Hemi-tongue Allograft Transplantation in Dogs

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Background: When complete recovery of tongue function following tumor excision is desired, reestablishment of the complex movements of the tongue is necessary. However, currently available methods for recovery of tongue function, such as flap surgery or prosthesis insertion, are inadequate. In the current study, we investigated the effects of transplantations of tongue allografts.
Methods: Hemi-tongue allotransplantation procedures were performed with 8 pairs of sex-blind and unrelated beagle dogs. In each donor, the right side of the tongue, including the lingual and hypoglossal nerves, extrinsic muscles of the tongue, mucous membrane of the oral floor, lingual artery, and vein were exposed. A vascularized transplantation method was used with manual anastomosis of the blood vessels and nerves.
Results: Survival of the grafted tongue was only noted in 1 dog that died 5 days after transplantation. We suspected that the death was due to nutritional deficiency or dehydration, rather than hyperacute rejection of the transplant or technical failure of the microsurgical anastomosis. The grafted tongue was partially connected to the side of the recipient tongue, and lymphocyte infiltration was observed in this dog.
Conclusions: Postoperative management is difficult in dogs. Even if tongue allograft including nerves and extrinsic muscles is performed, it seems to take a long time before the tongue recovers its functions. Furthermore, expansive tongue allograft was too invasive a treatment for animals. If we want to adapt this procedure to humans, the first trial in a human will be done without animal experiments, as was the case with face transplantations. *(Plast Reconstr Surg Glob Open 2020;8:e2767; doi: 10.1097/GOX.0000000000002767; Published online 10 April 2020.)*

INTRODUCTION

Patients who undergo tongue excision because of a tumor experience postoperative difficulties with various oral functions, such as eating, swallowing, and speaking, when the excision size is large. To improve the postoperative condition, flaps or a palatal augmentation prosthesis is typically used.[1–6] However, dysfunctional chewing and speaking often remain in many patients following such procedures, and swallowing difficulty recurs postoperatively due to atrophy of the flap. Suzuki et al[7] reported that the average percent change in flap volume after free-flap reconstructive surgery in patients with tongue cancer was

82.3% at 1 year postoperatively. In those patients, the quality of life became poor and satisfaction with treatments was low, despite the controlled tumor growth.

The tongue comprises muscle tissues and can change its shape or location freely by muscle movements. Furthermore, the movements of the tongue are delicate and enable precise control. The intrinsic muscles comprise bands of vertical, horizontal, and sagittal muscle fibers, which allow shape changes by cooperative movements. The extrinsic muscles play important roles in supporting and changing tongue position, such as thrusting forward and pulling back. The mammalian tongue also serves as a sensory organ that includes taste buds and anterior lingual salivary glands.

When complete recovery of tongue function is required following excision of a tumor, reestablishment of the complex movements of the tongue is necessary. However, currently available methods for restoring those movements are inadequate, and there are no known techniques available for reconstruction of the ordinal structure of the tongue or augmentation of the function of other parts of the tongue. If the regeneration of the dominant nerves, the intrinsic muscles, and the extrinsic muscles is be able

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to complete with tongue allograft, the whole tongue function can be recovered.

In the present study, we investigated the effects of tongue allograft transplantations (containing lingual and hypoglossal nerves, and intrinsic and extrinsic muscles) on the complete recovery of tongue function after hemisection following excision in beagle dogs.

MATERIALS AND METHODS

Animals

Allotransplantation procedures were performed using 8 pairs of sex-blind beagle dogs (n = 16; TOYO beagle; Oriental Yeast Co., Tokyo, Japan). The donors ranged from 9 to 14 months of age and weighed between 9 and 12 kg. The recipients were age- and weight-matched, unrelated beagles except for 2 pairs of animals. Furthermore, 2 pairs of these beagles did not have the dog erythrocyte antigen (DEA) type 1.1 in the trial, which is related to a specific immune response.[8–10] Except for these 2 pairs of

beagles, we did not check the DEA type because we have successfully completed mandibular allografts and submandibular gland allografts in dogs without determining the DEA type.[11–14] The animals were housed in separate cages and given access to solid food (Oriental Yeast Co., Tokyo, Japan) and water ad libitum. All experimental protocols were reviewed and approved by the Intramural Animal Care and Use Committee of Osaka University Graduate School of Dentistry before the experiments (approval number: 26-005-0).

