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Title: A Rat Graft Rejection Model of Intestinal Transplantation with Exteriorized Ileostomy for Longitudinal Prognosis Assessment

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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: **MM/DD/YYYY**

When you are ready to submit your video files of interviews, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 25

Number of Shots: 55

Introduction

- 1.1. **Koki Takase:** Due to stronger immunogenicity than other organs, intestinal transplantation shows unsatisfactory long-term outcomes and requires further advances in peri-operative intervention. We are trying to overcome this issue.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

- 1.2. **Yu-Tung Li:** While animal models of intestinal transplantation are necessary to assess potentially beneficial intervention, these models often involve complex surgical procedures, and attaining stable animal survival is challenging.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

What advantage does your protocol offer compared to other techniques?

- 1.3. **Koki Takase:** Graft procurement and implantation technique are simple enough to achieve around 80% long-term survival. We also construct ileostomy to ease time course image-based assessment of the transplantation outcomes.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee at the Graduate School of Medicine, The University of Osaka

Protocol

2. Donor Surgery to Extract the Graft

Demonstrator: Koki Takase

- 2.1. To begin, make an 8-centimeter midline incision to open the abdominal cavity of the donor rat and extend the incision cranially to the xiphoid process [1-TXT].
 - 2.1.1. JoVE intestinal transplantation_original_1 01:41-01:46 and 02:35-02:36.
TXT: Isoflurane anesthesia: 1.5 L/min
Induction: 2.5%
Maintenance: 1.5%
- 2.2. Dissect the Treitz ligament [1] and rotate the small intestine 180 degrees in a clockwise direction to reverse the intestinal rotation [2].
 - 2.2.1. JoVE intestinal transplantation_original_1 04:10-04:15.
 - 2.2.2. JoVE intestinal transplantation_original_1 05:20-05:30.
- 2.3. Observe the mesenteric vascular loops and select a graft site approximately 10 centimeters in length from the ileum with clear vascularity [1].
 - 2.3.1. JoVE intestinal transplantation_original_1 06:20-06:30.
- 2.4. Now, dissect the distal mesentery using scissors [1] and with silk sutures, ligate the superior mesenteric artery and superior mesenteric vein of the distal ileum [2].
 - 2.4.1. JoVE intestinal transplantation_original_1 08:40-08:50.
 - 2.4.2. JoVE intestinal transplantation_original_1 08:57-09:04 and .
- 2.5. Displace the stomach and spleen cranially to expose the portal vein clearly [1].
 - 2.5.1. JoVE intestinal transplantation_original_1 12:05-12:20.
- 2.6. Dissect and ligate the middle colonic artery and vein and the right colonic artery and vein on the left side of the superior mesenteric artery and vein [1].
 - 2.6.1. JoVE intestinal transplantation_original_1 15:22-15:28 and 16:08-16:12.

- 2.7. Then, dissect the mesentery on the right side of the superior mesenteric artery and vein and ligate the jejunal branch at a safe distance from the vein to avoid kinking [1].

2.7.1. JoVE intestinal transplantation_original_1 19:08-19:12 and 23:08—23:12.

- 2.8. Now, using a cotton swab, separate the base of the superior mesenteric artery [1] and ligate the mesentery between the aorta above the artery and the portal vein [2].

2.8.1. JoVE intestinal transplantation_original_1 24:50-24:55.

2.8.2. JoVE intestinal transplantation_original_1 27:55-28:05.

- 2.9. Next, ligate the right renal artery on the right side of the aorta with a suture [1].

2.9.1. JoVE intestinal transplantation_original_1 33:30-33:40.

- 2.10. After determining the final length of the graft, ligate any excess mesenteric branches and the straight arteries at both graft ends [1].

2.10.1. JoVE intestinal transplantation_original_2 06:50-07:05.

- 2.11. Now, ligate the aorta below the superior mesenteric artery first [1], then ligate the aorta above it [2], and excise the artery base with a circular patch of the aorta [3]. Cut the portal vein at the hepatic hilum [4].

2.11.1. JoVE intestinal transplantation_original_2 08:35-08:42.

2.11.2. JoVE intestinal transplantation_original_2 09:28-09:36.

2.11.3. JoVE intestinal transplantation_original_2 10:05-10:15.

2.11.4. JoVE intestinal transplantation_original_2 10:15-10:30.

- 2.12. Next, cut the proximal and distal vascular loops and the intestine [1-TXT]. Gently perfuse the graft with 15 milliliters of cold saline via the superior mesenteric artery [2-TXT].

