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Composite Mandibulectomy: A Novel Animal Model

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Abstract

Objectives—Segmental mandibular defects can result after the treatment of various pathologic processes, including osteoradionecrosis, tumor resection, or fracture nonunion with sequestration. The variety of etiologies and the frequency of occurrence make the reconstruction of segmental mandibular defects a topic of significant interest. Despite these incentives, a well-established small-animal model of the segmental mandibulectomy, including composite resection, does not exist. The objective of this study is the creation of a reliable animal model that can be used to study the reconstruction of en bloc mandibular defects. Surgical techniques and an array of reconstructive options are described.

Study design—Description of an animal model.

Setting—Animal laboratory at a quaternary care university medical center.

Methods—We present an Animal Research Oversight Committee–approved prospective analysis of survival operations in the rat model. A detailed, stepwise description of surgical technique and relevant intraoperative anatomy is presented. Postoperative management, early pitfalls, surgical complications, and future applications are discussed.

Results—A total of 72 operations were performed by a single individual between July and October 2010. Two intraoperative and 9 postoperative complications were recognized. There were 6 orocutaneous fistulas, 2 abscesses, and 1 seroma. There were 4 fatalities, which were attributed to anesthetic complications (2, intraoperative), hematoma formation (1, postoperative), and foreign-body aspiration (1, postoperative).

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Conclusion—This novel animal model reliably replicates the en bloc segmental mandibular defects seen in our patient population and can be manipulated to achieve a wide variety of research objectives.

Keywords

head and neck; composite; mandible; biomaterials

Several pathologic processes of the head and neck including benign and malignant neoplasms require the through-and-through resection of a segment of mandible as a component of their management.^{1,2} Occasionally, the resection of a segment of abnormal tissue in conjunction with adjacent bone is required. This procedure, termed *composite mandibulectomy*, invariably includes a large intraoral defect and, thus, direct communication with the site of mandibular resection. As a consequence, any reconstructive effort must take into consideration the increased potential for salivary contamination and postoperative complications that may result.^{3–6}

The primary reconstructive modality for composite mandibular resections is the free vascularized bone flap; however, several limitations to this procedure exist. Consequently, there is now a significant research effort focused on alternative methods of osseous reconstruction. A small-animal model that accurately represents the composite mandibulectomy has not been previously described.

We present a reliable small-animal model that can be effectively used to study the reconstruction of composite mandibular defects. A detailed, stepwise description of surgical technique and relevant intraoperative anatomy is presented. Intraoperative considerations, perioperative complications, and postoperative care are discussed.

Methods

This study was performed after approval by the UCLA Office of Animal Research Oversight from July to October 2010. Under our protocol, 72 four-month-old Sprague Dawley rats (*Rattus norvegicus*) underwent the procedure described below. Animals were killed 1 week (4 animals), 4 weeks (6 animals), 8 weeks (34 animals), and 12 weeks (28 animals) postoperatively.

Surgical Procedure

The procedure requires the exposure of the mandibular body and ramus from a ventral approach. With the animal supine, a curvilinear incision is carried through the dermis, extending from the mandibular symphysis to the posterolateral mandibular angle. Subdermal fascia is then dissected bluntly, and soft-tissue flaps are elevated and suspended superiorly and inferiorly (Figure 1). Battery-operated electrocautery is used to cauterize small and superficial venous structures as necessary. The masseter is released from its ventral and posterior attachments using a caudal elevator. When the mandible is exposed (Figure 2), a sterile marking pen is then used to mark a 5-mm segment on the ventral surface of the mandible. In keeping with the operative procedure performed in the human patient, an internal fixation device is required to stabilize the mandible after mandibulectomy. There are 2 possible approaches to fixation of the mandible, described separately below. For the purpose of this experiment, all animals included in this data set received internal fixation using a polypropylene splint (option 1 below). A modified internal fixation method (option 2 below) is also presented for completeness. This second method is recognized as an equally acceptable means by which mandibular fixation may be achieved and has become the preferred method used in our laboratory.

Option 1: polypropylene splint internal fixation—A prefashioned sterile polypropylene splint is inserted lateral to the mandible and affixed using 0.8-mm threaded K-wire at 4 points (Figure 3). The splint is previously marked with a 5-mm segment, and during fixation, this segment is aligned with the marked segment of mandible. With the splint in place, a 5-mm defect is created in the mandibular body with a high-speed cutting burr (Stryker, Kalamazoo, Michigan). (During pilot trials, use of an oscillating or reciprocating saw to create the mandibular defect frequently resulted in excessive collateral trauma to the mandible and surrounding soft tissue. A cutting burr was therefore found to be vastly more effective in creating a precision mandibular defect.) The mandibular segment is then removed, and a mucosal defect is created. The mucosal defect is created by placing a sterile Q-tip intraorally and palpating the Q-tip at the mandibulectomy site. A 5- × 2-mm elliptical incision is then created over the Q-tip with a #11 blade, resulting in a defect area of 15 mm.