Surgical Procedures and Immunosuppressive Regimen

All dogs were fasted, beginning 1 day before transplantation. Animals were anesthetized with intramuscular injections of medetomidine (0.02 mg/kg) and midazolam (0.3 mg/kg) and then received an intraperitoneal injection of sodium pentobarbital (25 mg/kg) 15 minutes after intramuscular injections. We performed tracheotomies on all animals that were fixed in the supine position, except for the first pair of dogs that had a ventilation tube passed through their mouths.
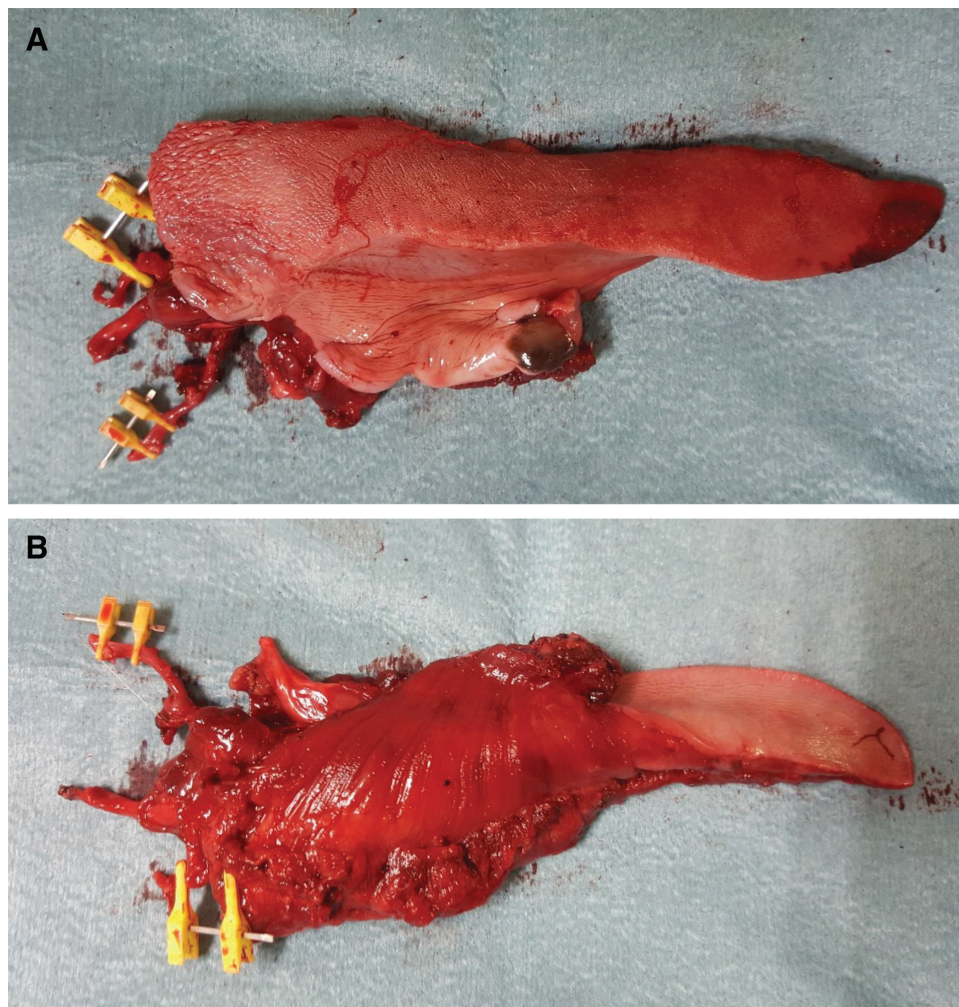

Fig. 1. Harvested hemi-tongue flap. In each donor dog, the right side of the tongue and the lingual and hypoglossal nerves, extrinsic muscles of the tongue, mucous membrane of the oral floor, lingual artery, and vein were harvested. A, Lateral side. B, Opposite side.

