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Title: Enhanced Gene Delivery and Expression Using Intraosseous Injection of Chitosan Nanoparticles Encapsulated Adenine Base Editor Plasmids

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

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

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

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **05/10/25**

When you are ready to submit your video files, please contact our China Location Producer, Yuan Yue.

Current Protocol Length

Number of Steps: 21
Number of Shots: 49

Introduction

1.1. **Min Li:** We propose a gene therapy method that directly delivers Adenine Base Editor-coated chitosan to the bone marrow through intraosseal injection **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.6.3*

What are the current experimental challenges?

1.2. **Min Li:** The transfection efficiency of exogenous plasmids entering experimental animals is relatively low, and the introduction of plasmids for long-term gene expression may be affected by the immune system **[1]**.

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

What research gap are you addressing with your protocol?

1.3. **Min Li:** We used chitosan to encapsulate Adenine Base Editor plasmids and directly delivered the complex to the bone marrow of mice through intraosseal injection, making it suitable for diseases caused by abnormal osteoclast function **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.8.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Min Li:** Our protocol offers high transfection efficiency, small biological damage and high gene expression efficiency **[1].**

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

How will your findings advance research in your field?

1.5. **Min Li:** Our novel strategy is not only suitable for diseases caused by abnormal osteoclast function, but also holds significant potential for advancing the field of gene therapy **[1]**.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.*

Ethics Title Card

This research has been approved by the Animal Health Committee at Anhui University

Protocol

2. Marrow-Derived Cell Processing and Chitosan-Mediated Genetic Delivery

Demonstrator: Min Li

2.1. To begin, place a euthanized mouse on a worktable **[1]**. Using surgical scissors and tweezers, remove the tibia and strip away all surrounding muscle tissue **[2]**. Soak the cleaned tibia in PBS and wash until only the bone remains **[3]**.

2.1.1. WIDE: Talent placing a euthanized mouse on the worktable. NOTE: Shot 2.1.1-2 mis-slated. The file name is correct.

2.1.2. Talent using scissors and tweezers to isolate the tibia and remove surrounding muscle tissue. NOTE: Shots 2.1.2-1 and 2.1.2-2 are mis-slated. The file names are correct.

2.1.3. Talent soaking the cleaned tibia in phosphate-buffered saline. NOTE: Shots 2.1.3-1 and 2.1.3-2 are mis-slated. The file names are correct.

2.2. Draw 1 milliliter of PBS into a syringe **[1]** and flush the bone marrow cells from one end of the tibia until the bone turns completely white **[2]**.

2.2.1. Talent filling a syringe with 1 mL PBS.

2.2.2. Shot of the tibia being flushed with PBS until it appears white.

2.3. Next, collect the cell suspension in a tube **[1]**. Centrifuge it at 800 *g* at 4 degrees Celsius for 5 minutes **[2]**.

2.3.1. Talent collecting the suspension in a tube.

2.3.2. Talent places the tube in a centrifuge and sets parameters to 800 × g at 4 degrees Celsius for 5 minutes.

2.4. After pipetting out the supernatant, add 500 microliters of red blood cell lysis buffer to the pellet **[1]**. After a minute, add 1 milliliter of separation buffer to terminate the lysis **[2]**.

2.4.1. Talent removing the supernatant and adding red cell lysis buffer.

2.4.2. Talent adding separation buffer after 1 minute.

2.5. Centrifuge the suspension again at 800 *g* at 4 degrees Celsius for 5 minutes **[1]**. Remove the supernatant and place the resulting cell pellet on ice for later use **[2].**

2.5.1. Shot of the tube in a centrifuge.

2.5.2. Talent placing the tube containing the pellet on ice.

2.6. For chitosan transfection, dissolve 4 milligrams of chitosan in 20 milliliters of acetic acid solution **[1-TXT]**. Adjust the pH to 5.5 using 10 molar sodium hydroxide **[2]**. Then dispense 500 microliters of the chitosan solution into individual microcentrifuge tubes **[3]**.

