Submission ID #: 61856
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18866128

Title: Preparation of Decellularized Kidney Scaffolds in Rats

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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**

   If **Yes**, can you record movies/images using your own microscope camera?

   No

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Leica, OH1-3096

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

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

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

Current Protocol Length

Number of Steps: 15
Number of Shots: 28

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Kim Eun Heui:** This protocol makes it possible to develop vascularized scaffolds using kidneys in a rat model. The kidney is the most suitable organ for scaffold of differentiated stem cells.

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

1.2. **Kim Eun Heui:** This is a highly successful protocol for decellularization of the kidney. It effectively removes the nuclei with minimal destruction of the ECM ultrastructure.

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

OPTIONAL:

1.3. **Kim Eun Heui:** This technique can be applied to transplantation medicine and tissue engineering.

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

Introduction of Demonstrator on Camera

1.4. **Kim Eun Heui:** Demonstrating the procedure will be Kim Joo Hyoung, a MD PhD associate professor of plastic surgery from my laboratory.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. Procedures involving animal subjects were approved by the administration of Pusan National University of Medicine and conducted in accordance with ethical guidelines for the use and care of animals.

Protocol

2. Preparation for Harvesting Rat Kidneys

2.1. To begin, place the 8-week-old Sprague-Dawley rat on a warming pad **[1]** and position a rectal thermometer probe in the rectum to monitor core temperature **[2]**.

2.1.1. WIDE: Establishing shot of talent putting the rat on the warming pad.

2.1.2. Talent positioning the thermometer.

2.2. After anesthetizing the rat, place it in a supine position **[1]** and secure the four limbs to the table with tape **[2]**. Shave and clean the abdomen with germicidal soap **[3]**.

2.2.1. Talent placing the rat in a supine position.

2.2.2. Talent securing the limbs with tape.

2.2.3. Talent shaving and/or cleaning the rat.

2.3. Apply 2% betadine for at least 1 to 2 minutes **[1]**, then wipe it off with a 70% ethanol solution. Repeat this sequence three times **[2]**.

2.3.1. Talent applying the betadine.

2.3.2. Talent wiping off the betadine, with the ethanol container in the shot.

2.4. Cover the operative field with a sterile fenestrated drape **[1]**. Make a vertical abdominal incision and expose the left kidney, ureter, abdominal aorta, and inferior vena cava **[2]**. Dissect the tissue just before cutting the pedicle **[3]**.

2.4.1. Talent covering the operative field.

2.4.2. Talent exposing the left kidney, ureter, abdominal aorta, and inferior vena cava.

2.4.3. Talent dissecting tissue. NOTE: this was filmed with SCOPE, for detail view ➔ During 2.4.3. , “the ligating the ureter”, which was in 4.1.2., was included

3. Transcardial Perfusion

3.1. Prepare 50 milliliters of perfusion solution per rat by mixing PBS with approximately 10 units per milliliter heparin **[1]**. Mix equal volumes of 8% PFA with PBS to make the 4% PFA solution **[2]**.

3.1.1. Talent mixing perfusion solution.

3.1.2. Talent mixing the 4% PFA.

3.2. Extend the vertical abdominal incision cranially, making sure to draw the scissors away from the organs when cutting to avoid damage. Continue the incision through the rib cage, and then cut through the diaphragm by lifting the sternum **[1]**.

3.2.1. SCOPE: Talent extending the incision and cutting through the diaphragm.

3.3. Retract the loose flap of skin out of the way with an Allis clamp **[1]**, and free the heart by tearing any connective tissue with the forceps **[2-TXT]**.

3.3.1. Talent pinning the skin. NOTE: The pinning was replaced with Allis clamp.

3.3.2. SCOPE: Talent freeing the heart. **TEXT: CAUTION: Do not use the scissors to free the heart**

3.4. Open the PBS line and ensure that it is flowing **[1]** before placing the needle into the left ventricle. Hold the heart gently with blunt forceps and use a hemostat to control the needle. The needle should be inserted no more than a quarter inch **[2]**. *Videographer: This step is important!*

3.4.1. Talent opening the PBS line.

3.4.2. SCOPE: Talent placing the needle into the left ventricle.

3.5. While supporting the heart with the needle and hemostat, locate the right atrium and snip through it with iridectomy scissors. Rest the hemostat on the rat's body, and make sure the needle is still positioned inside the heart **[1]**. *Videographer: This step is important!*

3.5.1. SCOPE: Talent snipping through the right atrium and resting the hemostat on the rat's body.

3.6. ~~**[1]**.~~ Continue perfusing PBS for 4 minutes or longer if there is still blood visible in the kidney and liver **[2]**. *Videographer: This step is difficult and important!*

3.6.1. ~~Talent unpinning the feet and skin flap~~.