In each donor dog, the right side of the tongue, including the lingual and hypoglossal nerves, extrinsic muscles, and mucous membrane of the oral floor, was exposed (Fig. 1). The lingual artery and vein formed the vascular trunk used for modular transplantation. In each recipient dog, the same side of the tongue was removed at the level of the circumvallate papilla, and the recipient vessels (lingual artery and vein), lingual and hypoglossal nerves, and extrinsic muscles of the tongue (genioglossus muscle, hyoglossus muscle, and styloglossus muscle) were exposed. A vascularized transplantation method was performed with manual anastomosis of the blood vessels and nerves (the donor's lingual artery and vein were anastomosed to the recipient's lingual artery and vein, and the donor's lingual and hypoglossal nerves were connected to the lingual and hypoglossal nerves). Each of the intrinsic and extrinsic muscles and oral floor mucosa were sutured together (Fig. 2).

To facilitate wound healing, the dogs' tongues had to remain at rest for 1 month. Therefore, all right upper and lower teeth were extracted, and the left nongrafted side of the tongue was fixed to the mandible with a nylon thread and a button by passing a puncture needle completely through the dorsal surface to the submandibular skin.

A gastrostoma was made using an endoscope (i-Vets 8.0; SCETI K., Tokyo, Japan), and an Elizabethan collar was placed on the neck to prevent contact with the gastrostoma (Fig. 3). Animals received 2 daily injections of Tube Diet Dog (5 g/kg/time) (KIDNA; Morinyu Sunworld, Tokyo, Japan) mixed with 300 ml of water through the gastrostoma for nutrients.

Animals were administered daily injections of tacrolimus (Astellas Pharmaceutical Co., Osaka, Japan) (0.16 mg/kg/d) for immunosuppression, which commenced 1 day before the operation and was maintained for as long as the animal survived. We used an immunosuppression protocol that was previously established by Eguchi.[11] As a trial, 1 pair of beagles received a double dose of tacrolimus because there was also a possibility that strong rejection symptom was appeared. The recipient dogs received approximately 800–1,000 ml/d of fluid containing an antibiotic agent (100 mg/kg/d ampicillin sodium; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) through a peripheral vein for 7 days after the transplantation procedures were finished.

Evaluation Methods

The transplanted tongues were observed in recipient dogs for as long as possible.

After 3 months, or when the animals died of various causes, histologic examination was performed using hematoxylin and eosin staining.