2.6.1. Talent weighing chitosan and adding it to acetic acid solution. **TXT: Acetic acid: 0.2 mg/mL**

2.6.2. Talent adjusting the pH using sodium hydroxide.

2.6.3. Talent pipetting the solution into microcentrifuge tubes.

2.7. Now add 2, 3, 4, and 5 micrograms of ABE *(A-B-E)* plasmids into individual tubes **[1-TXT]**. Dissolve the plasmids in 500 microliters of 30 millimolar sodium sulfate **[2]**.

2.7.1. Talent pipetting different plasmid concentrations into separate tubes. **TXT: ABE: Adenine Base Editor**

2.7.2. Shot of 500 µL 30 mM Na_2SO_4 being added to the tubes.

2.8. Then mix 500 microliters of plasmid solution with 500 microliters of chitosan solution in corresponding tubes **[1]**. Incubate the tubes in a water bath at 50 to 55 degrees Celsius for 15 minutes **[2]**. Vortex each tube for 15 to 30 seconds **[3]** and let them stand undisturbed for 30 minutes **[4]**.

2.8.1. Talent mixing chitosan and plasmid solutions.

2.8.2. Talent placing tubes in a water bath.

2.8.3. Talent vortexing the samples.

2.8.4. Talent placing the samples on a work bench.

2.9. For characterization, analyze the diameter and zeta potential of chitosan using dynamic light scattering **[1]**. Maintain the concentration of chitosan at 0.1 milligrams per milliliter in double-distilled water **[2-TXT]**.

2.9.1. Talent placing the samples in a DLS instrument.

2.9.2. Talent preparing chitosan samples for dynamic light scattering. **TXT: Run each sample 3x**

2.10. Next, cast 0.1 milligrams per milliliter chitosan samples onto a silicon chip **[1]**. Add 20 microliters of the resuspended samples onto 200-mesh grids and incubate **[2-TXT]**.

2.10.1. Talent pipetting the chitosan sample onto silicon chip.

2.10.2. Talent pipetting sample onto grid and incubating. **TXT: Incubation: RT, 10 min**

2.11. Now stain with 2% phosphotungstic acid for 3 minutes **[1]**. Remove excess liquid using filter paper before observing under a transmission electron microscope **[2]**.

2.11.1. Talent adds phosphotungstic acid to the grid.

2.11.2. Shot of excess liquid being removed with a filter paper.

3. Bone Marrow Cell Preparation and Flow Cytometric Evaluation

3.1. Centrifuge bone marrow cell suspension at 800 *g* at 4 degrees Celsius for 5 minutes **[1]**. Discard the supernatant, then add 500 microliters of red blood cell lysate **[2]**.

3.1.1. Talent placing bone marrow cell suspension in a centrifuge.

3.1.2. Shot of 500 µL RBC lysate being added.

3.2. After a minute, add 1 milliliter of separation buffer to stop the reaction **[1]**. Then count the number of cells with a cell counter **[2]**.

3.2.1. Talent adding separation buffer after 1 minute.

3.2.2. Talent placing the cell suspension in a cell counter. NOTE: Shot 3.2.2-2 mis-slated. The file name is correct.

3.3. Next, transfer 1 million cells into a new microcentrifuge tube **[1]**. After centrifuging again, pipette out the supernatant **[2]** and resuspend the cells in 500 microliters of PBS **[3]**.

3.3.1. Talent transferring cells into a fresh microcentrifuge tube.

3.3.2. Shot of supernatant being pipetted out.

3.3.3. Talent resuspending cells with phosphate-buffered saline.

3.4. Transfer the resuspended cells into a flow cytometry tube **[1]** and measure transfection efficiency using flow cytometry **[2]**.

3.4.1. Talent transferring the cell suspension into a flow cytometry tube.

3.4.2. Talent places the tube in a flow cytometer.

3.5. For bone marrow cavity injection, fix the anesthetized mouse in a supine position on the operating table **[1]**. Use adhesive tape to secure the front limb in place **[2]**.

3.5.1. Talent placing the anesthetized mouse on its back on the table.

3.5.2. Talent taping down the front limb of the mouse.

3.6. Disinfect the posterior tibia using an alcohol swab **[1]**. Then load the plasmid solution into a 1-milliliter syringe fitted with a 26-gauge needle **[2]**. Remove any air bubbles and keep the syringe ready for injection **[3]**.