2.12.1. JoVE intestinal transplantation_original_2 10:40-10:50. **TXT: Place the extracted graft into cold saline at 4 °C**

2.12.2. JoVE intestinal transplantation_original_3 02:05-02:15. **TXT: Wash the intestinal lumen; Remove fecal contents**

3. Recipient Surgery to Introduce the Graft

- 3.1. Placing the anesthetized recipient rat on a heating pad, and make a 5-centimeter midline incision to open the abdominal cavity [1-TXT].
 - 3.1.1. JoVE intestinal transplantation_original_4 00:30-00:45.
TXT: Isoflurane anesthesia: 1.5 L/min
Induction: 2.5%
Maintenance: 1.5%
- 3.2. Move the intestine and colon cranially and dissect the Treitz ligament [1]. Then, wrap the intestine and colon in damp gauze and place them on the rat's body [2].
 - 3.2.1. JoVE intestinal transplantation_original_4 01:45-01:52.
 - 3.2.2. JoVE intestinal transplantation_original_4 02:16-02:25.
- 3.3. Now, dissect the peritoneum over the inferior vena cava and aorta [1]. Identify and ligate the right gonadal artery and vein [2-TXT].
 - 3.3.1. JoVE intestinal transplantation_original_4 03:25-03:35.
 - 3.3.2. JoVE intestinal transplantation_original_4 02:43-04:53. **TXT: Locate the right ureter**
- 3.4. Rotate the rat 90 degrees counterclockwise so the head faces the operator's left [1] and clamp the inferior vena cava above the right renal vein using a curved Bulldog vascular clamp [2].
 - 3.4.1. JoVE intestinal transplantation_original_4 05:00-05:15.
 - 3.4.2. JoVE intestinal transplantation_original_4 08:10-08:25.
- 3.5. Now, incise the inferior vena cava wall with scissors to create an oval opening [1]. Flush with saline [2] and place a stay suture on the caudal edge of the inferior vena cava [3].
 - 3.5.1. JoVE intestinal transplantation_original_4 12:40-12:50 14:43.
 - 3.5.2. JoVE intestinal transplantation_original_4 13:28-13-32
 - 3.5.3. JoVE intestinal transplantation_original_4 14:08-14:20.
- 3.6. Then, position the graft on the rat's left side with the portal vein and superior mesenteric artery aligned near the inferior vena cava and aorta [1]. Orient the distal ileum toward the head. Wrap the graft in cold, damp gauze and apply ice to maintain a low temperature [2].

- 3.6.1. JoVE intestinal transplantation_original_4 15:05-15:15.
- 3.6.2. JoVE intestinal transplantation_original_4 15:20-15:35.
- 3.7. Pass the stay suture needle through the caudal edge of the portal vein and use a mosquito clamp to control suture tension if required [1].
 - 3.7.1. JoVE intestinal transplantation_original_4 16:10-16:30. **TXT: Use 8-0 synthetic polypropylene suture**
- 3.8. Suture and tie the cranial edge of the portal vein to the inferior vena cava [1] and anastomose the posterior portal vein wall [2]. Then, tie the stay suture at the right edge of the portal vein [3] and anastomose the anterior portal vein wall [4].
 - 3.8.1. JoVE intestinal transplantation_original_4 18:21-18:26 and 18:44-18:52 and 19:24-19:30
 - 3.8.2. JoVE intestinal transplantation_original_4 22:27-22:40 and 26:12-26:18
 - 3.8.3. JoVE intestinal transplantation_original_4 31:12-31:28
 - 3.8.4. JoVE intestinal transplantation_original_4 34:48-34:55 and 35:17-35:25
- 3.9. Before completing the anastomosis, clamp the graft portal vein and inject heparinized saline [1]. Tie the suture and release the inferior vena cava clamp [2]. Then, place a curved Bulldog clamp at the aorta above the iliac artery bifurcation clamp [3].
 - 3.9.1. JoVE intestinal transplantation_original_4 36:25-36:30 and 37:12-37:16
 - 3.9.2. JoVE intestinal transplantation_original_4 39:30-39:40 and 40:30-40:38
 - 3.9.3. JoVE intestinal transplantation_original_4 42:22-42:32.
- 3.10. After incising the aortic wall, place a stay suture on the cranial edge of the aorta and superior mesenteric artery [1]. Hold the stay suture with a mosquito clamp [2].
 - 3.10.1. JoVE intestinal transplantation_original_4 44:10-44:20.
 - 3.10.2. JoVE intestinal transplantation_original_4 44:35-44:40.
- 3.11. Pass another 8-0 (8-oh) needle through the caudal edge of the superior mesenteric artery and the aorta [1]. Now, tie and begin anastomosis on the rat's right side, then tie the stay suture [2].
 - 3.11.1. JoVE intestinal transplantation_original_4 46:10-46:20.
 - 3.11.2. JoVE intestinal transplantation_original_4 52:20-52:35.

