Microvascular Transplantation and Replantation of the Rabbit Submandibular Gland

Jeffrey H. Spiegel, MD; Daniel G. Deschler, MD; Mack L. Cheney, MD

Background: Xerostomia is a devastating complication of radiation therapy. Previous research has demonstrated that submandibular glands may be removed from the neck and transplanted using microvascular techniques, with good gland survival. However, microvascular transplantation and replantation has never been attempted on a composite tissue such as a salivary gland.

Objective: To evaluate the ability of a rabbit submandibular gland to undergo 2 successive microvascular transplantations.

Subjects and Design: Study rabbits underwent a midline neck incision with dissection of a submandibular gland to its arterial and venous pedicle. Microvascular techniques were then used to transplant the gland to the femoral system of the right groin. The incisions were re-opened later under surgical conditions. The transferred gland was examined for survival and patency of its artery and vein. Healthy glands were dissected and transferred to a suitable artery and vein within the neck, where they were again reanastamosed using microvascular surgical techniques. After additional time, the gland was again examined for survival and pedicle patency, then removed and evaluated for histopathological evidence of survival.

Results: Surgical technique evolved during the course of this work to avoid encountered pitfalls. After refining the technique, we have determined that the rabbit submandibular gland is able to withstand successive microvascular transplantation and replantation with good likelihood of long-term survival, according to histopathological criteria.

Conclusions: The rabbit submandibular gland is able to undergo microvascular transplantation and replantation with evidence of long-term survivability and preserved function. The body’s natural response to surgery and tissue transplantation makes replantation a technical challenge; however, methods delineated herein alleviate many of the potential pitfalls. Extending these results to humans, patients who are to undergo radiation therapy could have a disease-free gland removed from the neck, transferred outside of the field of radiation, and then returned to the neck at the completion of radiation therapy. This may enable them to maintain salivary gland function and maintain oral cavity function and comfort.


XEROSTOMIA IS a devastating complication of radiation therapy to the upper aerodigestive tract. Patients receiving as little as 1000 to 2000 rad (10-20 Gy) suffer substantial discomfort and functional loss, whereas those receiving 3500 rad (35 Gy) must endure a permanent loss of saliva, as less than 7% of normal salivary flow will remain.1-3 Typically, these patients can be expected to experience dry mouth, thick mucus, dysphagia, odynophagia, difficulty with speech, and a painful burning sensation.3,5 As the protective barrier of saliva is lost, dental caries result, as well as candidiasis, esophagitis, and increased gastroesophageal reflux.6-8 Treatment of xerostomia represents a significant challenge, and many treatment modalities have been tried. These include the use of artificial saliva substitutes, saliva storage, electrostimulation of salivary flow, and acupuncture.6-10 Medical treatments have focused on stimulation of remnant glandular tissue. Pilocarpine hydrochloride is the most widely available medical treatment for xerostomia, but unfortunately only seems to increase saliva for a limited time after administration, and most patients need to continue pilocarpine therapy simultaneously with other symptomatic treatments.1,11

As stimulation of residual glandular tissue has provided only moderate improvement, prevention of xerostomia has become a focus for some investigators. When possible, salivary glands are ex-
MATERIALS AND METHODS

Six female white rabbits (*Oryctolagus cuniculus*; weight approximately 4 kg each) were used in this study. All work was approved by our institutional research board and animal care committee.