3.6.1. Talent disinfecting the mouse tibia with an alcohol swab.

3.6.2. Talent filling the syringe with plasmid solution.

3.6.3. Talent checking for air bubbles and preparing for injection.

3.7. Next, touch and stabilize the shin of the mouse with your fingers **[1]**. Position the injection needle to enter the tibial shaft from the tibial plateau near the knee joint **[2]**.

3.7.1. Talent holding the shin of the mouse securely.

3.7.2. Talent aligning the needle at the tibial plateau.

3.8. Now rotate the needle parallel to the tibia **[1]**, insert into the bone marrow cavity **[2]**, and inject slowly over 3 seconds **[3]**.

3.8.1. Shot of the needle being rotated parallel to the tibia.

3.8.2. Shot of the needle being inserted into the bone marrow cavity.

3.8.3. Talent slowly injecting the plasmid solution into the cavity.

3.9. After the injection, slowly withdraw the needle over 3 seconds **[1]**. Immediately disinfect the puncture site using an alcohol swab to stop any bleeding **[2]**.

3.9.1. Talent pulling the needle out carefully.

3.9.2. Talent dabbing the puncture site with an alcohol swab.

3.10. Place the mouse in a warm environment at 20 to 26 degrees Celsius to facilitate its recovery **[1-TXT]**.

3.10.1. Talent placing the mouse into a recovery chamber or incubator.**TXT: Monitor mice after recovery from anesthesia**

Results

4. Results

4.1. Nanoparticles formed with the ABE and gRNA *(G-R-N-A)* plasmids had a uniform spherical morphology **[1]** and a narrow size distribution with an average size of 202.9 nanometers, a zeta potential of 2.77 millivolts, and a polydispersity index of 0.22 **[2]**.

4.1.1. LAB MEDIA: Figure 1C.

4.1.2. LAB MEDIA: Figure 1B. *Video editor: Highlight the central peak on the graph labeled "Size (d.nm)" around the 200 mark.*

4.2. Chitosan-embedded ABE plasmid significantly enhanced fluorescence signal in bone marrow cells, indicating higher transfection efficiency compared to the control group **[1]**.

4.2.1. LAB MEDIA: Figure 2B. Video editor: *Please emphasize the Bone marrow cell column of images corresponding to all rows with ABE plasmid*

4.3. Flow cytometry confirmed that chitosan-coated ABE plasmid achieved high transfection efficiency in bone marrow cells **[1]**, with the peak efficiency of 51.2% observed at a dose of 4 micrograms **[2]**, while 3 micrograms provided a balance of high efficiency of 47.2% **[3]**.

4.3.1. LAB MEDIA: Figure 3A. *Video Editor: Please highlight all columns corresponding to ABE*

4.3.2. LAB MEDIA: Figure 3B. *Video Editor: Please highlight the panel corresponding to 4 µg ABE plasmid*

4.3.3. LAB MEDIA: Figure 3B. *Video Editor: Please highlight the panel corresponding to 3 µg ABE plasmid*

4.4. Sanger sequencing and PCR of bone marrow cell genomic DNA revealed in vivo gene editing at target site A1 with 14.27% efficiency, and at site A2 with 10.69% efficiency **[1]**.

4.4.1. LAB MEDIA: Figure 4B. *Video editor: Zoom in on the sequence traces labeled "A1" and "A2" showing colored peaks. Please also highlight the A1 and A2 columns in the bar graph*

Pronunciation Guide:

1. Euthanized
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/euthanize
- **IPA:** /ˈjuːθəˌnaɪzd/
- **Phonetic Spelling:** yoo-thuh-nized

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2. Tibia
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/tibia
- **IPA:** /ˈtɪbiə/
- **Phonetic Spelling:** TIB-ee-uh

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3. Chitosan
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/chitosan
- **IPA:** /ˈkaɪtəsæn/
- **Phonetic Spelling:** KYE-tuh-san

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4. Phosphotungstic (acid)
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/phosphotungstic
- **IPA:** /ˌfɑsfoʊtʌnˈgstɪk/
- **Phonetic Spelling:** FAWS-foh-tung-STIK

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5. Polydispersity
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/polydispersity
- **IPA:** /ˌpɑliˌdɪˈspɜrsɪti/
- **Phonetic Spelling:** PAH-lee-dis-PUR-si-tee