Development of New Composite Tissue Allotransplantation Models using supermicrosurgery

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Abstract

Currently, the composite tissue allotransplantation (CTA) is used clinically. However, side effects due to the immunosuppressant agents not for critical grafting become a major issue. Immunological tolerance introduced by the bone marrow transplantation (BMT) is an innovative therapy enabling long survival of a graft without immunosuppressant agents. We developed a new CTA model in order to apply the immunological tolerance introduction to the CTA. We prepared a rat lower limb transplantation model, a toe transplantation model, a forearm transplantation model, and mouse thigh chimera CTA model using supermicrosurgical technique. Additionally, we prepared a swine mandible CTA model in order to apply the technology to humans showing it is useful and with a wide clinical application. The CTA models developed by us do not include bones containing a lot of marrow (i.e., femur, humerus). They are superior CTA models that can evaluate the effect of immune tolerance by BMT.

Keywords

Composite tissue allotransplantation, Supermicrosurgery, Immunotolerance induction, Bone marrow transplantation

Introduction

Quality of life (QOL) is significantly deteriorated due to disordered function and cosmetic reason if upper and lower extremities or face tissue are lost because of trauma and tumor. Various therapies are tried to reconstruct loss of tissue and some effects have been achieved. However, it is very difficult to restore complicated shape and function of

Introduction

Quality of life (QOL) is significantly deteriorated due to disordered function and cosmetic reason if upper and lower extremities or face tissue are lost because of trauma and

face and hands (Figure.1). Reconstruction by facial and limb transplant attracts attention as useful remedy for these lesions. Composite tissue allotransplantation such as facial and hand transplant are performed clinically. However, CTA including skin is highly likely to generate rejection and graft survival is unstable. Immunosuppressant agents are used for raising survival rate¹⁻⁴⁾. However, side effects of immunosuppressant agents such as infection and malignant tumor constitute a major problem because the transplantation is not for saving life.

Various pieces of research are being performed on reduction of side effects of immunosuppressant agents. Specially, introduction of immunological tolerance to implant grafts without using immunosuppressant agents is actively researched. In 2007, Kawai et al. reported a clinical example of adult renal transplant allowing long term graft survival by introducing immunological tolerance by concomitantly using BMT without using immunosuppressant agent^{5, 6)}.

For CTA, large animal models such as swine, mouse and rat lower limb transplantation model have been reported. All CTA models include femur containing a lot of bone marrow. Therefore, immunological tolerance by the difference of contents of bone marrow cannot be verified. 7-9 We developed a new CTA model in which effect of introduction of immunological tolerance by BMT can be verified. Additionally, we prepared a new CTA model using swine (large animal) aiming at clinical application for humans.

Method

We devised and prepared CTA models (bone, muscle, nerve and skin) that did not include femur, humerus, sternum and pelvis that did not include a lot of bone marrow using rats (200-350 g),

mouse (20-40 g) and swine (10-20 kg). We prepared a rat

lower limb transplantation model, a toe transplantation model, a forearm transplantation model, a mouse thigh chimera CTA model, and a swine mandible CTA model. We performed transplantations by the technique of supermicrosurgery in a synergetic manner. We evaluated the vascular diameter and patency rate of the rat and mouse models (small animal).

Rat lower limb transplantation model: We dissected the femoral artery, vein and nerve of the donor rat from the groin to the knee. We cut the lower limb at the knee joint and neurovascular bundle at the groin to take the lower limb graft. Likewise, we cut the lower limb of the recipient rat. We performed end-to-end anastomosis on femoral artery, vein and nerve and transplanted the lower limb graft on the back.

Rat toe transplantation model: We dissected the artery, vein and nerve from the groin to the great toe of the donor rat. We cut the great toe at the great toe MTP joint and removed the blood vessel and nerve at the level of knee to take a toe graft. We performed end-to-end anastomosis on superficial inferior epigastric artery, vein and nerve with the femoral artery, vein nerve (knee level). We transplanted the toe graft on the lower abdomen (Figure 2).

Rat forearm transplantation model: We dissected the brachial artery, vein and nerve of the donor rat from the axilla to cubital region. We cut the forearm at the cubital joint and removed blood vessel and nerve at the axilla to take the forearm graft. We cut the forearm of the recipient rat. We performed end-to-end anastomosis on artery vein and nerve and transplanted the forearm graft on the back.

Mouse femoral chimera CTA model: We raised superficial inferior epigastric artery and vein flap, femur cortical bone flap and adductor muscle flap as a whole using donor mouse's femoral artery and vein as common vascular pedicle. We removed femoral artery and vein and nerve to obtain thigh CTA graft. We performed end-to-end anastomosis of the femoral chimera graft to the femoral artery and vein and nerve in order to transplant the femoral chimera graft to the groin (Figure 3).

