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Title: Effective and Safe Gene Delivery to the Mouse Kidney via Slow Retrograde Renal Pelvis Injection of Adeno-Associated Virus Vectors

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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? **Yes**

   Videographer: Please capture the following shots using a SCOPE kit

   SCOPE: 2.8.1, 2.8.2, 2.8.3, 2.9.1, 2.9.2, 2.10.1, 2.10.2, 2.11.1, 2.11.2, 2.12.1, 2.12.2, 2.12.3, 2.12.4

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: 12
Number of Shots: 27

Introduction

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

1.1. **Anusha Sairavi:** Our goal is to develop effective ways to deliver genes to kidney cells using Adeno-Associated Virus vectors. This will advance basic kidney research and help develop novel gene therapies for kidney diseases.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.9.1*

What are the current experimental challenges?

1.2. **Ranjan Das:** A major challenge is the lack of effective methods to deliver genes to key kidney cell types such as renal tubules and podocytes due to the organ's structural complexity, limiting progress in kidney-targeted Adeno-Associated Virus gene therapy.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.10*

What significant findings have you established in your field?

1.3. **Anusha Sairavi:** We have shown that effective gene delivery to kidney cells, including renal tubules and podocytes, can be achieved by selecting the right Adeno-Associated Virus capsids and administration routes based on disease context.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Ranjan Das:** Our slow renal pelvis injection method increases local Adeno-Associated Virus vector concentration, thereby allowing efficient transduction of various kidney cell types that are otherwise difficult to target by systemic delivery.

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

What research questions will your laboratory focus on in the future?

1.5. **Anusha Sairavi:** By utilizing both the systemic and renal pelvis injection routes, we aim to identify optimal Adeno-Associated Virus capsids and administration routes for effective and selective transduction in target kidney cell types.

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.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Oregon Health & Science University (OHSU)

Protocol

Videographer's Note: The two files of scope footage does not have slating, but should be fairly clear what actions relate to each written step.

2. Surgical Method for Renal Pelvis Injection in Rodent Models

Demonstrator: Ranjan Das

2.1. Dilute the Adeno-Associated Virus vector solution using 5% sorbitol PBS **[1-TXT]**. Place the diluted solution on ice **[2]**.
- 2.1.1. WIDE: Talent pipetting sorbitol into the AAV vector tube and mixing.
- 2.1.2. Talent placing the tube on ice.

2.2. With a needle holder, bend and break off only the needle tip from a 30-gauge needle **[1]**. Under a stereomicroscope, connect the 30-gauge needle tip, 15 centimeters of PE-10 tubing, another 30-gauge needle, and a gas-tight glass syringe in order from the tip **[2]**.
- 2.2.1. Talent gripping a 30-gauge needle with a needle holder and snapping off the tip.
- 2.2.2. Talent connecting components sequentially under a stereomicroscope.

2.3. Now, set the connected gas-tight glass syringe onto an infusion syringe pump **[1]**. Using the infusion syringe pump, aspirate 90 microliters of the vector solution into the gas-tight glass syringe **[2]**.
- 2.3.1. Talent placing the assembled syringe into the infusion syringe pump holder.
- 2.3.2. Shot of the solution being aspirated into the syringe, displaying the volume.

2.4. Set the infusion mode on the syringe pump to a target volume of 50 microliters and a flow rate of 50 microliters per minute **[1]**.
- 2.4.1. Talent setting the infusion mode on pump to target volume to 50 microliters and flow rate to 50 microliters per minute.

2.5. Next, place an anesthetized mouse in a lateral position on a closed-loop heat pad connected to a heat therapy pump maintained at 37 degrees Celsius **[1-TXT]**. Cover the mouse with a sterile surgical drape **[2]**.
- 2.5.1. Talent laying the mouse laterally on a heat pad and turning on the heat therapy pump. **TXT: Anesthesia: 4% isoflurane inhalation**
- 2.5.2. Talent carefully laying a sterile surgical drape over the mouse.

2.6. Using tissue forceps, pinch the skin **[1].** Then use surgical scissors to make a 1-centimeter skin incision at the costovertebral angle level near the left kidney **[2]**.

2.6.1. Talent pinching the skin with forceps.
2.6.2. Talent cutting skin with scissors at the correct anatomical location.

