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Title: A Unique Mouse Model for Quantitative Assessment of Biofilm Formation on Surgical Implants in Subcutaneous Abscess

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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. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 06
Number of Shots: 18

Introduction

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

1.1. **Mitsuhiro Nishizawa:** Our research aims to develop innovative treatments for implant-related infections. To evaluate novel biomaterials with potential antimicrobial properties, we develop a more precise in vivo testing approach.
   1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.2*

What research gap are you addressing with your protocol?

1.2. **Mitsuhiro Nishizawa:** Prior animal models are typically limited to producing a single implant-related infection per animal, which can lead to significant variability in the average outcomes obtained from multiple animals.
   1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What advantage does your protocol offer compared to other techniques?

1.3. **Mitsuhiro Nishizawa:** Our mouse model allows a direct comparison of the two implants subjected to identical infectious conditions within a single animal, hence providing a precise assessment of the antibacterial activity.
   1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.2*

How will your findings advance research in your field?

1.4. **Mitsuhiro Nishizawa:** Our in vivo experimental methodology will accelerate research on potentially antimicrobial biomaterials, contributing to the development of new treatments for implant-related infections in the future.
   1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1.1*

What new scientific questions have your results paved the way for?

1.5. **Mitsuhiro Nishizawa:** Our experimental approach is replicable with standard equipment and simple procedures in a conventional research setting, hence enhancing comprehension of the mechanisms behind antibacterial activity.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.3.1*

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee at the University of California, San Francisco (UCSF)

Protocol

2. Creation of a Mature Subcutaneous Pouch in Mice

Demonstrator: Mitsuhiro Nishizawa

2.1. To begin, position the anesthetized mouse on the surgical bed **[1]**.
  2.1.1. WIDE: Talent placing the anesthetized mouse onto the surgical bed. **TXT: Anesthesia: 2% Isoflurane**

2.2. Fill a 10-milliliter syringe with sterile air **[1]** and attach a 27-gauge needle to the outlet **[2]**. Now, gently pinch and elevate the base of the mouse's neck to create space between the subcutaneous tissue and the fascia **[3]**. Place the needle into the midline between the mouse's scapulae **[4]** and inject 3 milliliters of sterile air subcutaneously to create the air pouch **[5]**.
  2.2.1. Talent filling a 10 milliliter syringe with sterile air.
  2.2.2. Shot of attaching a 27-gauge needle to the syringe.
  2.2.3. Talent pinching and elevating the base of the mouse's neck.
  2.2.4. Close-up of the needle being inserted between the scapulae.
  2.2.5. Shot of pushing the plunger and injecting sterile air into the animal.
  Videographer: Please obtain multiple reusable shots for this step. It will be used again **NOTE**: Vid took 5 takes

2.3. Then, return the mouse to a cage warmed with a thermal pad **[1]**. Inject 3 milliliters of sterile air every 2 days to maintain the cavity's inflation and create a mature pouch **[2]**.
  2.3.1. Talent placing the mouse back into a warmed cage.
  2.3.2. *Reuse 2.2.5*

3. Implantation of Connected Wires and Bacterial Inoculation

3.1. After anesthetizing the animal, inject it with bupivacaine subcutaneously **[1-TXT]**. Make a 3-millimeter midline longitudinal incision at the top of the pouch **[2]** and insert an 18-gauge needle containing the connected wires into the pouch through the hole **[3]**. Push out the wires using an inner syringe of a 25-gauge spinal needle **[4]**.
  3.1.1. Talent injecting bupivacaine subcutaneously to the animal. **TXT: Perform this step 7 days after the first air injection**
  3.1.2. Talent making a 3 millimeter midline incision.
  3.1.3. Talent inserting an 18-gauge needle containing the connected wires into the pouch through the hole. Videographer's **NOTE**: first take was better
  3.1.4. Shot of pushing out the wires. Videographer's **NOTE**: second take was better

3.2. Leaving the tip of the 18-gauge needle inside the pouch **[1]**, gently remove the inner cylinder and inject Xen36 *(zen-36)* culture using the syringe **[2-TXT]**. Carefully remove all needles **[3]**, close the skin using a wound clip **[4]**, and seal with topical skin adhesive **[5]**.

3.2.1. Shot of the needle tip placed inside the pouch. Videographer's **NOTE**: 3.2.1 and 3.2.2 were shot together

3.2.2. Talent injecting Xen36 culture inside the pouch. **TXT: Xen36: 3 mL of 1 x 10^5 CFU/mL per**

3.2.3. Talent removing needles. Videographer's **NOTE**: 3.2.3 and 3.2.4 were shot together

3.2.4. Talent closing the skin with wound clip.

3.2.5. Talent applying adhesive to the wound.

3.3. Finally, return the mouse to a cage warmed with a thermal pad for monitoring **[1-TXT]**.

3.3.1. Talent placing the mouse back into a warmed cage. **TXT: Extract the implants from the subcutaneous abscess; Quantify the formed biofilm**

Results

4. Results

4.1. Crystal violet staining showed consistent biofilm formation on both wires without observable differences **[1]**.
4.1.1. LAB MEDIA: Figure 7A.

4.2. Absorbance measurements from the dissolved crystal violet assay showed no statistically significant differences in bacterial load between the two wires **[1]**.
4.2.1. LAB MEDIA: Figure 7B

4.3. Colony-forming unit counting **[1]** and quantitative PCR analysis of 16S ribosomal RNA and LuxA *(lux-A)* genes showed no statistically significant differences in bacterial load between the two wires **[2]**.
4.3.1. LAB MEDIA: Figure 7C.
4.3.2. LAB MEDIA: Figure 7D.

Pronunciation guide:

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1. Bupivacaine

- **Pronunciation link**: https://www.merriam-webster.com/medical/bupivacaine
- **IPA**: /bjuːˈpɪv.əˌkeɪn/
- **Phonetic Spelling**: byoo-PIH-vuh-kayn(merriam-webster.com)

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2. Xen36

- **Pronunciation link**: No confirmed link found
- **IPA**: /zɛn θɜːti sɪks/
- **Phonetic Spelling**: zen THIR-tee-six

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3. Crystal Violet

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/crystal%20violet
- **IPA**: /ˈkrɪs.təl ˈvaɪ.ə.lət/
- **Phonetic Spelling**: KRIS-tuhl VY-uh-luht(merriam-webster.com)

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4. Colony-Forming Unit

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˈkɒl.ə.ni ˈfɔː.mɪŋ ˈjuː.nɪt/
- **Phonetic Spelling**: KOL-uh-nee FOR-ming YOO-nit

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5. LuxA

- **Pronunciation link**: https://www.howtopronounce.com/luxa
- **IPA**: /ˈlʌks.eɪ/
- **Phonetic Spelling**: LUKS-ay(howtopronounce.com, howtosay.co.in)