Option 2: titanium internal fixation—After marking the mandible, a 6-hole titanium plate (Upperface plate tray, Stryker Craniomaxillofacial, Portage, Michigan) is bent to fit the mandibular contour. The plate holes are then marked and drilled anterior and posterior to the proposed defect site. The segmental defect is created in the mandible with a high-speed cutting burr as described above. After creating bony cuts, the mandibular segment is removed with overlying retromolar mucosa. If the segment of mandible is removed without attached mucosa, the mucosal defect is created as described above. After closure of the mucosal defect, the titanium plate is affixed to the mandible using 1-mm titanium screws (Figure 4).

At this time, regardless of fixation technique, the rat has an open intraoral defect, an internally fixed mandible, and a segmental 5-mm mandibular defect. The mandibular defect is now available for use in the evaluation of various reconstructive or regenerative techniques. The intraoral defect is then closed using a 6-0 PDS suture in a horizontal mattress fashion. Care is taken to avoid constrictive closure and the possibility of subsequent tissue ischemia at the incision site. When a titanium plate is used, closure of the mucosal defect is ideally performed after the segmental mandibular resection but prior to plate placement. This allows for optimal visualization of the defect site and thus improved access for closure. The animal is allowed to emerge from anesthesia under direct observation.

All procedures are performed using 2.5× surgical loupes or an operating microscope.

Results

A total of 72 rats received a composite mandibulectomy between July and October 2010. There were 9 (12.5%) nonfatal postoperative complications. These include 6 (8.3%) fistulas, 1 (1.3%) seroma, and 2 (2.7%) abscesses. Four (5.6%) fatal complications were recorded. Of these, 2 (2.8%) deaths occurred intraoperatively. Two (2.8%) deaths occurred within 24 hours of the operation and were attributed to hematoma formation (1) and aspiration (1; Table 1).

Fistula

Four of the 6 animals that developed fistulas were recognized within 1 month of the operative date. The other 2 fistulas developed greater than 6 weeks postoperatively. Evidence of fistula development included small wound dehiscence with salivary extravasation and/or the development of a fibrinous cap with subsequent scaffold extrusion.

Hematoma and seroma

Two animals developed postoperative fluid collections. The first animal developed an immediate fluid collection within 6 hours of the procedure. The second animal was discovered deceased on postoperative day 1. Hematoma was evident on necropsy.

Abscess

Two animals developed an abscess at the mandibular defect site. Incision and drainage was performed; however, the animals subsequently suffered weight loss and failure to thrive, and they were euthanized.

Aspiration

A single animal was noted to have acute-onset stridor beginning 3 hours postoperatively in conjunction with a small serous fluid collection at the incision site. The serous fluid collection was drained; however, there was only mild improvement in the intensity of stridor. A single dose of intravenous decadron was given. On postoperative day 1, the animal was found deceased. Necropsy demonstrated a wood chip at the level of the subglottis with what appeared to be complete tracheal obstruction.

Discussion

Segmental defects of the human mandible may occur secondary to several pathologic processes requiring surgical intervention, including congenital malformations, benign and malignant neoplasms, and trauma. Mandibular reconstruction after surgical resection is not an absolute reconstructive necessity. The absence of reconstruction, however, can have significant life-altering consequences. Mandibular reconstruction serves to provide oral competence, improve occlusion, and restore facial contour.^{3,5-9} Any disruption of mandibular continuity is associated with both the loss of an occlusal surface and the frequent creation of a soft-tissue defect. These anatomic changes can cause significant pain in the temporomandibular joint, restrict the patient to a soft or liquid diet, and cause catastrophic communication dysfunction.^{1-3,6}

En bloc, composite mandibulectomies are often associated with a large soft-tissue defect. Today, there are several approaches that can provide bony reconstruction of the mandible itself, including cancellous marrow grafts and cranial bone grafts.^{5,7,9} When a significant soft-tissue defect is created, the standard of care frequently involves the use of vascularized bone graft for reconstruction.^{7,10,11} Unfortunately, vascularized osseous grafts are technically demanding, have associated donor-site morbidity, and may negatively influence the intraoperative risk profile for many patients.^{6,7}

The established shortcomings of the current standard of care have stimulated interest in new sources of tissue for mandibular reconstruction. As a result, significant research efforts have been focused on alternative methods of reconstruction, including the use of biomimetic scaffolds impregnated with growth factors and mesenchymal stem cell—impregnated grafts.¹²⁻¹⁴

