SHOTLIST: APPROVED FOR FILMING

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Title: A Mouse Model to Evaluate the Long-Term Structural and Functional Outcomes After the Reversal of Prolonged Unilateral Ureteric Obstruction

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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: 13
Number of Shots: 31

Introduction

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

1.1. **Mark de Caestecker:** My laboratory works on the mechanisms regulating long-term functional tissue repair after kidney injury. We are currently focusing on the mechanisms of tissue repair after reversal of urinary obstruction **[1]**.

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

What significant findings have you established in your field?

1.2. **Mark de Caestecker:** We have developed a new mouse model of reversible unilateral ureteric obstruction (R-UUO) that can be used to evaluate the functional consequences of reversing prolonged urinary obstruction on the kidney **[1]**.

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

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

1.3. **Mark de Caestecker:** We are planning to use this model to study the mechanisms regulating renal medullary repair, and to develop therapies to improve functional recovery after reversal of urinary obstruction **[1]**.

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

What advantage does your protocol offer compared to other techniques?

1.4. **Mark de Caestecker:** Other models require multiple surgeries, or microsurgical expertise that most laboratories do not have. In addition, since the obstruction is unilateral, most models do not allow functional assessment of recovery **[1]**.

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

What are the experimental challenges using this model?

1.5. **Mark de Caestecker:** There are really only two. First, consistent placement of a vascular clamp on the proximal ureter, and second, removal of the clamp a few days later without damaging the ureter **[1]**.

   1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B. roll: 2.4.1*

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. **Mark de Caestecker, Professor of Medicine**: (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 Vanderbilt University Medical Center

Protocol

Videographer's NOTE: Some of the shots have a "tail slate" instead of a slate at the beginning

2. Reversible Ureter Obstruction and Delayed Nephrectomy in Mouse Model

Demonstrator: Rachel Delgado

2.1. To begin, prep the surgical site of an anesthetized mouse with antiseptics **[1].** Make a 1.5-centimeter longitudinal dorsal incision along the midline through the skin and subcutaneous layers using scissors and forceps **[2]**.

2.1.1. WIDE: Talent wiping the incision site with betadine/alcohol.

2.1.2. Shot of a midline dorsal incision being made with scissors, lifting the skin using forceps.

2.2. Make a small incision through the left flank muscle and fascia above the kidney **[1].** Then, with a pair of forceps, exteriorize the left kidney **[2]**.

2.2.1. Talent incising left flank muscle and fascia.

2.2.2. Shot of the kidney being pulled out with a pair of forceps.

2.3. Using blunt forceps, carefully dissect the fat at the lower pole and some connective tissue near the ureter **[1]**. Locate the ureter region **[2]**. Then, separate the ureter along with its connective tissue from the renal pedicle to avoid including the renal vein and artery with the clamp **[3]**.

2.3.1. Talent dissecting lower pole fat with blunt forceps. **Videographer's NOTE:** 2.3.1 to 2.3.3 are combined in one shot.

2.3.2. Close-up of the ureter region with connective tissue intact.

2.3.3. Talent separating the ureter from the renal pedicle using forceps.

2.4. Use clamp appliers to open the vascular clamp and position it directly below the renal pelvis on the ureter **[1]**. Use the markings on the clamp to ensure uniform pressure is applied across all mice **[2-TXT]**.

2.4.1. Talent using clamp appliers to open and place the clamp on the ureter.

2.4.2. Close-up of markings on the clamp aligned properly. **TXT: Do not reposition clamp once placed, to prevent ureter damage**

2.5. Now, use a saline-soaked sterile cotton swab to gently push the kidney with the clamp back into the retroperitoneal space **[1]**. Then suture the muscle layer using an absorbable suture **[2]**. Use skin clips to close the skin layer **[3]**.

2.5.1. Talent pushing the clamped kidney back inside using a moistened cotton swab.

2.5.2. Talent stitching the muscle layer with absorbable thread.

2.5.3. Talent applying skin clips to close the dorsal skin incision.

2.6. To remove the ureteric clamp, remove the wound clips, then clean the surgical area without re-shaving, using the original incisions to access the clamp **[1]**.

2.6.1. Shot of the skin surface being cleaned near the incision site.

2.7. Reopen the muscle layer **[1]** and expose the retroperitoneal space **[1].** Use forceps to carefully locate the clamp in the retroperitoneal space without exteriorizing the kidney **[3]**. With clamp appliers, gently open the clamp while pulling the surrounding tissue away using forceps to safely remove it **[4]**. **NOTE:** Only sentence numbers are adjusted to accommodate the added shot.

Added shot: 2.7.1. Talent reopening the skin

2.7.1. Talent reopening the muscle layer and expose the retroperitoneal space without exteriorizing the kidney. **Videographer's NOTE:** Slated as 2-7-1x

2.7.2. Shot of the clamp being located in the retroperitoneal space using forceps.

2.7.3. Talent using clamp appliers and forceps to release and remove the clamp from the ureter.

2.8. Now, exteriorize the kidney to inspect the renal pelvis for obstruction **[1]**. A swollen renal pelvis confirms hydronephrosis **[2]**.