3.6.2. Perfusion continuing.

4. Kidney Harvesting and Decellularization

4.1. Harvest the left kidney with the abdominal aorta and inferior vena cava **[1]**. Ligate the ureter, thoracic aorta, superior vena cava, and branches of the abdominal aorta **[2]**.

4.1.1. SCOPE: Talent harvesting the tissue.

4.1.2. SCOPE: Talent ligating the ureter, thoracic aorta, superior vena cava, and branches of the abdominal aorta. NOTE: 4.1.1 and 4.1.2 shot together, ligation of the ureter can be found in shot 2.4.3.

4.2. Keep the organ hydrated in DPBS in a 10-centimeter Petri dish **[1]**. Cannulate the abdominal aorta and inferior vena cava with a 23 Gauge catheter **[2]**. *Videographer: This step is important!*

4.2.1. Talent putting the organ in the Petri dish.

4.2.2. SCOPE: Talent cannulating the abdominal aorta and inferior vena cava.

4.3. To remove residual blood, connect the cannula with a peristaltic pump **[1]** and wash the organ with 500 milliliters of DPBS and 16 units per milliliter heparin for 90 minutes at 5 rpm and 37 degrees Celsius **[2]**. *Videographer: This step is important!*

4.3.1. Talent connecting the cannula with the peristaltic pump.

4.3.2. Organ washing.

4.4. To decellularize the kidney, perfuse it with 1% Triton X-100 for 3 hours and then with 0.75% SDS solution for 6 hours at a constant pressure of 40 millimeters of mercury **[1]**. *Videographer: This step is important!*

4.4.1. Talent perfusing the kidney with 1% Triton X-100.

4.5. To remove residual SDS, perfuse the sample with 1% penicillin in distilled water for 18 hours and then with sterile DPBS and 16 units per milliliter heparin for 90 minutes **[1]**. *Videographer: This step is important!*

4.5.1. Talent perfusing the kidney with 1% penicillin.

Author NOTE: Before the shooting, we prepared the kidneys that had completed the previous steps and add the close shoots. I hope this is useful.

Results

5. Results: Rat Kidneys Subjected to Renal Arterial Perfusion Decellularization

5.1. The gross morphology of rat kidneys was dark red **[1]**. After decellularization, the kidney became pale and translucent **[2]**.

5.1.1. LAB MEDIA: Figure 1 A.

5.1.2. LAB MEDIA: Figure 1 D.

5.2. Residual genomic DNA was quantified in decellularized kidney scaffolds and compared with that in native kidneys, confirming that tissue genomic DNA was almost eliminated after decellularization **[1]**.

5.2.1. LAB MEDIA: Figure 2.

5.3. From 14 cases, the average DNA contents were 115.05 nanograms per microliter for the control **[1]** and 1.96 nanograms per microliter for the decellularized scaffold. In total, 98.3% of DNA was removed **[2]**.

5.3.1. LAB MEDIA: Figure 2. *Video Editor: Emphasize the control bar.*

5.3.2. LAB MEDIA: Figure 2. *Video Editor: Emphasize the decell bar.*

5.4. The three-dimensional structure was maintained, and acellular glomeruli were preserved in the cortical parenchyma **[1]**.

5.4.1. LAB MEDIA: Figure 3.

Conclusion

6. Conclusion Interview Statements

6.1. **Kim Eun Heui:** When inserting the needle into the left ventricle, the needle should be inserted no more than 1/4 inch. Any further insertion may come out the other side.

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

6.2. **Kim Eun Heui:** We have confidence that this technique will open a new prospect in the field of tissue engineering.

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