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Scriptwriter Name: Swati Madhu
Supervisor Name: Anastasia Gomez
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Title: Partial Heterotopic Hindlimb Transplantation Model in Rats

Authors and Affiliations:

Marion Goutard[1,2,3], Mark A. Randolph[1,2,3], Corentin B. Taveau[1,2,3,4], Elise Lupon[1,2,3], Laurent Lantieri[4], Korkut Uygun[1,2,3], Curtis L. Cetrulo Jr.[1,2,3]*, Alexandre G. Lellouch[1,2,3,4]*

[1]Division of Plastic and Reconstructive Surgery, Massachusetts General Hospital, Harvard Medical School
[2]Vascularized Composite Allotransplantation Laboratory, Center for Transplantation Sciences, Massachusetts General Hospital, Harvard Medical School
[3]Shriners Hospital for Children
[4]Service de Chirurgie Plastique, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris (APHP), Université Paris

*C. L. Cetrulo Jr. and A. G. Lellouch contributed equally to this work.

☐ All author names and affiliations are correct.

Corresponding Authors:

Alexandre G. Lellouch alellouch@mgh.harvard.edu

Email Addresses for All Authors:
mgoutard@mgh.harvard.edu
marandolph@mgh.harvard.edu
corentin.taveau@gmail.com
elupon@mgh.harvard.edu
laurentlantieri@gmail.com
korkut.uygun@gmail.com
ccetrulo@mgh.harvard.edu
alellouch@mgh.harvard.edu

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 M525 F40 surgical microscope

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

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page as soon as possible.

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.

   ☐ Interviewees self-record interview statements. JoVE can provide support for this option.

   ☐ Interview Statements are read by JoVE's voiceover talent.

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

   If **Yes**, how far apart are the locations? **Click to enter distance between locations.**

To ensure that your script can be filmed in one day, the Protocol section is restricted to **55 shots** (shots are the 3-digit numbers like 2.1.1, 2.1.2…etc)

Current Protocol Length

Number of Steps: 16
Number of Shots: 54

NOTE to Video Editor: Authors used the script draft for filming instead of the final script

Introduction

1. Introductory Interview Statements

1.1. **Alexandre G. Lellouch:** We present a reliable and reproducible surgical model of heterotopic hindlimb transplantation in rats.

1.2. **Alexandre G. Lellouch:** This technique is ideal for short-term studies on preservation or immune rejection in VCA. This step-by-step description will allow trainees to rapidly master this surgery.

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Massachusetts General Hospital.

Protocol

2. Donor Right Partial Hindlimb Procurement

2.1. To begin, make a circumferential incision on the skin above the ankle at the distal third of the leg **[1-TXT]**. Skeletonize and cauterize the saphenous artery and the terminal branch of the popliteal artery using bipolar forceps **[2]**. Cauterize and cut the gastrocnemius, soleus, tibialis anterior, and biceps femoris muscles until the tibial bone is exposed **[3]**.

2.1.1. WIDE: Talent making an incision. **TEXT: Anesthesia induction: 5% isoflurane; Anesthesia maintenance: 1.5–3% isoflurane inhalation through a breathing cone**

2.1.2. Talent skeletonizing and cauterizing the arteries.

2.1.3. Added shot: Talent cauterizing and cutting the tissues.

2.2. Make a 2.5-centimeter incision at the right inguinal crease **[1]** and dissect the inguinal fat pad **[2]**. Use a fishhook retractor to grasp the inguinal ligament **[3]**.

2.2.1. Talent making incision. NOTE: most of the procurement shots may have been SCOPE

2.2.2. Talent dissecting the fat pad.

2.2.3. Talent grasping the inguinal ligament.

2.3. Hold the inguinal fat pad distally with clamping forceps **[1]** and retract the fat pad to expose the femoral vessels **[2]**. Dissect the femoral vessels **[3]**, individualize the murphy branches **[4]**, and ligate with 8/0 nylon ties **[5]**.