Grafted tongue flap

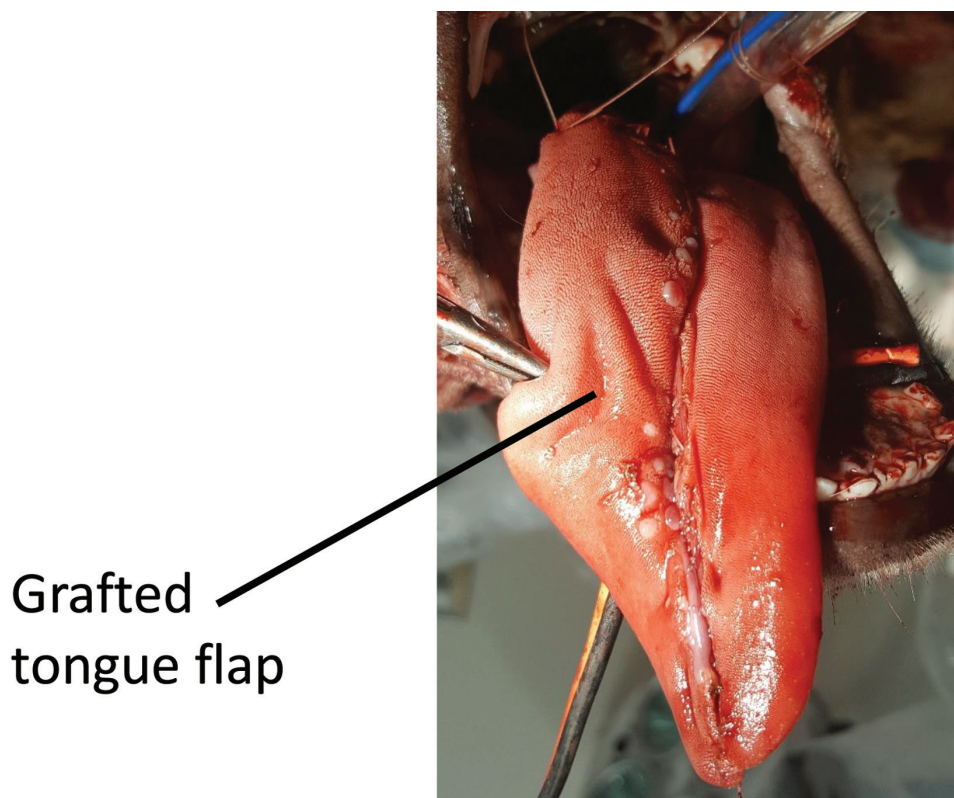**Fig. 2.** Grafted tongue flap at 1 day after transplantation. In each recipient dog, the same side of the tongue was removed at the level of the circumvallate papilla and then the recipient vessels (lingual artery and vein), lingual and hypoglossal nerves, and extrinsic muscles of the tongue were exposed. A vascularized transplantation method was performed using manual anastomosis of blood vessels and nerves.
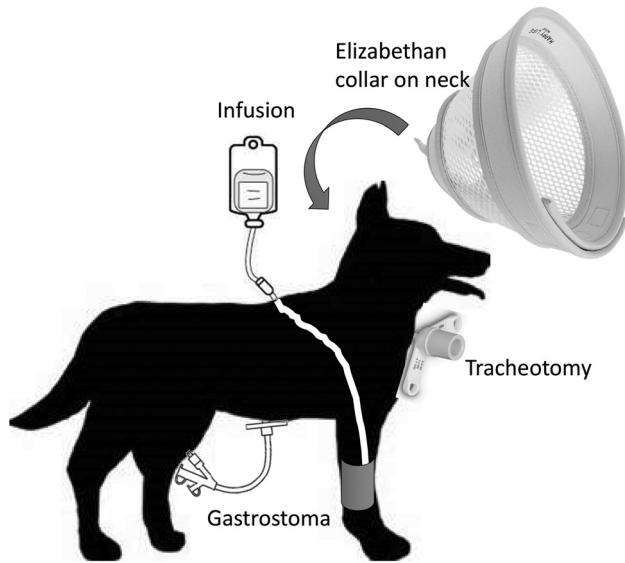
Fig. 3. Schematic drawing of management after allograft transplantation. To allow for wound healing for 1 month, a gastrostoma was made using an endoscope, and an Elizabethan collar was placed on the neck to prevent contact with the gastrostoma. Nutrition was mixed with 300 cc of water and injected through the gastrostoma twice a day. The dogs received approximately 800–1,000 ml of fluid each day, along with antibiotics through the peripheral veins, starting 7 days after transplantation.

RESULTS

Only 1 grafted tongue in the 8 recipient dogs survived, and that animal died 5 days after transplantation either from nutritional deficiency or dehydration (No. 4, Table 1). We made a gastrostoma and gave fluids intravenously. However, infusion through both could not keep for a long time, because the dogs could not keep their body still so long time. Thus, total amount of water seemed lacking absolutely. In the other recipients, the grafted tongue showed necrosis 1–3 days after the operation. Two dogs died of suspected aspiration, 1 died of an unknown cause, and 1 died of general debility. In addition, 3 of the grafted tongues became detached; therefore, we removed the tracheotomy tube and gastrostoma early and then allowed the dogs to eat orally.

When the animal with the surviving graft died, we removed the grafted tongue and the opposite side of the tongue. This tissue was histologically examined (Fig. 4). The grafted portion was partially connected to the recipient side of the tongue at 5 days after transplantation. We could not distinguish a boundary line between the grafted and nongrafted sites but observed infiltrating plasma cells and lymphocytes in the grafted site, which indicated slight inflammation.