Tissue engineering methods are frequently evaluated in vivo, using animal models of osseous defects. Common models used to test novel tissue-engineering techniques include the mouse calvarial defect and the rat spinal fusion model.^{12,13} More representative models, in which the mandible of the animal is resected, also exist. Unfortunately, these models are predominantly performed in larger animal models and do not have an associated soft-tissue intraoral defect. For example, the canine, primate, and rabbit model have been described and appear to be effective representatives of the mandibular resection itself.¹⁴⁻¹⁷ Unfortunately, a

significant feature of many segmental resections of the mandible is the presence of intraoral continuity at the defect site prior to mucosal closure. Despite meticulous mucosal closure or soft-tissue reconstruction, perioperative salivary contamination may potentially occur if an intraoral defect is created during the course of the procedure. Although watertight closure is frequently accomplished, the risk of fistula formation for patients requiring closure of a contiguous or composite defect exceeds the risk otherwise associated with a patient in which this defect is not created. The implicit significance of salivary exposure or bacterial contamination lies in the potential for suboptimal healing and infectious sequelae, which may understandably affect outcomes of proposed reconstructive techniques.¹⁸ The lack of an intraoral defect is an important feature that has been previously neglected in most animal models used to evaluate segmental mandibulectomy defect sites.

The model described here effectively uses a small animal to represent the defect seen in the human patient after composite mandibulectomy. Important similarities include the requirement for rigid fixation across the defect site with potential hardware-related sequelae and the potential for salivary exposure. In addition, the ability to perform this experiment in a small animal model has positive financial and ethical implications. Specifically, animal husbandry expenses are frequently reduced in comparison to the larger animal model; small animal models, when appropriate, are preferred.

The animal model described here was developed in concert with our evolving understanding of the pertinent anatomy, perioperative behavior, and potential complications or obstacles that this model may pose to the researcher. We have outlined these details below.

Rat anatomy and surgical implications

Of significant importance in the design and augmentation of a stable animal model is a sound knowledge of relevant anatomic structures. For simplicity, we have limited our discussion of anatomy to those anatomic considerations pertinent to the procedure being described herein.

Muscular anatomy

The masseter can be identified overlying the mandibular body and ramus, with the primary component of muscular bulk seated lateral to the mandible. In our experience, manipulation of the masseter and release from the mandible should be performed in a suprapariosteal plane. The masseter itself is a frequently adequate size for coverage of the mandibulectomy defect. In addition, the sternomastoid or cleidomastoid muscles may be used as a superiorly based flap with adequate length to cover the mandible if necessary.

Vascular anatomy

The primary vascular structures encountered during the course of this dissection include branches of the external carotid artery. When considering intraoperative dissection, the most frequently encountered vessel of importance is the supplementary mandibular artery, which traverses a foramen that is located more ventral and anterior than is the foramen mandibularis.^{19,20} This formidable branch of the facial artery lies at the posterior-most border of the segmental mandible defect and is at great risk of avulsion during dissection of the masseter and in performing osteotomies. Avulsion of this vessel can be controlled with direct pressure and electrocautery or suture ligation. We have not appreciated any gross differences in mandibular or muscular appearance on necropsy in those animals that have required arterial ligation.

Osseous anatomy

The rat mandible has a comparatively long anteroposterior axis with a tall retromolar dimension. Creation of a segmental mandibular defect requires transection of the mandible anterior to the condyle, coronoid process, and most of the ramus. When using a synthetic splint and K-wires, the bulk and nonconforming rigidity of the fixator is a limiting factor. Because the segmental defect must be created between the drill holes, the potential locations for osteotomies are limited to a short anatomic region. With a polypropylene splint in place, this drilling is performed perpendicular to the mandible, and visualization of the entire mandible is often inhibited. Although all animals included in the data set for this experiment received a polypropylene splint, the above-mentioned shortcomings associated with this fixation technique can be overcome with the use of a titanium miniplate. This allows for the drilling of the mandible to be performed prior to internal fixation, thereby providing an improved view of the lateral cortex. Finally, although the mandibulectomy defect invariably transects the posterior segment of the incisor, loss of tooth viability has not been appreciated in our animals.

Animal Behavior and Postoperative Care—There are several limitations to one's control over postoperative behavior in the laboratory rat. The functional deficit after the creation of a segmental mandibular defect is significant; however, the laboratory animal will continue to attempt normal behavior. Many of these behaviors appear to directly inhibit the healing process.

One of the most significant obstacles to overcome postoperatively is excessive use of the mandible. A soft, high-calorie diet was provided to avoid excessive strain across the site of internal fixation. This must be offered in the absence of standard hard-pellet feed, as the animal will choose familiar items over newly introduced food. In addition to mandible overuse, the laboratory rat is known to chew and ingest various objects, including feces, bedding, and plastic. These behaviors are increasingly common in times of stress. In the first 10 animals, standard woodchip bedding was provided. The bedding was subsequently changed to a paper composite to avoid aspiration in the setting of restricted mandibular mobility postoperatively. In an attempt to combat infectious complications, all animals are maintained on oral antibiotics for 5 days postoperatively.