2.7. Cut the underlying muscle layer along the same length as the skin incision **[1]**. Now gently press the abdomen with fingers, avoiding direct contact with the kidney **[2]**, to expose the kidney through the incision site **[3]**.
2.7.1. Shot of the muscle layer under the skin being cut with scissors.
2.7.2. Talent gently pressing the abdomen.
2.7.3. Shot of the exposed kidney.

2.8. Carefully remove minimal surrounding fat until the renal pelvis appears as a small white area **[1]**. Now, use a straight-type micro vessel clip to clamp the ureter first **[2]**, then clamp the renal artery and vein together with a curved-type micro vessel clip **[3]**.
Videographer: Please capture videos of the shots labelled SCOPE, using a SCOPE kit
2.8.1. SCOPE: Shot of the fat being removed around the kidney to expose the renal pelvis.
2.8.2. SCOPE: Shot of the ureter being clamped with a straight-type micro vessel clip.
2.8.3. SCOPE: Shot of the artery and vein being clamped with a curved-type micro vessel clip.

2.9. Use curved forceps to insert the 30-gauge needle about 3 millimeters into the pelvic cavity **[1]**. Start the injection of 50 microliters of vector solution using the infusion syringe pump over 1 minute at a flow rate of 50 microliters per minute **[2]**.
2.9.1. SCOPE: Talent guiding the needle with forceps.
2.9.2. SCOPE: Talent initiating the injection on the syringe pump.

2.10. Keep the needle and clamps in place for 5 minutes to enhance kidney exposure to the vector **[1]**. Then, remove both the ureter and vessel clamps **[2]**.
2.10.1. SCOPE: shot showing the needle and clamps maintained in place with timer overlay.
2.10.2. SCOPE: Talent releasing the clamps from the ureter and vessels.

2.11. Slowly withdraw the needle **[1]** while pressing a cotton-tipped applicator over the injection site to apply pressure **[2]**.
2.11.1. SCOPE: Talent pulling out the needle.
2.11.2. SCOPE: Shot of the cotton-tipped applicator being used to put pressure on the injection site.

2.12. Now, gently reposition the kidney back into the peritoneal cavity **[1]**. Then suture the muscle layer with 6-0 *(Six-zero)* absorbable suture **[2]** and then the skin with 5-0 monofilament suture **[3]**. Finally, inject 1 milliliter of saline subcutaneously into the mouse's back for fluid support **[4]**.
2.12.1. SCOPE: Talent using forceps to gently tuck the kidney back into the body.

2.12.2. SCOPE: Shot of the muscle layer being sutured with 6-0 suture.
2.12.3. SCOPE: Shot of the skin being sutured with 5-0 monofilament suture.
2.12.4. Talent injecting saline under the skin on the mouse's back.

Results

3. Results

3.1. The concentration of vector genomes in crude lysates was significantly higher in AAV9 *(A-A-V-nine)* than KP3 *(K-P-Three)* preparations **[1]**, while the final centrifugally ultrafiltered-purified AAV9 and KP3 preparations showed high vector genome titers of 3.2 into 10^{13} and 8.8 into 10^{12} vector genomes per milliliter, respectively **[2]**.

3.1.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the columns labelled "1" in both AAV9 and KP3*

3.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the columns labelled "8" in both AAV9 and KP3*

3.2. Blot analysis of silver-stained cesium chloride -purified AAV9 and KP3 revealed three distinct bands for VP1 *(V-P-one)*, VP2, and VP3 with minimal background, indicating high purity **[1]**, while centrifugally ultrafiltered-purified samples loaded at high amounts displayed protein smearing **[2]**, but at lower loads, VP bands became distinct **[3]**.

3.2.1. LAB MEDIA: Figure 3A.

3.2.2. LAB MEDIA: Figure 3B.

3.2.3. LAB MEDIA: Figure 3C.

3.3. Two weeks after renal pelvis injection, the centrifugally ultrafiltered KP3 vector transduced renal proximal tubules effectively **[1]**, while the centrifugally ultrafiltered AAV9 vector showed limited tubular transduction and was mostly confined to glomerular mesangial cells **[2]**.

3.3.1. LAB MEDIA: Figure 4A. *Video editor: Please highlight the CU-KP3 row*

3.3.2. LAB MEDIA: Figure 4A. *Video editor: Please highlight the CU-AAV9 row*

3.4. Quantitative PCR revealed a 73-fold higher vector genome copy number in centrifugally ultrafiltered KP3-injected kidneys **[1]**.