DISCUSSION

In addition to organ transplantation in humans, facial transplantations are commonly reported, and several facial allograft transplants have been performed worldwide.[15–20] However, no successful cases of tongue transplantation in humans have been reported. Tongue dysfunction is associated with a number of complications, and partial loss of tongue control can lead to near-fatal aspiration. Compared with flap transplantation procedures, tongue allografts are regarded as the best method to restore normal tongue usage in patients.

When performing animal experiments, postoperative management following transplantation is very difficult, especially in regard to the control of pneumonia, dehydration, anomalotrophy, grafted tissue trauma, venous thrombosis, and rejection. For example, Kulahci et al[20–22] conducted a study that involved composite hemifacial/mandible/tongue allograft procedures in rats; they noted hemifacial transplantation in femoral areas, but not in the original facial area. In addition, Day et al[23] reported total excision and replantation of tongues in dogs. However, those animals were only monitored for 16 hours postoperatively under anesthesia and were then euthanized.

Haughey et al[24] exchanged hemi-tongues in 10 pairs of dogs and reported 5 successful allotransplantations. They also examined recovery of motor function using electromyography.[24] Notably, the extrinsic muscles of the tongue were not included in the transplantation so that noninvasive exchanges could be made between dogs in each pair. They used a simple postoperative management protocol. Moderate sedation was used for 2–3 days postoperatively; cervical esophagostomy was also performed because it is suitable for experiments with dogs and eliminates the need for tube gastrostomy. However, our facility does not have access to equipment that can manage dogs while they are under sedation. Thus, we performed tracheotomies, inserted gastrostoma tubes, and fixated the tongues in the current study.

Table 1. Outcomes of Tongue Allograft Transplantation in 8 Recipient Dogs

| No.	Date of Transplantation	Distinctive Features	Tongue Flap Survival	No. Survival Days after the Transplantation	Suspected Cause of Death
1	August 16, 2015	No trachea	Unknown	1	Vomit and aspiration
2	November 17, 2015	—	Nonsurvival	3	Vomit and aspiration
3	March 27, 2016	—	Nonsurvival	30	Unknown
4	April 24, 2016	—	Survival	5	Nutritional deficiency or dehydration
5	June 12, 2016	—	Nonsurvival	—	—
6	July 31, 2016	—	Nonsurvival	—	—
7	September 4, 2016	Negative DEA	Nonsurvival	—	—
8	October 8, 2016	Negative DEA; double dose of tacrolimus	Nonsurvival	4	Debilitation due to operation

Grafted site   Normal site

500µm
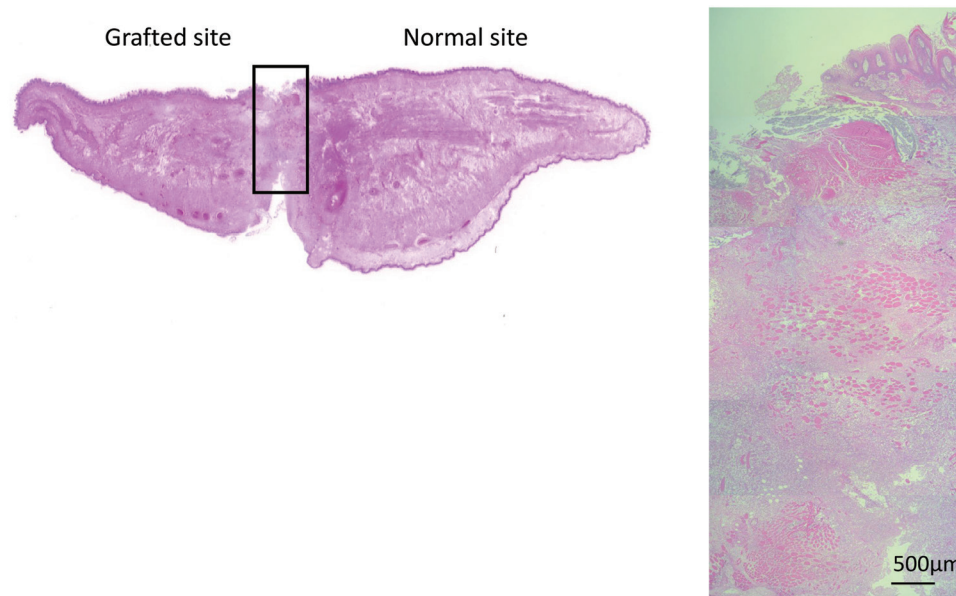
Fig. 4. Histologic results of normal and grafted tongues 5 days after transplantation. The grafted tongue was partially connected to the side of the recipient tongue. We could not identify a boundary line between the grafted and nongrafted sites; however, infiltrating plasma cells and lymphocytes, which indicate inflammation, were slightly observed.

