

2.7.2. Talent cleaning the vertebrae.

2.7.3. Shot of IVDs in Falcon tube with PBS+1% P/S on a shaker.

2.7.4. Talent aspirating the PBS+10% P/S from the Falcon tube, and adding PBS+1% P/S.

3. IVD culture and loading

3.1. Transfer discs to IVD chambers containing 5 milliliters of IVD culture medium **[1]** and place them in the bioreactor system within an incubator at 37 degrees Celsius with 85% humidity and 5% carbon dioxide **[2].**

3.1.1. Talent placing IVD in chamber.

3.1.2. Talent placing the chambers in the bioreactor.

3.2. Culture the discs for 4 days within a bioreactor system by maintaining different loading conditions according to the experimental groups **[1].** In the physiological control group **[2]**, use a loading protocol of 0.02 to 0.2 Millipascals and 0.2 Hertz for 2 hours per day **[3]**. *Videographer: This step is important!*

3.2.1. Culture chambers in the bioreactor system with different conditions.

3.2.2. IVD cultured under physiological loading

3.2.3. Computer screens showing the dynamic force and frequency curves of physiological loading condition

3.3. In the pathological group, maintain degenerative loading **[1]** conditions at 0.32 to 0.5 millipascal and 5 Hertz for 2 hours per day **[2]**. Between the loading procedures, place the IVDs in six-well plates with 7 milliliters of IVD culture medium for free-swelling recovery **[3].** *Videographer: This step is important!*

3.3.1. Shot of IVD under degenerative loading

3.3.2. Computer screens showing the dynamic force and frequency curves of degenerative loading condition.

3.3.3. Talent placing IVDs for free swelling recovery.

3.4. Measure the disc height daily with a caliper after the free swelling period and after dynamic loading for the experimental duration **[1].**

3.4.1. Talent measuring disc height with a caliper.

4. Intradiscal TNF-α injection

4.1. After the first dynamic loading cycle on day 1, directly place the IVDs in a Petri dish in a vertical position **[1]** and stabilize them with a tweezer **[2].** Inject recombinant TNF-α *('T-N-F-alpha')* with a 30-gauge insulin needle, slowly at a speed of approximately 70 microliters per minute into the IVDs of the pathological group **[3-TXT].** *Videographer: This step is important!*

4.1.1. Talent placing IVDs in a petri dish.

4.1.2. Talent stabilizing the IVD with tweezer.

4.1.3. Talent injecting TNF-α into the tissue. **TEXT: 100 ng TNF-α in 70 µL of PBS per IVD**

4.2. After injecting, pull the syringe halfway back **[1]** and pull the plunger to create a vacuum that prevents the injected solution from leaking, then remove the syringe completely from the IVD **[2].** *Videographer: This step is important!*

4.2.1. Talent pulling the syringe halfway back within the IVD.

4.2.2. Talent pulling the plunger and then slowly removing syringe from IVD.

Results

5. Results: **Assessment of intervertebral disc degeneration caused by TNF-α injection**

5.1. Degenerative loading combined with limited nutrition supply and TNF-α *(pronounce 'T-N-F-alpha')* injection caused a significant increase in the gene expression of proinflammatory markers interleukin 6 and interleukin 8 in NP tissue after 4 days of culture **[1].**

5.1.1. LAB MEDIA: Figure 2. *Video Editor: emphasize on the left graph for NP inflammatory genes.*

5.2. The interleukin 8 protein release into the conditioned medium showed a marked increase in the pathological group on day 2 and day 4 **[1].**

5.2.1. LAB MEDIA: Figure 3. *Video Editor: emphasize on bars for day 2 after load and day 4 after FS.*

5.3. Disc height reduction after loading was higher in the pathological group compared to the physiological group **[1]** and the differences were more pronounced after days 2 and 3 compared with day 1, indicating a progressive effect of the degenerative and inflammatory conditions **[2].**

5.3.1. LAB MEDIA: Figure 4. *Video Editor: emphasize on bars below the axis.*

5.3.2. LAB MEDIA: Figure 4. *Video Editor: emphasize on bars below the axis labelled as Day 2 after load and Day 3 after load.*

Conclusion

6. Conclusion Interview Statements

6.1. **Babak Saravi:** Standardized dissection technique is important for reproducible IVD whole organ culture models. A bioreactor is required to apply physiological or degenerative loading on IVDs to simulate various in vivo biomechanical conditions.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1 for dissection technique and 3.2.1 for bioreactor.*

6.2. **Zhen Li:** This technique can be applied on human IVD explants, which is of high clinical relevance. It is a novel preclinical model for developing effective treatments on early-stage disc degeneration.

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