Each rabbit was anesthetized by pretreatment with acepromazine maleate, 1 mg/kg, followed by ketamine hydrochloride, 30 mg/kg, and xylazine hydrochloride, 7 mg/kg, given intramuscularly. Animals were then shaved in the neck and groin bilaterally and prepared for surgery using povidone-iodine (Betadine) solution. Each animal received 300,000 U of intramuscular penicillin G benzathine before incision. A paramedian incision was made along the right groin and the inferior epigastric vessels were identified. These were used to locate the femoral artery and vein, which were then dissected free of surrounding tissues using magnification from an operating microscope. Injury to the femoral nerve was avoided. The distal artery and vein were ligated, and the proximal ends were then clamped with size 2V microvascular clamps and divided. A midline incision was then made along the ventral surface of the neck, and self-retaining retractors were placed. A microscope was used for subsequent dissection. The right salivary gland was dissected to identify its duct, artery, and vein. The vessels were followed to their origins at the external carotid artery and internal jugular vein. A battery-powered, handheld cautery was used to assist with hemostasis. These larger vessels were selected, as the rabbit facial artery and vein have diameters of less than 1 mm. At this point, the distal external carotid artery and internal jugular vein and then the proximal aspects of these vessels were ligated and divided. The duct was divided without ligation. This provided a free gland that could be removed from the neck. The neck was irrigated with isotonic sodium chloride solution and closed using running polypropylene (Prolene; Ethicon, Inc, Somerville, NJ) suture. With the use of microvascular techniques, 10-0 nylon suture was used to reanastomose the femoral artery to the external carotid artery and the femoral vein to the internal jugular vein. Care was taken to avoid twisting the pedicle. When anastomoses were complete, the vascular clamps were released and the anastomoses were inspected. Good flow was ensured and small leaks were repaired using 10-0 nylon suture. When a successful transfer was obtained, the gland was sutured to the muscles of the leg to maintain good vessel position, and the wound was irrigated, then closed using running polypropylene suture. Animals were then returned to the animal care facility for recovery.

After a period ranging from 3 to 14 days, the animals were anesthetized in the same manner and the transplant site was reopened. The transferred gland was evaluated grossly as to overall viability, function (presence of a sialocele), and patency of the pedicle vessels. The midline neck incision was then reopened, and the gland on the contralateral side was dissected to the external carotid artery and internal jugular vein. The distal ends of these vessels were ligated and divided after placing 2V vascular clamps on the proximal ends. With the recipient bed thus prepared, the femoral artery and vein at the groin transfer site were ligated proximally and then cut for transfer of the gland back to the neck.

Once the gland was transferred to the neck, a microvascular anastomosis was performed using 10-0 nylon suture of the femoral artery to the external carotid artery, and of the femoral vein to the internal jugular vein. Clamps were then removed and blood flow through the gland was assessed. When blood flow was deemed adequate, the wound was irrigated and the gland was sutured to the deep neck musculature to ensure proper vessel orientation. The neck and groin incisions were then closed using 3-0 polypropylene suture.

To preserve salivary gland function, Jha et al have reported transferring a submandibular gland into the anterior submental space to move the gland outside the radiation field. Sixteen patients with carcinoma of the larynx, oropharynx, or hypopharynx without previous irradiation underwent selective neck dissection of 1 submandibular gland, which was left pedicled on the distal aspects of the facial artery and vein. The gland, remaining attached to the submandibular ganglion, was then rotated anteriorly beneath the anterior belly of the digastric muscle, where it received nutrients via retrograde flow. Clips were placed to mark the gland during radiation treatment planning. These researchers found that xerostomia did not develop in their patients and that they had a more mild overall radiotherapy course, with decreased weight loss and mucositis that was less than usual.

Other approaches to the treatment of xerostomia have focused on the restoration of salivary gland function to the oral cavity. Krause et al injected a suspension of immortalized parotid acinar cells beneath the oral mucosa of rats and noted cell survival after 30 days. Un-
Rabbit Surgical Course and Outcome