Unfortunately, the tongue allograft procedure that was used in the present study was invasive for dogs, and it was more difficult to manage the animals after transplantation than we had anticipated. A tongue has a protuberant shape, which makes it difficult to keep the tongue at rest compared with other organs surrounded by the soft tissue. We expected the recipient dogs to eat the grafted tongues; therefore, we fixed unaffected tongues to the oral floor. However, tongue fixation can lead to aspiration pneumonitis, and a few of the dogs in the present study may have died of this complication. Some of the dogs in the present study also removed their own gastrostoma; thus, it became impossible to provide adequate nourishment and water to these animals. In addition, a veterinarian suggested that debilitation was a possible cause of death in this sample of dogs.

Haughey et al[24] used DEA type unmatched dog pairs. We used to achieve success allograft of the mandibular or the submandibular gland with unmatched dog pairs also. However, the tongue allografts in the present cases failed, so we suspected that it was potentially due to the rich blood flow in the tongue, relative to that of the salivary glands. We attempted allograft procedures with DEA 1.1-negative dogs as donors in 2 pairs of beagles, but these grafts did not survive. Thus, DEA type may not related to rejection. Furthermore, a double dose of tacrolimus did not aid in survival following allograft transplantation.

Therefore, we believe that the failed transplantations were due to a poor postoperative regimen, not hyperacute rejection. As noted above, the management of dogs following transplantation was difficult. Although face transplantations were also performed recently not only clinical but also preclinical study, they were performed with fresh cadavers, not live animals.[25] This use of cadavers might

have been due to the difficulty of the management of animals after transplantation. Therefore, sedative techniques should be employed to aid in the recovery of anastomosis following transplantation. In human medicine, patient cooperation may make the procedure more accessible. Sedation can also be provided, which facilitates wound maintenance. Therefore, we presume that tongue transplantations may be possible in humans.

Recently, the development of cancer has been reported in face transplant patients.[26] A recent report noted that 36 patients underwent face transplantation before 2016; of these, 3 developed cancer, of which 2 died because of cancer. However, this incidence is low in the context of immunosuppression. Other case reports described cancer in organ allografts; this was treated successfully in both cases.[27,28] Furthermore, bone allograft reconstruction after malignant bone tumor resection (eg, scapular, femur, or tibia) was performed in a few cases and showed good functional results.[29–31] These reports suggest the potential for successful transplantation of tongue allografts after tumor resection.

Fang et al[32] reported that skin allografts promoted the generation of antitumor effector memory T cells in C57BL/6 mice with B16 melanoma, which emphasizes the strong promise of effector memory T cell stimulation using allograft transplants in effective tumor immunotherapy. Land et al[33] reported that allograft rejection and cancer immunotherapy shared a common mechanistic basis of regulated necrosis and inflammation. Performance of allograft transplantation in patients with cancer may have risk of recurrence of cancer due to immunosuppression. Thus, we suggest that transplantation of a tongue is better to use for reconstruction secondary rather than immediately; wait for 5 years after

an operation of cancer, and do allograft after checking that there is no recurrence.

Considering the limitation of the flap reconstruction after the tongue resection, tongue allografts may be an expectant method for recovery of tongue function in long term. Even if tongue allograft including nerves and extrinsic muscles is performed, it seems to take a long time before the tongue recovers its functions. Furthermore, expansive tongue allograft was too invasive treatment for animals. If we want to adapt to human, first trial in human will be done without animal experiments as like as face transplantations.

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