2.3.1. Talent holding the inguinal fat pad.

2.3.2. Talent retracting the fat pad.

2.3.3. Talent dissecting the femoral vessels.

2.3.4. Talent individualizing the murphy branches..

2.3.5. Talent ligating. *Videographer: This shot will be used again at 3.3.1.*

2.4. Heparinize the donor rat by injecting the heparin in the penile dorsal vein using a 27.5-gauge needle **[1-TXT]**. Complete the skin incision around the hip **[2]**. Use the bipolar forceps to cauterize the biceps femoris and gluteus superficialis muscles **[3]**, then cauterize and cut the sciatic nerve at mid femur length **[4]**.

2.4.1. Talent injecting heparin in the penile dorsal vein. **TEXT: Heparin-100 IU/kg**

2.4.2. Talent making the incision around the hip.

2.4.3. Talent cauterizing the muscles.

2.4.4. Talent cauterizing and cutting the nerve.

2.5. Expose the femur proximally at the level of the posterior femoral crest **[1]** and ligate the femoral vessels with nylon ties at the level of the inguinal ligament **[2]**. Perform an arteriotomy on the femoral artery just below the ligature **[3]** and dilate to insert the 24-gauge angio-catheter **[4]**.

2.5.1. Talent exposing the femur.

2.5.2. Talent ligating the femoral vessel.

2.5.3. Talent performing the arteriotomy.

2.5.4. Talent dilating the femoral artery and inserting the angio-catheter.

2.6. Cauterize and cut the remaining muscle underneath the pedicle to expose the anterior side of the femur **[1]**. Use the bone cutter to cut the tibia proximally **[2]** and cut the femur as distally as possible **[3]**. Flush the partial hindlimb with 2 milliliters of heparin saline **[4-TXT]** to obtain a clear venous outflow **[5]**.

2.6.1. Talent cauterizing and cutting the remaining muscles.

2.6.2. Talent cutting the tibia proximally.

2.6.3. Talent cutting the femur as distally as possible.

2.6.4. Talent flushing the partial hindlimb with heparin saline. **TEXT: Heparin-100 IU/mL**

2.7. Place the hindlimb on ice in a sterile gauze until the microvascular transfer **[1]**.

2.7.1. Talent placing the hindlimb on the ice.

3. Recipient Surgery

3.1.1. Talent making an incision. NOTE: Authors deleted VO narration, please show this shot along with the narration for the next step.

3.2. Dissect the inguinal fat pad **[1-TXT]** and recline the inguinal fat pad distally to expose the femoral vessels **[2]**. Use a hook to retract the inguinal ligament **[3]** and clamping forceps to hold the inguinal fat pad distally **[4]**.

3.2.1. Talent dissecting the inguinal fat pad. **TEXT: Analgesia: buprenorphine 0.01–0.05 mg/kg subcutaneously**

3.2.2. Talent retracting the inguinal ligament.

3.2.3. Talent reclining the inguinal fat pad.

3.3. Dissect the femoral vessels, individualize the murphy branches, and ligate with nylon ties, as demonstrated **[1]**. Ligate both vessels above the epigastric vessels using nylon ties **[2]**. Place the approximator clamps proximally **[3]**, dilate the vessel ends **[4]**, and rinse with heparin saline **[5]**.

3.3.1. *Use 2.3.5*

3.3.2. Talent ligating the vessels.

3.3.3. Talent place the approximator clamps.

3.3.4. Talent dilating the vessel ends.

3.3.5. Talent rinsing with heparin saline.

3.4. Make an incision on the left flank above the hip **[1]** and create a subcutaneous pocket with a subcutaneous tunnel to the inguinal crease **[2]**. Place the proximal part of the partial limb and the inguinal fat pad through the subcutaneous tunnel for microvascular transfer **[3]**.

3.4.1. Talent making an incision.

3.4.2. Talent creating a subcutaneous pocket with a subcutaneous tunnel.

3.4.3. Talent placing the partial limb and the inguinal fat pad through the subcutaneous tunnel.

3.5. Perform the venous and arterial anastomoses using nylon sutures **[1-TXT]**. Remove both approximator clamps **[2]** and observe the revascularization of the limb **[3]**. Perform a milking test on both vessels to assess the patency of each anastomosis **[4]**.