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Transplantation to Groin</th>
<th>Examination of Groin Transplant</th>
<th>Retransplantation</th>
<th>Examination of Retransplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
<td>Aborted due to injured pedicle</td>
<td>…</td>
<td>Aborted due to injured pedicle</td>
<td>…</td>
</tr>
<tr>
<td>2‡</td>
<td>Successful</td>
<td>10 d, viable, sialocele</td>
<td>Successful, to contralateral groin</td>
<td>4 d, viable, sialocele</td>
</tr>
<tr>
<td>3</td>
<td>Successful</td>
<td>14 d, viable, sialocele</td>
<td>Aborted due to injured pedicle</td>
<td>…</td>
</tr>
<tr>
<td>4‡</td>
<td>Successful</td>
<td>14 d, viable, sialocele</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>5§</td>
<td>Successful</td>
<td>3 d, nonviable</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>6</td>
<td>Successful</td>
<td>3 d, viable, sialocele</td>
<td>Successful, to neck</td>
<td>4 d, viable, sialocele</td>
</tr>
</tbody>
</table>

*§Surgical modifications resulting from experience include avoiding dissection of small gland vessels and focusing dissection at larger external carotid and internal jugular system. Ellipses indicate no data available or applicable.
†Surgical modifications resulting from experience include anastomosing gland to distal aspect of femoral vessels and minimizing dissection at proximal femoral system. Ellipses indicate no data available or applicable.
‡Surgical modifications resulting from experience include placing small segment of latex rubber beneath gland and between vessels and thigh muscles to minimize scarring. Ellipses indicate no data available or applicable.
§Surgical modifications resulting from experience include increasing the number of sutures between gland and thigh muscles to maintain gland position and vessel geometry. Ellipses indicate no data available or applicable.

Unfortunately, duct formation did not occur and these cells proved to be of little functional benefit. Similar work performed by Greer et al.16 found that hamster salivary gland tissue placed as a free autogenous graft into the cheek pouch can demonstrate histological evidence of viability. Again, these authors note that subsequent practical function of such glands is unclear.

Similarly, sublingual glands have been transferred as a free graft (no microvascular reanastomosis) to the fornix of the eye of rabbits as treatment for xerophthalmia.17 These glands atrophied, and only minimal acinar fornix of the eye of rabbits as treatment for xerophthalmia. They reported 88% gland viability of transplanted gland remaining at least 50% functional after transplantation. MacLeod and Robbins27 followed this with a similar procedure in 8 human patients, 7 of whom reported significant improvement in their xerophthalmia, several with complete cessation of epiphora and 2 patients had success receiving enhanced corneal humidity.

This success prompted Kumar et al.18 to perform a rabbit study to evaluate submandibular gland transplantation for the treatment of xerophthalmia. Their treatment group experienced decreased corneal ulceration, and 75% of transplanted gland remained at least 50% functional after transplantation. MacLeod and Robbins20 followed this with a similar procedure in 8 human patients, 7 of whom reported significant improvement in their xerophthalmia, several with complete cessation of other treatments. Viability of transplanted salivary glands has also been demonstrated in a rat model.20

Lauer et al.21 and Geerling et al.22 have each described the same 20 to 22 patients who underwent microvascular submandibular gland transplantation for the treatment of xerophthalmia. They reported 88% gland survival at 3 months and 75% survival after 1 year. Of those patients with viable transplants, 75% were able to stop using eye drops, and epiphora even developed in some, requiring surgical reduction of gland size.

Follow-up reports by Sieg and Geerling23 and Geerling and Sieg24 contain details of 30 transplants with 25 viable glands. Epiphora developed in 10 patients, which resulted in micropycotic epithelial edema. This was thought to be due to the relative hypotonicity of saliva vs normal tears; nonetheless, the authors conclude that salivary gland transfer is suitable treatment for severe keratoconjunctivitis sicca.

