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Title: Animal Model of Implant-Associated Infections in Mice

Authors and Affiliations:
Jiawei Mei*, Quan Liu*, Xianli Hu*, Wenzhi Wang*, Ruixiang Ma*, Wanbo Zhu, Chen Zhu, Zheng Su

Department of Orthopedics, Centre for Leading Medicine and Advanced Technologies of IHM, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China

***These authors contributed equally to this work**

Corresponding Authors:
Wanbo Zhu (zhuwanbo@sjtu.edu.cn)
Chen Zhu (zhuchena@ustc.edu.cn)
Zheng Su (suz924@ustc.edu.cn)
Email Addresses for All Authors:
Jiawei Mei (meijiawei@mail.ustc.edu.cn)
Quan Liu (doctorliuq@mail.ustc.edu.cn)
Xianli Hu (hjb999999@mail.ustc.edu.cn)
Wenzhi Wang (wangwenzhi@mail.ustc.edu.cn)
Ruixiang Ma (maruixiang@mail.ustc.edu.cn)
Wanbo Zhu (zhuwanbo@sjtu.edu.cn)
Chen Zhu (zhuchena@ustc.edu.cn)
Zheng Su (suz924@ustc.edu.cn)

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. **Filming location:** Will the filming need to take place in multiple locations?
Yes. The First Affiliated Hospital of USTC is 11 kilometers away from the Institute of Health and Medicine, Hefei Comprehensive National Science Center.

Current Protocol Length
Number of Steps: 14
Number of Shots: 27

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Chen Zhu:** Establishing stable animal models provides reliable platforms for testing implant-associated infection therapies while investigating pathological status and immune responses within the infection microenvironment.

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

:

What technologies are currently used to advance research in your field?

1.2. **Zheng Su:** Emerging techniques like bioluminescent bacteria, visualization, and gene editing provide enhanced tools for studying bacterial lifecycles and traits in implant-associated infection.

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

What are the current experimental challenges?

1.3. **Ruixiang Ma:** The current challenge was constructing a stable, reproducible model that mimics the complex *in vivo* microenvironment and clinical reality of implant-associated infections.

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

What significant findings have you established in your field?

1.4. **Wanbo Zhu:** Superior to subcutaneous abscess models, implant-associated infection models offer enhanced clinical relevance, sustained infections, improved reproducibility, and greater biosafety.

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

NOTE: Please add missing media title card

What research gap are you addressing with your protocol?

1.5. **Jiawei Mei:** This modeling method is highly safe and reliable, enables comprehensive investigation of pathophysiological mechanisms, and supports the development of diagnostic criteria with targeted therapeutic strategies.

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

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer:

- ***Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.***
- ***Also, kindly note that testimonial statements will be presented live by the authors, offering their spontaneous perspectives.***

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Dr. Chen Zhu, Director of Orthopedic Department, Chief Physician, Professor** :
(authors will present their testimonial statements live)

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Dr. Zheng Su, Associate Researcher**(authors will present their testimonial statements live)

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Institute of Health and Medicine, Hefei Comprehensive National Science Center

Protocol

2. Preparation of *Staphylococcus aureus* Suspension for Subcutaneous Implant Infection Model

Demonstrator: Quan Liu

2.1. To begin, obtain cultures of *Staphylococcus aureus* **[1].** Remove a loopful of the culture with an inoculation loop **[2],** streak the suspension onto a blood agar plate and incubate **[3-TXT]**.

2.1.1. WIDE: Talent holding tubes containing *Staphylococcus aureus* colonies.

2.1.2. Shot of a loopful of culture being taken out with an inoculation loop.

2.1.3. Talent streaking the suspension on a blood agar plate. **TXT: Incubation: 37 °C, 24 h**

2.2. Select a single, round and smooth independent colony with golden yellow pigmentation and a clear hemolysis zone **[1]**. With a sterile loop, inoculate it into a 15-milliliter sterile centrifuge tube containing 5 milliliters of sterile tryptic soy broth **[2]**. Place the tube in a shaking incubator set to 37 degrees Celsius and shake at 200 revolutions per minute for 12 hours **[3]**.

2.2.1. Shot of the single colony displaying typical morphology, on the agar plate.

2.2.2. Talent transferring the colony into a centrifuge tube containing tryptic soy broth.

2.2.3. Talent placing the tube into a shaking incubator and closing the lid.

2.3. Dilute the bacterial suspension with tryptic soy broth at a 1 to 50 ratio and incubate again **[1]**. Then use a spectrophotometer to measure and record the optical density at 600 nanometers to confirm the bacteria have reached the logarithmic growth phase **[2-TXT]**.

