Hand Transplantation in the Rat: Technical Refinements of the Rat Forelimb Vascularized Composite Allotransplantation Model

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Introduction

The worldwide experience in upper limb transplantation has now reached nearly 150 limbs in 100 patients with overall promising mid-term results[1]. Until recently, most limb transplant research done on rats consisted of either orthotopic or heterotopic hind limb transplantation[2]. This approach, based on anastomosis of the femoral vessels, has been technically achievable since at least the 1970s[3]. With the improvement of microsurgical techniques over the years and the need for a better functional model of limb transplantation, our team was the first to report successful orthotopic forelimb vascularized composite allotransplantation in a rat model[4]. Since this publication, we have developed technical refinements of the model which we hypothesize will lead to better allograft and animal survival rates, allowing for better study of immunologic and functional outcomes.

Methods

After IACUC approval, we performed 60 orthotopic forelimb transplants from Brown Norway rats to Lewis rats. Transplantation was performed at the mid-humeral level on rats 8-10 weeks old weighing an average of 250g. For the donor operation, under deep Isoflurane inhalational anesthesia, the brachial vessels, radial, median, and ulnar nerves were dissected and the nerves were divided with as much length as possible (Figure 1). The muscle was cut with bipolar cautery and the bone sectioned with a rotary saw. The vessels were then divided and the limb was stored in moist gauze on ice. The recipient operation was similar to the donor, except the lateral thoracic vein was also dissected and transposed into the field as a larger recipient vein (instead of the external jugular vein as in our previous work). In addition, instead of using the common carotid artery for the recipient artery as done previously, we used the axillary artery. Transplantation was then performed with a 25gauge needle for humerus fixation as an intramedullary rod (Figure 2), followed by muscle repair, microvascular arterial, vein, and nerve repairs (Figure 3). The skin was then closed (Figure 4). The animals were treated

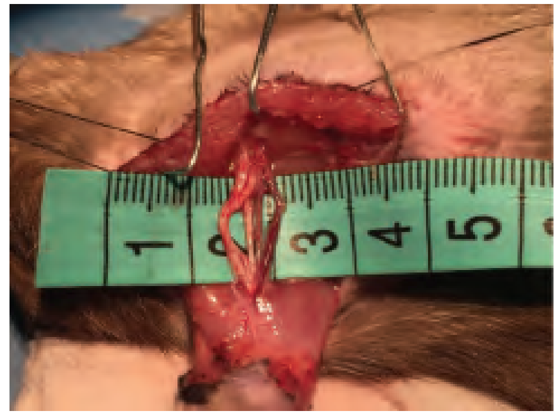

Figure 1. Donor limb neurovascular bundle of the right forelimb with head to the left. The median, ulnar, and radial nerves are to the left and the brachial artery and vein to the right.

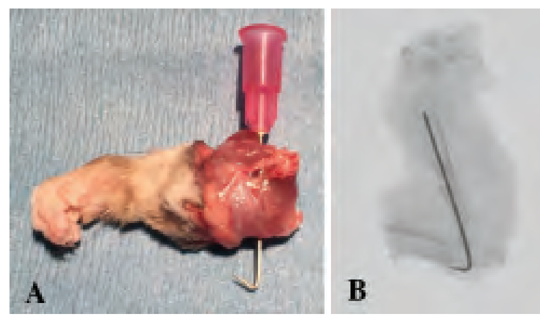

Figure 2. Bone fixation in the rat forelimb transplant. **(A)** Donor limb with a 25 g needle through the distal humerus as an IM rod. **(B)** X-ray of the rat forelimb transplant with the needle IM rod showing adequate bone contact between donor and recipient. This is reinforced by the surrounding muscle repairs.

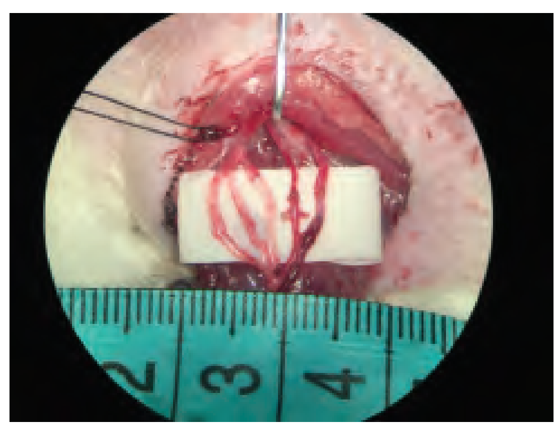

Figure 3. Demonstration of anastomosis of brachial artery to brachial artery and brachial vein to lateral thoracic vein with 11-0 suture. And approximation of the median, ulnar and radial nerves with 10-0 nylon interrupted epineurial sutures.

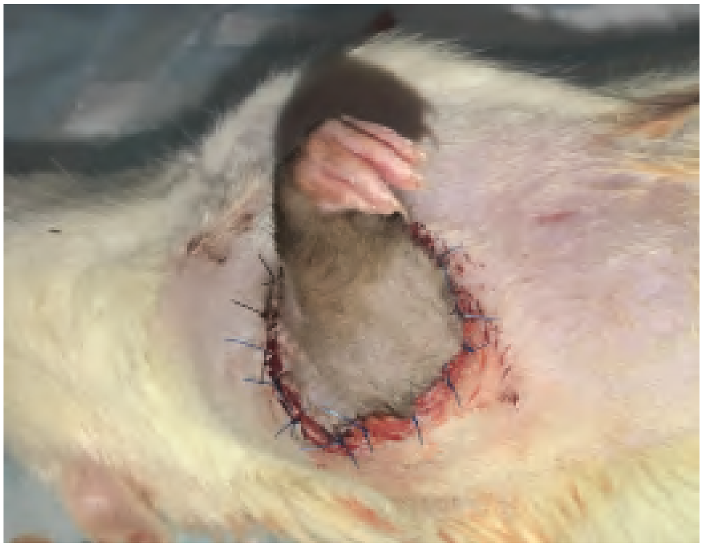

Figure 4. Immediate postoperative photo of the rat forelimb transplant from Brown Norway rat (donor) to a Lewis rat (recipient).

with analgesics, and some with an implantable capsule for sustained release of rapamycin 1mg/kg/day. Allograft and animal survival rates were recorded.

Results

Surgery time was 2.79 ± 0.58 hours with an ischemia time of 1.98 ± 0.48 hours. One animal died during surgery making for 98.3% survival. The allograft survival rate was 91.7% with 5 vascular complications including venous thrombosis in 2, arterial thrombosis in 2, and 1 animal death. The average artery size was 0.72 ± 0.17 mm and the vein size was 1.22 ± 0.24 mm. The allograft survived less than 13 days without rapamycin and up to 35 days with rapamycin.

Conclusions

Although technically demanding, rat orthotopic forelimb transplantation is feasible with standard microsurgical techniques leading to high allograft survival rates. We were able to improve on our previous work by using the lateral thoracic vein for outflow instead of the external jugular vein, and the axillary artery for inflow instead of the common carotid artery. This saves time by preventing dissection in the neck and mitigates risk of brain injury in the animals. This model may prove to be good for studying functional outcomes after forelimb transplantation and the impact of immunomodulation/immunosuppression on nerve regeneration.

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