As inferred by the work of Jha et al.,14 a single preserved submandibular gland provides adequate protection against xerostomia. This is consistent with studies of gland physiology, which show that the paired submandibular glands produce more than 70% of total saliva volume.25-27 The submandibular gland receives its blood supply through branches of the facial artery and vein and parasympathetic innervation via the submandibular ganglion off the lingual nerve. Sympathetic innervation is along a plexus following the facial artery. The gland drains into the oral cavity through a duct, and salivary flow is maintained with advancing age (when flow volume is controlled for medication use).30,31

On the basis of work initiated in a rat model, Spiegel et al.20 hypothesized that xerostomia could be prevented by protecting a submandibular gland outside of head and neck radiation fields during the course of radiotherapy, and then by replacing that gland at the conclusion of treatment. Microvascular transplantation of the gland to the groin, followed by reimplantation to the neck, would ensure survival of the gland during the period of cancer treatment.

Although microneurovascular tissue transplantation is used for reconstruction of many surgical, traumatic, and congenital defects, microsurgical techniques to permit the heterotopic protective storage of tissue have not been explored. This study was designed to evaluate the viability of the rabbit submandibular gland after 2 successive microvascular transplantations. Technical challenges have encouraged the evolution of a more precise surgical method that permits successful transfer and reimplantation.

RESULTS

Six rabbits were used in this experiment, and they underwent operation successively, rather than simultaneously. This serial approach proved very important, as the surgical technique required frequent modification to achieve eventual success. The Table describes the surgical course and outcome of each rabbit.

Rabbit 1 underwent an aborted first transplantation, as the small arterial branches of the submandibu-
lar artery (diameter, <0.5 mm between the facial artery and gland) were damaged during dissection. This occurred as the arterial and venous branches were separated to follow them back to the external carotid artery and internal jugular vein. Surgical procedure was thus modified at this point to eliminate dissection of the feeder vessels and to begin dissection with the external carotid artery and internal jugular vein, and then to keep the smaller branches off these as an undissected bundle. Only the duct was dissected as a separate structure.

Rabbit 2 underwent a successful transplantation of the right gland to the right groin. After 10 days, the groin was reopened and a large sialocele was identified originating from the free end of the submandibular gland duct. The gland appeared grossly viable. However, during dissection of the pedicle for transplantation back to the neck, the submandibular artery was injured. This occurred during dissection through inflamed and scarred tissue to identify the site of initial anastomosis. As a result of this development, surgical technique was modified to anastomose the gland to a very distal point along the femoral vessels. This was done in anticipation of being able to work with the previously undissected proximal portions of the femoral vessels for the replantation, and thus to obviate the need to dissect around the submandibular branches.

Rabbit 3 underwent successful transplantation of the right gland to the right groin, as evidenced by a grossly viable gland and a moderate sialocele on inspection at 14 days. The left salivary gland was damaged at the time of removal of the right gland, and was thus removed at the time of the initial procedure. On 14-day inspection, again, significant scarring was present around the femoral vessels, but the proximal vessels were isolated. Dissection of the gland from the muscle bed proved very difficult in the inflamed scar tissue, but the gland was nonetheless removed. Reanastomosis to the left groin femoral vessels was performed, as we believed that damage to the remaining left submandibular gland would make finding vessels in the neck difficult. After 4 days, the gland was reevaluated, appeared viable, and was removed for pathological evaluation.

Rabbit 4 underwent successful transplantation of the right gland to the right groin, and after 14 days, it had a viable gland with moderate sialocele and significant scarring. Despite planning to use the proximal femoral vessels, the scarring was too significant and the femoral vessels were damaged during gland harvest.

At this point, it was determined that scarring and inflammation needed to be curtailed to allow for a successful return transfer. These problems were magnified by the need to dissect through thigh muscle to isolate the femoral vessels, and by the lack of duct drainage outside the wound. Previous rat experimentation demonstrated that a successful transplant was feasible without duct repair; however, in the rabbit model, this proved to cause too much inflammation to permit successful repeated dissection.20 Thus, the surgical technique was modified to place a segment of sterile latex glove beneath the gland and between the femoral vessels and the thigh muscle bed. This prevented adherence of the gland and its vasculature to the underlying tissues. In addition, the time between transplant and return was decreased. Three days has proved to be adequate time to determine the success of a microvascular transplant.20

