FINAL SCRIPT: APPROVED FOR FILMING

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Title: PET/CT With [^{68}Ga]-NOTA-FAP-2286 for Imaging of Tendon Injuries in Rat Achilles Tendon Injury Models

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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? **Yes**

Videographer: Please film the screen for the following shots labelled SCREEN
SCREEN: 2.6.1-2.6.3, 2.7.1-2.7.1, 2.8.1-2.8.2

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

Current Protocol Length
Number of Steps: 09
Number of Shots: 22

Introduction

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

1.1. **Qianhe Xu:** Our research investigates the potential of FAP-targeted PET/CT imaging using [68Ga]-NOTA-FAP-2286 for accurate detection and evaluation of tendon injuries **[1]**.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.4.2*

What are the most recent developments in your field of research?

1.2. **Qianhe Xu:** The latest advances in my research is the use of FAP-targeted PET/CT for precise imaging of tendon injuries **[1]**.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1.2*

What are the current experimental challenges?

1.3. **Qianhe Xu:** Current experimental challenges include optimizing tracer specificity, minimizing background signals, and correlating imaging findings with histopathology **[1]**.

1.3.1. 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.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at First Affiliated Hospital of Zhejiang University School of Medicine

Protocol

2. Imaging Achilles Tendon Injury in Murine Model

Demonstrator: Qianhe Xu

2.1. To begin, secure an anesthetized rat onto a fixation board **[1-TXT]**. Sterilize the surgical instruments with alcohol **[2]**. Then disinfect the surgical site thoroughly **[3]**.

2.1.1. WIDE: Talent placing the unconscious rat on a fixation board and securing it while applying the gas mask. **TXT: Anesthesia: Isoflurane-oxygen inhalation**

2.1.2. Talent sterilizing surgical instruments by wiping them with alcohol.

2.1.3. Talent disinfecting the hind limb using appropriate disinfectant.

2.2. With a scalpel, make a longitudinal incision to expose the Achilles tendon **[1]**. Compress the tendon using hemostatic forceps until it is flattened **[2]**. Now close the incision with sutures **[3]**.

2.2.1. Talent making an incision along the hind limb to reveal the Achilles tendon.

2.2.2. Talent compressing the Achilles tendon with hemostatic forceps.

2.2.3. Talent suturing the incision site.

2.3. To establish the Achilles tendon rupture model, grasp the Achilles tendon with hemostatic forceps **[1]**. Use surgical scissors to create a full-thickness midline incision in the tendon **[2]**. Then, suture the severed tendon and close the skin incision **[3]**.

2.3.1. Talent holding the tendon using hemostatic forceps.

2.3.2. Talent performing a full-thickness cut on the tendon using surgical scissors.

2.3.3. Talent suturing both the tendon and the overlying skin.

2.4. For PET *(P-E-T)* imaging, secure the rat in a restrainer **[1]**. Administer 500 microcurie of ^{68}GA-NOTA-FAP2286 *(G-A-sixty-eight-Not-Ah-Fap-Two-Two-Eight-Six)* via the tail vein to both groups of rats **[2]**.

2.4.1. Talent placing the rat into the restrainer.

2.4.2. Talent injecting ^{68}GA-NOTA-FAP2286 through the tail vein.

2.5. Position the fully anesthetized rat in the scanning field with the Achilles tendon aligned at the center **[1]**. Turn on the computer connected to the small animal PET *(P-E-T)* system **[2]**.

2.5.1. Talent positioning the rat in the scanning field ensuring proper alignment.

2.5.2. SCREEN: Powering on the PET system computer.
Videographer: please capture the screen of the instrument for all shots labelled SCREEN

2.6. Now click on **CT** *(C-T)* **Acquisition** to begin the preheating of the computed tomography system **[1]**. Once the preheating is complete, select the **scanning protocol [2]**. Then open the corresponding scanning workflow which has a preset **scan** duration of **10 min** *(minutes)* **[3]**.
Videographer: please capture the screen of the instrument for all shots labelled SCREEN

2.6.1. SCREEN: Click on CT Acquisition and verify the preheating process is in progress.

2.6.2. SCREEN: Selecting appropriate scanning protocol from the menu.

2.6.3. SCREEN: Opening the scanning workflow and verifying the preset scan time.

2.7. Click on **Scout View** to verify the Achilles tendon is at the center of the scanning field **[1]**. If the positioning is accurate, click **Start Workflow** to initiate the PET and CT scans **[2]**.
Videographer: please capture the screen of the instrument for all shots labelled SCREEN

2.7.1. SCREEN: Clicking on Scout View and verifying Achilles tendon alignment.

2.7.2. SCREEN: Clicking on Start Workflow to begin imaging.

2.8. Set the scan parameters **NCT** *(N-C-T)* **bed view** from **0** to **36.2 to 76.4 mm** *(millimeters)*, **CT scan time** of **150 s/one bed** *(seconds per bed),* and **PET Acquire by time** set to **600s** *(seconds)* **[1]**. Then set the **Photopeak Energy Level** to **511 KeV** *(kiloelectronvolt),* **Lower-Level Discrimination** to **350 KeV**, **Upper-Level Discrimination** to **650 KeV**, and **Timing Window** to **3.432 ns** *(nanoseconds)* **[2]**.

2.8.1. SCREEN: The NCT bed view, CT Scan time and PET Acquire by Time parameters are being set.

2.8.2. SCREEN: The Photopeak Energy Level, Lower-Level discrimination, Upper-level discrimination and Timing Window parameters are being set.

Results

3. Representative Results

3.1. ^{68}GA-NOTA-FAP-2286 showed a high radiochemical purity exceeding 95% **[1]**. Tracer uptake began at the site of Achilles tendon rupture or injury 30 minutes post injection and intensified significantly over 90 minutes, showing higher signals compared to the surrounding muscle and the normal tendon **[2]**.

3.1.1. LAB MEDIA: Figure 4. *Video editor: Highlight the sharp tall peak around the 10-minute mark on the top graph where the line spikes upwards.*

3.1.2. LAB MEDIA: Figure 5. *Video editor: Highlight the red and yellow areas on the right leg of each mouse in images A to F. Keep emphasis on C*

3.2. Computed tomography images failed to visually differentiate between injured-ruptured and normal Achilles tendons, showing similar grayscale appearances across all samples **[1]**.

3.2.1. LAB MEDIA: Figure 6. *Video editor: Show the images labeled "rupture" (A to C) and "injury" (D to F),*

3.3. Maximum intensity projection imaging demonstrated that ^{68}GA-NOTA-FAP-2286 uptake on the injury or rupture side increased from the first week and peaked at the second week, indicating elevated fibroblast activation protein expression during tendon repair **[1]**.

3.3.1. LAB MEDIA: Figure 7. *Video editor: Please sequentially highlight the images from A to F. Keep emphasis on B*