3.4.1. LAB MEDIA: Figure 4D. *Video editor: Please highlight the column corresponding to CU-KP3*

3.5. The KP3 vector also transduced collecting duct cells, as shown by co-localization with aquaporin-2 staining **[1]**, while AAV9 primarily targeted thick ascending limb cells co-stained with NKCC2 *(N-K-C-C-Two)* **[2]**.

3.5.1. LAB MEDIA: Figure 5A. *Video editor: Please highlight the green part of 1st image in KP3 row (left most)*

3.5.2. LAB MEDIA: Figure 5B. *Video editor: Please highlight the green part of 1st image in AAV9 row (left most)*

3.6. Off-target liver transduction was noticeably reduced with KP3 compared to AAV9 **[1]**.

3.6.1. LAB MEDIA: Figure 5C

3.7. No apparent histological damage in kidneys injected with the KP3 vector was observed **[1]**.

3.7.1. LAB MEDIA: Figure 6. *Video editor: Please highlight the KP3 row*

Pronunciation Guide:
□ Adeno-Associated Virus (AAV)
Pronunciation link: https://www.merriam-webster.com/dictionary/adeno-associated (if available)
IPA: /ˌædɪˌnoʊ əˈsoʊsieɪtɪd ˈvaɪrəs/
Phonetic: ad-eh-noh uh-SOH-shee-ay-tid VY-rus
— And for the acronym: "A-A-V" = /ˌeɪˌeɪˈvi/ (ay-ay-vee)
□ Stereomicroscope / Stereomicroscope / SCOPE
Pronunciation link: https://www.merriam-webster.com/dictionary/stereomicroscope
IPA: /ˌstɛrioʊˈmaɪkrəˌskoʊp/
Phonetic: stere-OH-MY-croh-scope
□ Renal pelvis
Pronunciation link: https://www.howtopronounce.com/renal-pelvis — shows IPA and audio. howtopronounce.com
IPA: /ˈriːnəl ˈpɛlvɪs/
Phonetic: REE-nul PEL-vis
□ Capsid
Pronunciation link: https://www.merriam-webster.com/dictionary/capsid
IPA: /ˈkæpsɪd/
Phonetic: KAP-sid
□ Transduction
Pronunciation link: https://www.merriam-webster.com/dictionary/transduction
IPA: /ˌtrænzˈdʌkʃən/
Phonetic: tranz-DUK-shun
□ Glomerular / Glomeruli
Pronunciation link: https://www.merriam-webster.com/dictionary/glomerulus / glomeruli
IPA (singular): /gləˈmɛrjələr/
Phonetic (singular): glo-MEH-ruh-ler
IPA (plural "glomeruli"): /gləˈmɛrjʊlaɪ/
Phonetic (plural): glo-MEH-rooh-lye
□ Mesangial
Pronunciation link: https://www.merriam-webster.com/dictionary/mesangial
IPA: /məˈzæn.dʒiəl/
Phonetic: muh-ZAN-jee-ul
□ Proximal / Distal
Pronunciation link: https://www.merriam-webster.com/dictionary/proximal, https://www.merriam-webster.com/dictionary/distal
IPA (proximal): /ˈprɑksɪməl/ — phonetic: PROX-ih-mul
IPA (distal): /ˈdɪstəl/ — phonetic: DIS-tul
□ Ultrafiltered / Ultrafiltration
Pronunciation link: https://www.merriam-webster.com/dictionary/ultrafiltered / ultrafiltration
IPA (ultrafiltered): /ˌʌltrəˈfɪltərd/

Phonetic: UL-truh-FILL-terd
IPA (ultrafiltration): /ˌʌltrəfɪlˈtreɪʃən/
Phonetic: UL-truh-fil-TRAY-shun
Histological
Pronunciation link: https://www.merriam-webster.com/dictionary/histological
IPA: /ˌhɪstəˈlɑdʒɪkəl/
Phonetic: his-tuh-LOJ-ih-kul
Vector genome copy number

- **Vector: /ˈvɛktər/ (VEK-ter)**
- **Genome: /ˈdʒiːnoʊm/ (JEE-nohm)**
- **Copy: /ˈkɑpi/ (KAH-pee)**
- **Number: /ˈnʌmbər/ (NUM-ber)**