Rabbit 5 underwent successful transplantation of the right gland to the right groin. After 3 days, the wound was inspected and the distal end of the wound had been opened. Apparently, the rabbit chewed out the distal-most sutures and opened approximately one third of the wound. In addition, the rabbit had chewed through the distal sutures that held the gland to the thigh musculature. On inspection, the gland appeared to be nonviable. No sialocele was present. The glove segment was present and permitted dissection and inspection of the anastomoses. These were intact with good flow. However, with inadequate suturing to the thigh bed, the gland had twisted and kinked the submandibular vessels off the external carotid artery. Surgical technique was again modified to suture the gland more securely to the surrounding tissues (over the glove segment), to prevent twisting and vascular compromise.

Rabbit 6 underwent successful transplantation of the right gland to the right groin. After 3 days, this rabbit underwent evaluation, and again the distal segment of the wound (approximately one third) was devoid of sutures and open. Nonetheless, the gland was grossly viable and a moderate sialocele was present. The latex glove segment that had been placed beneath the gland was removed and a clear plane was visible. This greatly facilitated dissection of the gland and more proximal femoral vessels. The neck was then reopened, and the left salivary gland was dissected to its external carotid artery and internal jugular vein pedicle. The left gland was removed, and then the former right gland was anastomosed to the left-side vasculature on transplantation from the right groin. After 4 days, the neck was reopened and a large sialocele was visible. The gland appeared grossly viable and the vessels demonstrated good flow. This gland was then removed and sent for pathological evaluation.

Thus, 5 rabbits had at least 1 transferred gland. Of these, all were initially successful, but only 4 were viable on reinspection (1 gland twisted and had kinked vessels). Presence of a sialocele is interpreted as gross evidence of preserved gland function. Three rabbits had significant scarring at the groin donor site, and only 1 of these underwent a successful replantation. Two rabbits had placement of a glove segment to decrease scarring between the gland and recipient bed. Of these, both had easily dissected glands and good flow through the anastomoses. However, 1 segment twisted, so only 1 of the 2 underwent successful replantation to the neck.

Histopathological evaluation of hemotoxylineosin stains was performed on all removed glands. Rabbit 1 showed normal architecture consistent with gland excision. Rabbits 2 and 4 demonstrated mild inflammation and minimal ischemic injury, as manifested by some acinar loss and vacuolization. Rabbit 5 showed marked ischemic injury with inflammatory infiltrate; acini seen were atrophic and necrotic. Evaluation demonstrated that the 2 glands with 2 successful transplantations (rabbits 3 and 6) showed good preservation of gland architecture with some inflammation (Figure). Acinar structure was maintained with minimal damage. Ducts remained patent, and no evidence of endothelial injury was
seen within the microvasculature. This is thought to be consistent with, at most, mild ischemic damage and predictive of good long-term survival of the transplanted organ.

COMMENT

Transplantation of the submandibular gland by using microvascular techniques is feasible and can be shown in rat and rabbit models to maintain a cellular structure predictive of long-term gland survival. The surgical techniques are straightforward and consistent with those used throughout microsurgical procedures. However, to our knowledge, replantation of a transplanted organ has not been reported and requires several variations in surgical technique.

Several problems encountered are particular to the animal model. The very small vessel size for the submandibular vasculature in the rabbit necessitates dissection of the larger vessels from which they originate. However, the small vessels remain susceptible to injury during initial dissection and all subsequent manipulation. In humans, the submandibular gland vasculature (facial artery and vein) is larger and more easily handled. Nonetheless, minimizing dissection of gland vessels close to the parenchyma of the organ would seem prudent.

In addition, animals are often able to chew through sutures and open surgical incisions. The presence of thick fur makes placement of an occlusive dressing over the wound difficult. In future studies, the use of surgical skin staples may better protect closure of the groin incision. An increased number of sutures between the gland and the groin wound bed can better protect the gland from changing position should the skin incision open.