3.5.1. Talent performing the venous and arterial anastomoses. TEXT: **TEXT: Nylon sutures: 10/0**

3.5.2. Talent removing the approximator clamps.

3.5.3. Revascularized limb.

3.5.4. Talent performing the milking test.

3.6. Make a longitudinal skin incision on the medial side of the transplanted limb **[1]** and insert the graft **[2]**. Remove the excess skin of the graft **[3]** and close the wound with separate sutures and a running suture using absorbable 4/0 sutures **[4-TXT]**.

3.6.1. Talent making an incision.

3.6.2. Talent inserting the graft.

3.6.3. Talent removing the excess skin of the graft.

3.6.4. Talent closing the wound with suture. **TEXT: 4/0 absorbable sutures.**

3.7. Suture together the inguinal fat pads of the transplanted limb and the recipient using two separate absorbable sutures **[1]** and close the inguinal crease after the last checkup of the microvascular anastomoses **[2]**.

3.7.1. Talent suturing the fat pad and the recipient.

3.7.2. Talent closing the inguinal crease.

Results

4. Results: Analysis of Transplanted Syngeneic Heterotopic Partial Limb

NOTE: Numbering was wrong in the script that the authors used for the shoot, it was fixed during postshoot stage for VO recording purposes.

4.1. The evolution of the heterotopic hindlimb model was monitored until the end of the study **[1]**. The hair regrowth was observed during the first postoperative week **[2]**, and the cutaneous retraction appeared after 2 weeks **[3]**.

4.1.1. LAB MEDIA: Figure 3

4.1.2. LAB MEDIA: Figure 3 *Video editor: Please emphasize POD7 image in the figure.*

4.1.3. LAB MEDIA: Figure 3 *Video editor: Please emphasize POD14 image in the figure.*

4.2. Vascularized composite allotransplantation failure can occur during the first postoperative week due to microvascular thrombosis **[1]**. Venous thrombosis was the cause of early euthanasia in 20% of the cases, all of which occurred before postoperative day 5 **[2]**. The skin appeared blue and became darker each day **[3]**.

4.2.1. LAB MEDIA: Figure 4

4.2.2. LAB MEDIA: Figure 4

4.2.3. LAB MEDIA: Figure 4 *Video editor: Please emphasize POD2 image in the figure.*

4.3. Self-mutilation is a serious concern in non-sensate grafts and often occurs between postoperative day-2 and 7 **[1]**. If limited to less than a third of the graft surface and concerns only skin **[2]**, surgical debridement and suture using non-absorbable sutures can be discussed with the staff veterinarian **[3]**.

4.3.1. LAB MEDIA: Figure 5

4.3.2. LAB MEDIA: Figure 5A

4.3.3. LAB MEDIA: Figure 5B and 5C

4.4. Prevention of self-mutilation relies on using an E-collar stitched to the neck until postoperative day-7 and cleaning any blood or crust on the animal's surgical wounds. Severe autophagia of multiple layers of the graft leads to euthanasia of the animal **[1]**.

4.4.1. LAB MEDIA: Figure 5D

4.5. Dermal cysts appeared after postoperative day-14 **[1]**, sometimes with a cutaneous necrotic center before the fistula **[2]**.

4.5.1. LAB MEDIA: Figure 6 *Video editor: Please highlight the area marked as "A" in the image.*

4.5.2. LAB MEDIA: Figure 6 *Video editor: Please highlight the area marked as "B" in the image.*

Conclusion

5. Conclusion Interview Statements

5.1. **Marion Goutard:** (3.4, 3.7) It is crucial to ensure that the graft placement will not prevent the animal from being ambulatory and that the pedicle is not kinked or tight during the graft inset. NOTE: Suggested B-roll 3.7.1.
NOTE: Numbering was messed up in the results section, these interviews should be slated as 5.1.1. and 5.2.1, but may also be slated as 4.6 and 4.7.

5.2. **Marion Goutard:** Our team performed preservation studies on the procured limbs before transplantation. The effects of different durations of static cold storage have been evaluated as well as different limb perfusion protocols.