Video Article

Orthotopic Hind-Limb Transplantation in Rats

Robert Sucher[1,*], Rupert Oberhuber[1,*], Christian Margreiter[1], Guido Rumberg[1], Rishi Jindal[2], WP Andrew Lee[2], Raimund Margreiter[1], Johann Pratschke[1], Stefan Schneeberger[1], Gerald Brandacher[1]

[1]Department of Visceral, Transplant, and Thoracic Surgery, Daniel Swarovski Research Laboratory, Innsbruck Medical University
[2]Department of Surgery, Division of Plastic and Reconstructive Surgery, University of Pittsburgh Medical Center

[*]These authors contributed equally

Correspondence to: Robert Sucher at robert.sucher@i-med.ac.at

URL: http://www.jove.com/details.php?id=2022

DOI: 10.3791/2022

Citation: Sucher R., Oberhuber R., Margreiter C., Rumberg G., Jindal R., Lee W.A., Margreiter R., Pratschke J., Schneeberger S., Brandacher G. (2010). Orthotopic Hind-Limb Transplantation in Rats. JoVE. 41. http://www.jove.com/details.php?id=2022, doi: 10.3791/2022

Abstract

Composite tissue allotransplantation (CTA) now represents a valid therapeutic option after the loss of a hand, forearm or digits and has become a novel therapeutic entity in reconstructive surgery. However, long term high-dose multi-drug immunosuppressive therapy is required to ensure graft survival, bearing the risk of serious side effects which halters broader application. Further progression in this field may depend on better understanding of basic immunology and ischemia reperfusion injury in composite tissue grafts.

To date, orthotopic hind limb transplantation in rats has been the preferred rodent model for reconstructive transplantation (RT), however, it is an extremely demanding procedure that requires extraordinary microsurgical skills for reattachment of vasculature, bones, muscles and nerves.

We have introduced the vascular cuff anastomosis technique to this model, providing a rapid and reliable approach to rat hind limb transplantation. This technique simplifies and shortens the surgical procedure and enables surgeons with basic microsurgical experience to successfully perform the operation with high survival and low complication rates. The technique seems to be well suited for immunological as well as ischemia reperfusion injury (IRI) studies.

Protocol

Donor Operation and Hind-Limb Harvest

1. For the operative procedure a sterile set of microsurgical instruments is utilized. The surgeon should wear proper attire including sterile gloves, which have to be changed prior to the start of the surgical procedure.
2. The rat is anesthetized with an intraperitoneal injection of pentobarbital (50mg/kg IP) and placed supine on a heated operation field, which facilitates a stable animal body temperature throughout the entire surgical procedure.
3. Prior to skin incision the filed of operation is sanitized three times each with both, a disinfectant and alcohol. To prevent the animal's corneas from drying out, eyes are lubricated with an ophthalmic ointment (Vidisic) prior to surgery.
4. A skin incision is made across the right groin approximately at the level of the inguinal ligament. The femoral vessels are dissected free with bipolar cautery and blunt dissection; deep branches are ligated with 8-0 silk.
5. 300µl (50 IU) of heparin are injected via the penile vein for anti-coagulation.
6. Femoral vessels are ligated at the level of the inguinal ligament and cut distal to the ligature.
7. The hind limb is flushed with ice-cold (4°C) HTK solution (5ml) via the femoral artery over 5 minutes.
8. All muscle groups are sharply divided with scissors and osteotomy is performed with a rotating electrical saw at the level of the distal third of the femur.
9. The harvested limb is then submerged in 20mL of ice-cold HTK solution for preservation and cold storage until transplantation.