- 3.12. Next, rotate the rat 180 degrees so the head faces the operator's right [1] and complete the superior mesenteric artery-to-aorta anastomosis on the left side [2]. Before the final suture, clamp the graft portal vein and inject heparinized saline [3]. Tie the suture [4]. Now, release the aortic and portal vein clamps to allow reperfusion [5].
- 3.12.1. JoVE intestinal transplantation_original_4 53:40-53:50 01:03:22.
- 3.12.2. JoVE intestinal transplantation_original_4 54:40-54:50.
- 3.12.3. JoVE intestinal transplantation_original_4 01:02:35-01:02:45.
- 3.12.4. JoVE intestinal transplantation_original_4 01:03:05-01:03:15.
- 3.12.5. JoVE intestinal transplantation_original_4 01:04:10-01:04:25.
- 3.13. Make two 8-millimeter skin incisions on the right abdomen and exteriorize the proximal and distal ileal graft ends [1-TXT]. Finally, suture the skin and graft serosa using 5-0 (5-oh) polydioxanone suture [2] and close the midline incision with the same suture to complete the surgery [3-TXT].
- 3.13.1. JoVE intestinal transplantation_original_5 00:30-00:40. **TXT: Rotate the rat 90° so the head faces the opposite side**
- 3.13.2. JoVE intestinal transplantation_original_5 09:50-10:00.
- 3.13.3. JoVE intestinal transplantation_original_5 15:50-16:00. **TXT: Assess the intestinal graft post-operatively**

Results

4. Results

- 4.1. The ileostomy grafts turned visibly whitish around post-operative day 45, indicating rejection in allogeneic recipients [1], while syngeneic grafts maintained a healthy pink tone through post-operative day 127 [2].

4.1.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the POD45 image.*

4.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the POD127 (syngeneic) image.*

- 4.2. Quantitative scoring using an RGB-based regression model showed a progressive decline in graft health scores over 45 days in allogeneic grafts [1].

4.2.1. LAB MEDIA: Figure 2C.

- 4.3. Histological imaging at post-operative days 28 and 45 revealed a loss of epithelial villi and crypt structures in allogeneic grafts [1], accompanied by an expanded submucosal matrix rich in collagen and laminin [2].

4.3.1. LAB MEDIA: Figure 3A. *Video editor: Zoom in on the merged panel at POD45 and POD 28.*

4.3.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the collagen and pan-laminin images for POD28 and POD45*

- 4.4. Sirius Red staining on post-operative day 45 confirmed prominent extracellular matrix deposition in the allogeneic group [1], in contrast to well-defined mucosal layers in syngeneic grafts [2].

4.4.1. LAB MEDIA: Figure 3B. *Video editor: Highlight image 'allogeneic'.*

4.4.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the left-side image 'syngeneic'*

- 4.5. Alternatively, graft health could be assessed by monitoring homeostatic intestinal macrophages expressing Cx3cr1 (*C-X-3-C-R-1*), Mrc1 (*M-R-C-1*), and Timp2 (*T-I-M-P-2*), which were distinctly localized within a specific cluster on UMAP [1].

4.5.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the cluster pointed by the 'black arrows' across the "Cx3cr1, Mrc1, and Timp2" plots*

4.6. MIKA-based scoring confirmed strong S1 identity for these homeostatic macrophages [1].

4.6.1. LAB MEDIA: Figure 4B. *Video editor: Highlight S1 panel.*