Swine mandible CTA model: We raised the mandible CTA graft including mandible bone body and skin using the facial artery and vein as vascular pedicle. We

Figure 2.
 (Left) The toe graft is raised by using femoral artery (above-knee) 0.3 mm and femoral vein (above-knee) 0.5 mm as vascular pedicle. (Right) The graft is transplanted to the lower abdomen by performing end-to-end anastomosis to the superficial inferior epigastric artery 0.3 mm and vein 0.5 mm.

Figure 3. Mouse femoral chimera CTA mode
 The chimera graft including cortical bone, muscle and skin is raised by using femoral artery 0.3 mm and femoral vein 0.5 mm as common vascular pedicle and transplanted in an orthotopic manner.

confirmed that we could adjust the content of the bone marrow by removing the cancellous bone. After cutting facial artery and vein, we transplanted the mandible graft at the same position (Figure 4).

R esult

Result

Table 1 shows the diameters of blood vessel of each transplantation model.

For the rat lower limb transplantation model, toe transplantation model, forearm

transplantation model, mouse femoral CTA model, swine

mandible CTA model, the patency test showed good blood flow immediately after anastomosis. No graft necrosis was observed and patency rate was 100%. CTA requiring microneurovascular anastomosis (less than 0.5 mm) was surely possible by using the technique of supermicrosurgery.

D iscussion

Discussion

If tissue of limbs and face is lost by trauma or tumor resection, QOL is significantly reduced due to dysfunction and reduced cosmetic condition. Various local

Figure 4. Swine mandible CTA model

(Left) Design of mandible CTA graft. (Right) The graft is raised by using the facial artery D1.5 mm and facial vein D2.0 mm as vascular pedicle. After cutting the vascular pedicle, it is anastomosed again. The graft is transplanted in an orthotopic manner.

	Donor artery / vein [diameter : mm]	Recipient artery / vein [diameter : mm]
Rat lower limb	Femoral artery [0.5mm]/vein [0.7mm]	Femoral artery [0.5mm]/vein [0.7mm]
Rat toe	Femoral artery [0.3mm]/vein [0.5mm]	Superficial inferior epigastric artery [0.3mm]/vein [0.5mm]
Rat forearm	Brachial artery [0.4mm]/vein [0.6mm]	Brachial artery [0.4mm]/vein [0.6mm]
Mouth thigh	Femoral artery [0.3mm]/vein [0.5mm]	Femoral artery [0.3mm]/vein [0.5mm]
swine mandible	Facial artery [1.5mm]/vein [2.0mm]	Facial artery [1.5mm]/vein [2.0mm]

Table 1. Vascular diameter of donor and recipient in each transplantation model

and free flaps have been developed for treatment of loss of tissue and achieved a certain effect. However, it is very difficult to reconstruct the original condition for regions having complicated shape and function such as face and hand. In the disputed areas and war zones, there are lots of limb deficiencies due to trauma irrespective of military personnel and civilian. Development of excellent therapy is desired from a viewpoint of health economics, welfare, politics and military affair. For these cases, reconstruction by facial transplant and limb transplant is attracting attention as useful therapy.

CTA such as facial transplant and hand transplant is effective for reconstruction of large tissue defect and regions having complicated function and shape. Large issue is the side effect of immunosuppressant agents not for critical care medicine but for transplantation^{1, 2, 4)}. Immunological tolerance by bonemarrow graft is applied in a clinical setting of kidney transplantation. It is an

attractive method to produce immunological tolerance also in CTA. Various CTA models such as swine (large animal) models and mouse and rat lower limb transplantation models have been devised⁵⁻⁸⁾. However, all models are CTA models including femur containing a large amount of bone marrow. Therefore, it is impossible to discuss the effect of immunological tolerance introduced by the amount of bonemarrow graft.

We succeeded in developing new CTA models by using bone tissue containing little bone marrow. We can discuss the amount of bone-marrow graft by using these models. We showed that we could transplant composite tissue including radius, ulna, tibia, fibula, and phalanx with little bone marrow by using supermicrosurgery. For rat lower limb transplantation, toe transplantation, forearm transplantation, mouth thigh chimera CTA and swine mandible CTA, secure patency was obtained (patency rate 100%). They were supposed to be useful for CTA model.

We also prepared a new CTA model of swine (large animal) that can adjust content of bone marrow. We showed clinical application to human was possible from technical basis.

Conclusion

We developed new CTA models containing little bone marrow. Secure vascular anastomosis is enabled by super-micro-surgery realizing a useful CTA model.

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