2.8.1. Talent gently pulling the kidney out of the incision using forceps.

2.8.2. Close-up of a visibly swollen renal pelvis indicating hydronephrosis.

2.9. Push the kidney back into the retroperitoneal space using a saline-soaked sterile cotton swab **[1]**, and suture the incision shut, as demonstrated **[2] [3].**

2.9.1. Talent repositioning the kidney into the body using a moistened cotton swab.

Added shot: 2-9-2 shows suturing

2.9.2. Shot of the sutured incision. **Videographer's NOTE:** Slated as 2-9-2b

2.10. To perform contralateral nephrectomy, reopen the dorsal site using the original skin incision **[1].** Incise the right flank muscle and fascia above the kidney **[2]**. Then, exteriorize the right kidney using forceps **[3].**

2.10.1. Talent reopening the dorsal incision on a prepped animal.

2.10.2. Shot of the right flank muscle and fascia being incised.

2.10.3. Talent exposing the right kidney with forceps after muscle incision.

2.11. Hold the right kidney with smooth, curved forceps and dissect the upper and lower poles free from the surrounding tissue **[1] [2-TXT]**.

2.11.1. Shot of the right kidney being grasped with smooth curved forceps. **Videographer's NOTE:** 2.11.1 to 211.2 are a single continuous action

2.11.2. Talent dissecting connective tissue around the kidney with curved forceps. **TXT: Do not remove the adrenal gland**

2.12. After freeing the kidney, tie a 4-0 *(Four-Oh)* silk suture around the renal vessels and ureter using a double surgical knot **[1]**. Wait for approximately 30 seconds until the kidney darkens **[2]**, then hold it with the smooth, curved forceps **[3]** and remove it by cutting distally to the knot using curved scissors **[4]**.

2.12.1. Talent tying off renal vessels and ureter with a silk suture.

2.12.2. Close-up of kidney turning darker in color.

2.12.3. Shot of the kidney being held with smooth curved forceps.

2.12.4. Talent cutting the kidney with scissors after the knot.

2.13. Gently push the remaining renal pedicle back into the retroperitoneal space using a saline-soaked sterile cotton swab **[1]**. Then close the muscle and skin layers using sutures and clips **[2]**.

2.13.1. Talent using cotton swab to reposition the pedicle.

2.13.2. Talent suturing muscle and applying skin clips to close the surgical site.

Results

3. Results

3.1. Typical survival and renal function measurements were taken 84 days after reversible unilateral ureteral obstruction **[1]**.

3.1.1. LAB MEDIA: Figure 1B–E.

3.2. Functional studies compared reversible unilateral ureteral obstruction with nephrectomy alone for blood urea nitrogen **[1]**, transdermal glomerular filtration rate **[2]**, and urinary osmolarity after 18 hours of water deprivation **[3]**. Urinary osmolarity values were markedly lower than nephrectomy control mice at the same time points **[4].**

3.2.1. LAB MEDIA: Figure 1C. *Video editor: Please highlight the RUUO column*

3.2.2. LAB MEDIA: Figure 1D. *Video editor: Please highlight the RUUO column*

3.2.3. LAB MEDIA: Figure 1E.

3.2.4. LAB MEDIA: Figure 1E. *Video editor: Please highlight the RUUO column*

Pronunciation Guide:

anesthetized
Pronunciation link (M-W): https://www.merriam-webster.com/dictionary/anesthetize
IPA: /əˈnɛsθəˌtaɪzd/
Spelling: uh-NESS-thuh-tized

longitudinal
Pronunciation link (M-W): https://www.merriam-webster.com/dictionary/longitudinal
IPA: /ˌlɑndʒəˈtjudənəl/
Spelling: lon-jih-TEW-di-nuhl

subcutaneous
Pronunciation link (M-W): https://www.merriam-webster.com/dictionary/subcutaneous
IPA: /ˌsʌbkjuˈteɪniəs/
Spelling: sub-kyoo-TAY-nee-uhs

fascia
Pronunciation link (M-W): https://www.merriam-webster.com/dictionary/fascia
IPA: /ˈfæʃə/
Spelling: FASH-uh

ureter
Pronunciation link (Cambridge):
https://dictionary.cambridge.org/us/pronunciation/english/ureter
IPA: /jʊˈriːtɚ/
Spelling: yoo-REE-ter YouTube+13How To Pronounce+13YouTube+13SciorаCambridge Dictionary+15Cambridge Dictionary+15Cambridge Dictionary+15

retroperitoneal
Pronunciation link (M-W): https://www.merriam-webster.com/dictionary/retroperitoneal
IPA: /ˌrɛtroʊpərɪtəˈniəl/
Spelling: re-troh-per-ih-TOH-nee-uhl Merriam-Webster+9Merriam-Webster+9YouTube+9

hydronephrosis
Pronunciation link (M-W Med): https://www.merriam-webster.com/medical/hydronephrosis
IPA: /ˌhaɪdroʊnəˈfroʊsɪs/
Spelling: HY-droh-nuh-FROH-sis

nephrectomy
Pronunciation link (M-W): https://www.merriam-webster.com/dictionary/nephrectomy
IPA: /nɪˈfrɛktəmi/

Spelling: nih-FREK-tuh-mee YouTube+11Merriam-Webster+11How To Pronounce+11How To Pronounce+2OpenMD+2How To Say Guide+2

renal
Pronunciation link (M-W): https://www.merriam-webster.com/dictionary/renal
IPA: /ˈriːnəl/
Spelling: REE-nuhl

pedicle
Pronunciation link (M-W): https://www.merriam-webster.com/dictionary/pedicle
IPA: /ˈpɛdɪkəl/
Spelling: PED-ih-kuhl

connective
Pronunciation link (M-W): https://www.merriam-webster.com/dictionary/connective
IPA: /kəˈnɛktɪv/
Spelling: kuh-NEK-tiv