The most significant difficulty encountered was the robust scar tissue formation at the groin transplant site. This was eventually circumvented by placement of a segment of latex glove beneath the gland. Several factors contribute to this scar tissue formation, but perhaps the most significant reason is no drainage port for saliva and dissection through muscle.

The small size of the rabbit submandibular gland duct prevented us from restoring drainage by suturing the duct to the external groin skin. In addition, previous experience with rat submandibular gland transplant demonstrated that the gland could withstand the presence of a local sialocele. Although this may be the case when evaluating a single transfer, replantation requires a more pristine recipient bed. Second, a vigorous scar reaction developed along the raw muscle edges formed during preparation of the femoral vessels for involvement in the anastomoses. Vessels surrounded by fat or fascia (eg, the rabbit external carotid system) were less likely to exhibit dense scar formation.

In humans, the inferior epigastric vessels could be selected for an initial transplantation site for a submandibular gland to be banked during head and neck radiation therapy. These vessels are typically surrounded by fat and thus should be more easily dissected on subsequent evaluation. In addition, the human submandibular gland duct should be spatulated and sutured to the groin skin to permit drainage of saliva outside the wound. This would also provide a monitor of gland function. On subsequent replantation to the oral cavity, a small segment of abdominal wall skin could be taken around the duct drainage site to preserve the length of the duct and to make reanastomosis of the duct drainage port to the oral cavity easier. Still, placement of a sterile segment of Silastic sheeting around the gland and vessels may greatly facilitate dissection for replantation to the neck.

The paired submandibular glands produce 70% of the total saliva. Previous work has demonstrated that a transferred and deinnervated gland produces adequate salivary flow to protect the cornea. Furthermore, if additional saliva was necessary, light pressure on the transferred gland resulted in expression of the necessary moisture. It is certainly technically possible to tag the submandibular ganglion at the time of gland resection and to plan on reanastomosis during replantation, but the success and necessity of this maneuver is unknown.

Xerostomia is a very distressing problem for affected patients and is commonly the most frequent complaint of patients who have undergone head and neck radiation therapy. As described, current therapeutic options provide limited relief. The proposed procedure of microvascular transplantation and replantation of a salivary gland to protect it from the radiation field is technically challenging. However, identifying the vascular pedicle of the human submandibular gland is a common procedure for otorhinolaryngologists (a standard part of submandibular gland excision), and the vessels are of relatively large diameter.

Microvascular tissue transfer is often considered to be a very time-consuming and thus expensive undertaking. Much of the time involved in microvascular free-tissue transfer is spent properly inseting the flap or contouring the tissue to provide the best possible anatomic and functional reconstruction. In addition, closing the donor site can be slow and often requires obtaining a split-thickness skin graft. In comparison, the proposed procedure can be predicted to be relatively quick. Resection of the submandibular gland is not particularly time-consuming, and the recipient vessels in the groin are readily identified. In addition, no complicated inset
CONCLUSIONS

Histological findings consistent with a prediction for long-term survival in multiple transplanted glands are encouraging. To our knowledge, no previous work on multiple transplantations of composite organs via microvascular techniques have been published. This rabbit pilot study has provided many insights into problems that may develop during multiple transplantations of a single organ. Many of these problems are unique to an animal model.

Xerostomia remains a difficult sequela of head and neck radiation therapy. Removal of a gland to a site distant from the head and neck will effectively protect the organ from radiation damage. Return of the gland at the completion of treatment can be predicted to provide adequate saliva to eliminate the devastating effects of xerostomia.

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Corresponding author: Jeffrey H. Spiegel, MD, Department of Otolaryngology-Head and Neck Surgery, Boston University School of Medicine, D-616, 88 E Newton St, Boston, MA 02118 (e-mail: jeffrey.spiegel@bmc.org).

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