2.3.1. Talent diluting the bacterial suspension with TSB at a 1:50 ratio .

2.3.2. Talent placing the bacterial suspension cuvette into a spectrophotometer and reading the OD600 value on the screen. **TXT: Correlate the optical density values to the CFU/mL**

2.4. Next, pipette 3 milliliters of the bacterial suspension into a tube **[1].** Mix it with 3 milliliters of cold sterile PBS **[2-TXT]**.

2.4.1. Talent pipetting and mixing bacterial suspension in a centrifuge tube.

2.4.2. Shot of cold phosphate-buffered saline being added to the suspension. **TXT: Bacterial concentration: 2 x 10^5 CFU/mL**

2.5. Centrifuge the mixture at 3,000 *g* for 10 minutes at 4 degrees Celsius **[1]**. Using a pipette, discard the supernatant **[2]** and gently resuspend the bacterial pellet in PBS **[3-TXT]**.

2.5.1. Talent placing the centrifuge tube into a centrifuge and closing the lid.

2.5.2. Talent pipetting out the supernatant.

2.5.3. Talent resuspending the bacterial pellet with a pipette. **TXT: Perform this step in duplicate**

2.6. Resuspend the washed bacterial pellet in PBS for further experimentation **[1]**.

2.6.1. Talent gently pipetting the pellet in phosphate-buffered saline to ensure even resuspension.

3. Mouse Surgical Procedure and Postoperative Evaluation in an Implant-Associated Infection Model

Demonstrator: Jiawei Mei and Wenzhi Wang

3.1. Randomly divide 20 C57BL/6J *(C-Fifty-Seven-B-L-Bar-Six-J)* wild-type mice into two groups of the implant-associated infection group and the subcutaneous abscess group **[1]**. Apply distinct tail markings to each mouse for individual identification **[2]**.

3.1.1. Talent assigning mice into two separate labelled cages for each group.

3.1.2. Talent using a marker or dye to apply identification marks on each mouse's tail.

3.2. After anesthetizing and preparing the skin of the animals, use sterile surgical blades to make a 1-centimeter incision in the dorsal region of each mouse **[1]**. Then insert a sterile titanium implant into the subcutaneous pocket created by the incision **[2]**.

3.2.1. Talent making a dorsal incision on an anesthetized mouse with a sterile surgical blade.

3.2.2. Talent inserting a titanium implant into the subcutaneous space.

3.3. Now use a sterile 1-milliliter syringe to inject 100 microliters of *Staphylococcus aureus* bacterial suspension directly onto the titanium surface **[1-TXT]**. Then, close the incision with 3-0 *(Three-Oh)* non-absorbable sutures **[2]**.

3.3.1. Talent injecting bacterial suspension onto the titanium implant using a syringe. **TXT: Bacterial concentration: 2 x 10^5 CFU/mL**

3.3.2. Talent closing the incision with sutures.

3.4. For the subcutaneous abscess group, create, disinfect, and suture the incision directly without implant insertion **[1]**. Inject 100 microliters of *Staphylococcus aureus* suspension into the incision site using a sterile 1 milliliter syringe **[2-TXT]**.

3.4.1. Talent creating, disinfecting, suturing the incision on the subcutaneous abscess group.

3.4.2. Talent injecting bacterial suspension into the incision of another mouse model using a sterile syringe. **TXT: Bacterial concentration: 2 x 10^5 CFU/mL**

3.5. To evaluate the infections in peripheral tissue, place the harvested tissue sample into a sterile tube **[1]**. Add an equal mass of sterile PBS and three sterile steel grinding beads to each tube **[2]**.

3.5.1. Talent placing a dissected tissue sample into a sterile tube.

3.5.2. Talent adding phosphate-buffered saline and grinding beads into the tube.

3.6. Homogenize the tissues in three cycles at 70 hertz for 60 seconds each, with a 20 second pause between cycles **[1]**. After homogenization, vortex the samples for 5 minutes **[2]**.

3.6.1. Talent placing the tubes into a tissue homogenizer and starting the grinding cycles.

3.6.2. Talent vortexing the homogenized samples.

3.7. Now, prepare serial dilutions of the tissue homogenate with PBS **[1-TXT]**. Using a micropipette, drop 15 microliters of each diluted homogenate onto designated sections of blood agar plates **[2]**. Then incubate the blood agar plates at 37 degrees Celsius without shaking for 24 hours **[3]**.

3.7.1. Talent pipetting homogenate into dilution tubes and performing serial dilutions. **TXT: Dilutions: 10^1 to 10^5**

3.7.2. Talent carefully dispensing drops of diluted homogenate onto different areas of blood agar plates.

3.7.3. Talent placing agar plates inside an incubator and closing the door and counting.

Results

4. Results

4.1. The implant-associated infection group developed visible wound rupture by day 3 **[1]**. On day 10, both groups of mice showed signs of wound recovery **[2]**, with the subcutaneous abscess group showing more pronounced recovery than implant-associated infection group on day 14 **[3].**

4.1.1. LAB MEDIA: Figure 3. *Video editor: Highlight the day 3 image of the implant-associated infection row*

4.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight the day 10 images of both rows*

4.1.3. LAB MEDIA: Figure 3. *Video editor: Highlight the day 14 images of both rows.*

4.2. Bacterial cultures from infected tissue showed sustained high bacterial growth in the implant-associated infection group at all time points **[1]**, whereas the subcutaneous abscess group showed a progressive reduction in bacterial colonies from day 3 to day 14 **[2]**.