Despite preventive measures, postoperative behavior of our laboratory animals is likely counterproductive to mandibular regeneration and mucosal healing. Watertight intraoral wound closure and sound mandibular fixation are therefore of paramount importance.

Complications—This animal model was associated with both intraoperative and postoperative complications. The most frequent postoperative complication involved the development of a postoperative fistula at the defect site, followed in frequency by abscess formation. The introduction of saliva and/or bacteria at the intraoral defect site is likely the most significant factor contributing to fistula and/or abscess development in our animals. Other potential contributing factors include the proportionally large mandibular defect and potential variability in the effectiveness of internal reduction between animals. In humans, an increased length of segmental resection has been attributed to an increased incidence of fistulas in some reports. This was demonstrated in human patients in a 1999 report by Arden et al,⁴ who demonstrated a 14% fistula rate (31 patients), with an increased incidence in patients who had a segmental defect exceeding 5 cm in length.⁴ In actuality, the development of a fistulous tract in the animal model is likely a multifactorial process. For the animals described here, the rate of fistula formation was 8.3% at the time of writing. Although not directly comparable, the incidence of fistula and abscess formation associated with composite mandibular resection in the human patient at this institution is <2% and <1%,

respectively. Other large series, excluding laryngopharyngeal reconstruction, quote fistula rates ranging from 5% to 15%.^{21–24}

During the course of this study, 2 animals developed a fluid collection (hematoma or seroma) postoperatively. The first animal was recognized within 6 hours of the procedure, and the fluid was effectively evacuated. This event stimulated close observation of all animals in the laboratory for 6 to 8 hours postoperatively. Despite this scrutiny, a second animal developed a fluid collection >10 hours postoperatively. This was recognized on postoperative day 1; however, the animal was deceased. Necropsy demonstrated a large collection of blood in the bilateral deep neck spaces. Although the potential for fluid collection must be appreciated, meticulous intraoperative hemostasis and close postoperative observation are essential. Notably, hemostasis is most effectively performed using 5-0 silk suture ligation for select vessels or battery-operated electrocautery. Chemical cautery should be avoided; we have demonstrated gross and histologic muscular necrosis and apparent systemic toxicity with the judicious application of silver nitrate in a separate animal.

Abscess development and foreign-body aspiration were low-frequency events; however, each should be considered important complications, as they ultimately led to the death or sacrifice of the animal in our series. Although uncommon in this series, abscess development emphasizes the importance of daily observation of animal behavior and inspection of surgical sites. Aspiration in the laboratory rat is an extremely rare phenomenon because of the upper aerodigestive and laryngeal anatomy of the animal. The event reported here was likely secondary to a combination of surgery-induced swallowing dysfunction and residual postoperative sedation; however, the etiology remains unknown.

Finally, 2 intraoperative deaths occurred within 10 minutes of anesthesia induction and shortly after skin incision. Animals were noted to have respiratory arrest followed by subsequent cardiac arrest despite resuscitation efforts. While the etiology of the events is unknown, complications secondary to inhalational anesthetic are suspected.

Conclusion

Composite segmental mandibulectomy is a complicated intervention that is used to treat several pathologic processes of the head and neck. Current reconstructive methods are effective; however, many potential complications exist, and some patients are not candidates for this operation. As a result, a significant body of research has been established in an effort to identify alternative methods of mandibular reconstruction. Prior to this investigation, an effective small animal model for the en bloc segmental mandibulectomy has not been described. We present a novel animal model that reliably replicates this complex reconstructive challenge and allows for the investigation of a wide variety of tissue-engineering techniques and research objectives.

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Figure 1. Supine animal under general anesthesia. A curvilinear incision has been made at the left neck. The masseter (M) is visualized after retracting the overlying fascial flap (F). Note that the submandibular gland (*) has been elevated and retracted.

Figure 2.
Supine animal under general anesthesia. The ventral mandible is exposed after lateralization of the masseter.

Figure 3.
Supine animal under general anesthesia. A synthetic splint (S) has been affixed to the lateral mandible with threaded K-wires (*) in 4 points.

Figure 4. Supine animal under general anesthesia. A titanium miniplate is affixed to the mandible (*) after segmental mandibulectomy. The masseter (M) can be visualized laterally. The strap musculature (S) remains in the midline.

Table 1

Fatal and Nonfatal Complications

	Complication	Number (%) of Patients
Nonfatal	Fistula	6 (8.4)
	Abscess	2 (2.8)
	Seroma	1 (1.4)
Fatal: intraoperative	Unknown/presumed anesthetic reaction	2 (2.8)
Fatal: postoperative	Aspiration (bedding)	1 (1.4)
	Hematoma	1 (1.4)