Recipient Operation and Transplantation

1. The rat is anesthetized with an intraperitoneal injection of pentobarbital and placed supine on the operation field.
2. A skin incision is made across the right groin approximately at the level of the inguinal ligament. The femoral vessels are dissected free with bipolar cautery and blunt dissection.
3. Femoral vessels are ligated as distal as possible, but proximal to the epigastric vessel takeoff. Vascular clamps are placed at the level of the inguinal ligament on artery and vein.
4. A polyethylene cuff is pulled over the artery (inside cuff diameter: 0.912 mm, cuff-wall thickness: 0.010 mm, cuff length: 2.0 mm) and vein (inside cuff diameter: 1.151 mm; cuff-wall thickness: 0.010 mm, cuff length: 2.0 mm). The edge of the vessel lumen is then pulled with a vessel dilator and the femoral artery and vein are everted over the whole body of the cuff (approximately 2mm) and fixed with a circumferential 8-0 silk ligature.
5. To make room for the donor limb the recipient limb is removed with sharp dissection of muscle groups and osteotomy is performed at the mid-femur.
6. The donor limb is removed from the HTK solution and transplantation begins with osteosynthesis of the femur using an 18-gauge needle (length: 1.5-2.0 cm) as an intramedullary rod.
7. Ventral muscle groups are closely approximated with 4-0 Vicryl or Prolene interrupted sutures.
8. Vascular anastomoses are performed by pulling the donor vein and artery over the recipient's vein and cuff complex. A circumferential 8-0 silk ligature is again placed to secure the anastomosis.
9. The venous clamp is released followed by the arterial clamp ,and reperfusion is visualized by return of colour to the distal limb and foot, as well as pulsatile flow through the artery.

10. The remaining dorsal muscle groups are re-approximated with 4-0 Vicryl or Prolene interrupted sutures.
11. The skin is closed with 4-0 vicryl or prolene interrupted sutures.
12. The recipient rat is placed on a heating pad and monitored closely until recovery.
13. Recipient animals receive prophylactic antibiotics (Cefazolin, 20 mg/day, IM) and Buprenex (0.1 mg/kg, SC) for three posttransplant days.

Discussion

Decades of experimental research have preceded and enabled successful human hand transplantation in the recent past. In 1978, Shapiro et al. developed an orthotopic hind limb transplant model in rats, well suited to study various pathological, physiological, and immunological aspects as well as functional outcome of composite tissue grafts. We have modified the most challenging part of the procedure, namely the vascular anastomosis, by introducing a non-suture cuff technique. When compared to the conventional suture technique it simplifies and shortens the surgical procedure and enables surgeons with basic microsurgical experience to carry out the procedure with a high survival rate (80-90%).

Without the variability of sutured anastomoses, the risk of bleeding, thrombosis, and kinking of the pedicle are diminished. Constriction or turbulence at the site where blood initially collides with the lumen passing through the cuff is a new potential risk of the cuff technique, although this can be minimized with appropriate cuff size selection. Furthermore, small size mismatches can easily be handled with a cuff anastomosis, again reducing the risk inherent to suturing vessels of different diameters. Some of the most significant considerations for a successful cuff anastomosis are given below:

1. A single injection of Pentobarbital (50 mg/kg IP) should be sufficient to anesthetize the animal for 90 minutes. In case the operation lasts more than 70-80 minutes an additional dose of 15mg/kg IP should be applied. Depth of anesthesia can be tested intraoperatively by repeating the toe-pinch reflex test.
2. The osteotomy of the donor hind limb/recipient hind limb should be performed at the mid-femur to alleviate osteosynthesis during transplantation.
3. A careful selection of cuff sizes is of crucial importance for successful revascularization.
4. To avoid thromboses, heparin (50IU) should be administered to the donor 1 minute prior to graft perfusion.

Disclosures

No conflicts of interest declared.

References

1. Shapiro, R.I. & Cerra, F.B., A model for reimplantation and transplantation of a complex organ: the rat hind limb. *J Surg Res* 24 (6), 501-506 (1978).
2. Petruzzo, P. *et al.*, The international registry on hand and composite tissue transplantation. *Transplantation* 86 (4), 487-492 (2008).
3. Schneeberger, S. *et al.*, First forearm transplantation: outcome at 3 years. *Am J Transplant* 7 (7), 1753-1762 (2007).