4.2.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the three culture plates from the implant-associated infection row*

4.2.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the red curve*

4.3. Scanning electron microscopy showed increasingly dense bacterial coverage on the titanium sheets in the implant-associated infection group from day 3 to day 14 **[1]**.

4.3.1. LAB MEDIA: Figure 5. *Video editor: Sequentially highlight the images from Day 3 to Day 14*

4.4. Giemsa staining revealed a marked decrease in bacteria in the subcutaneous abscess group by day 14 **[1]**, whereas the implant-associated infection group retained high bacterial presence throughout the period **[2]**.

4.4.1. LAB MEDIA: Figure 6. Video editor: Highlight the sparse red-arrow-marked regions in the subcutaneous abscess row on day 14.

4.4.2. LAB MEDIA: Figure 6. Video editor: Highlight the dense red-arrow-marked regions in the implant-associated infection row on days 3, 7, and 14.

4.5. Hematoxylin and eosin staining demonstrated that inflammatory cell infiltration in the subcutaneous abscess group diminished significantly by day 14 **[1]**, while the implant-associated infection group showed persistently dense cellular infiltration **[2]**.

4.5.1. LAB MEDIA: Figure 7. *Video editor: Highlight the image in the "subcutaneous*

abscess" row on day 14.

4.5.2. LAB MEDIA: Figure 7. *Video editor: Highlight the images of the bottom show " implant-Associated Infections"*

4.6. Histological examination of heart, liver, spleen, lung, and kidney tissues showed no visible lesions or abnormalities in the implant-associated infection group compared with the control group **[1]**.

4.6.1. LAB MEDIA: Figure 8. *Video editor: Highlight the bottom row (implant-associated infection)*

Pronunciation Guide

1. **Staphylococcus**
   Source: Merriam-Webster
   IPA: /ˌstæf.ə.ləˈkɑː.kəs/
   Spelling: staff-uh-luh-KAH-kus
   listening.comGrammarVocab *(dictionary for "species," but pronunciation style consistent with bacterial terms—see context)*
2. **Microbiology**
   Source: Merriam-Webster
   IPA: /ˌmī-krō-bī-ˈä-lə-jē/
   Spelling: my-kroh-bye-OL-uh-jee
   Merriam-Webster+1Word Finder
3. **Bacteria**
   Source: Merriam-Webster
   IPA: /bækˈtɪəriə/ or /bækˈtɪriə/
   Spelling: bak-TEE-ree-uh
   Word Finder+15Merriam-Webster+15Merriam-Webster+15Indian Chemistry+4GrammarVocab+4iew.com+4
4. **Genus**
   Source: Merriam-Webster
   IPA: /ˈjē-nəs/
   Spelling: JEE-nuhs
   Wikipedia+1Word Finder+3Merriam-Webster+3GrammarVocab+3
5. **Species**
   Source: Merriam-Webster
   IPA: /ˈspē-shēz/ or /-sēz/
   Spelling: SPEE-sheez
   Word Finder+15Merriam-Webster+15Merriam-Webster+15iew.com+1
6. **Subspecies**
   Source: Merriam-Webster
   IPA: /ˈsəb-ˌspē-shēz/ or /-sēz/
   Spelling: sub-SPEE-sheez
   Merriam-WebsterMerriam-Webster
7. **Phylum**
   Source: Merriam-Webster
   IPA: /ˈfī-ləm/
   Spelling: FY-luhm
   Merriam-Webster+1iew.com
8. **Animalcule**
   Source: Merriam-Webster
   IPA: /ˌan-ə-ˈmal-kyül/
   Spelling: an-uh-MAL-kyool
   Merriam-Webster+2YourDictionary+2Word Finder

9. **Binomial Nomenclature**
   Source: Merriam-Webster
   IPA: /bī-ˈnō-mē-əl nō-mə-ˈklā-chər/
   Spelling: bye-NOH-mee-uhl noh-muh-KLAY-cher
   Word Finder+15Merriam-Webster+15Merriam-Webster+15
10. **Cytokinesis** (commonly used in cell/microbial growth contexts)
    Source: YourDictionary (includes phonetic spelling)
    IPA: /ˌsaɪ.t̬oʊ.kɪˈniː.sɪs/
    Spelling: sigh-toe-kih-NEE-sis